INTRODUCTION

In unresectable Stage III non-small cell lung cancer (NSCLC), several randomized trials have shown that combinations of chemotherapy and thoracic radiotherapy (TRT) have improved survival compared with radiotherapy alone (1–4). It is important to identify optimal regimens of combined chemotherapy and radiotherapy and to evaluate the feasibility and efficacy of such combinations.

Irinotecan (CPT-11) is an antitumor agent that inhibits the nuclear enzyme topoisomerase I (5). CPT-11 has played a significant role in the development of chemotherapy for NSCLC since the initial reports of its efficacy as a single agent (6,7). Combination chemotherapy of CPT-11 and cisplatin (CDDP), which is also a commonly used agent for NSCLC, is a promising regimen for NSCLC, since its high antitumor activity and manageable toxicity have been reproducibly reported (8,9). One critical but uncommon toxicity of CPT-11 was reported to be pulmonary toxicity (7), and it is necessary to clarify how the chemotherapy regimen should be combined with TRT in patients with Stage III NSCLC.

In addition to combined chemoradiotherapy using full dose of anticancer drugs, concomitant treatment with low doses of anticancer drugs as radiosensitizers has also been investigated in patients with Stage III NSCLC. Schaake-Koning et al. (10). reported that daily low-dose CDDP combined with TRT improved the local control of tumors in a randomized study. CPT-11 has also been investigated as a radiosensitizer (11), and a phase I/II study of weekly administration of low dose CPT-11 combined with TRT has been reported (12). Esophagitis, pneumonitis and diarrhea were the dose-limiting toxicities of weekly irinotecan combined with TRT. The maximum tolerated dose and the recommended dose were 60 and 45 mg/m^2/week, respectively.

Therefore, in order to improve therapeutic outcomes in patients with unresectable Stage III NSCLC, a phase II study of a regimen of two courses of CDDP plus CPT-11 as induction chemotherapy, followed by TRT with weekly low-dose CPT-11 administration, was conducted. The recommended dose of CPT-11 with concomitant TRT was reconsidered and set at 30 mg/m^2/week in order to avoid radiation pneumonitis in this study setting.

PATIENTS AND METHODS

Patient Selection

Patients with histologically or cytologically confirmed, unresectable, Stage III NSCLC who had not received cancer therapy were enrolled in this study. Staging for entry criteria was performed according to the lung cancer staging system of the International Union against Cancer. Staging procedures included chest X-ray, computed tomography (CT) scan of the chest, CT scan or magnetic resonance imaging of the brain, CT scan or ultrasound of the abdomen and isotope bone scanning. *N*-status was mainly based on size criteria on the chest CT scan. Patients with pleural or pericardial effusions were excluded from the study. Each patient was required to meet the following criteria: Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; ≤70 years of age; predicted area of radiation field less than half of one lung; adequate hematological, pulmonary, renal and hepatic functions, i.e. white blood cell (WBC) count ≥4000/μl and ≤12 000/μl, hemoglobin level ≥9.5 g/dl, platelet count ≥100 000/μl, PaO_2 ≥70 torr, %DLco ≥60%, serum creatinine level no higher than the upper limit of normal, serum total bilirubin level ≤1.5 mg/dl and serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase levels less than twice the upper limit of normal. Patients with synchronous or metachronous malignancy, uncontrolled heart failure or diabetes, interstitial pulmonary fibrosis or chronic obstructive pulmonary disease that restricts thoracic radiation, or a history of myocardial infarction in the last 3 months were excluded from the study. Female patients who were pregnant or lactating when chemotherapy was to be given were also excluded. All patients gave their written informed consent.

Treatment Schedule

The treatment schema was shown in Fig. 1. After enrollment in the study, the patients received chemotherapy consisting of intravenous infusion of 80 mg/m^2 of CDDP on day 1 and 60 mg/m^2 of CPT-11 on days 1, 8 and 15. The induction chemotherapy was repeated 4 weeks after the start of the first course, as long as the patients had recovered sufficiently from toxicity. The induction chemotherapy of CPT-11 and CDDP was to be performed for two courses, unless unacceptable toxicity or disease progression occurred.

CPT-11 on day 8 or 15 was skipped if the WBC count was <3000/μl, the platelet count was <100 000/μl, or Grade 2 or higher diarrhea or abdominal pain developed. During chemotherapy, if the WBC count fell to <2000/μl or the neutrophil count dropped to <1000/μl, daily granulocyte colony-stimulating factor (G-CSF) was administered subcutaneously until the WBC count increased to ≥10 000/μl or was no longer clinically indicated. Radiotherapy with concomitant use of G-CSF was contraindicated. When the second course of CDDP plus CPT-11 was started, each patient was required to meet the following

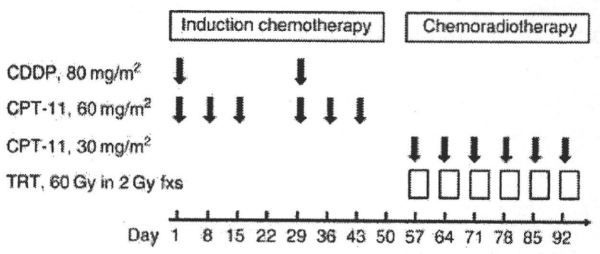

Figure 1. Treatment schema for this study.

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criteria: WBC count $\geq$4000/μl, platelet count $\geq$100 000/μl, serum creatinine level no higher than the upper limit of normal and no episodes of diarrhea. If the second course was delayed 2 weeks or more due to toxicity, chemotherapy with CDDP plus CPT-11 was terminated, and only radiotherapy was given. Based on toxicities during the first course of chemotherapy, the doses of CDDP and CPT-11 were reduced by 20 and 10 mg/m^2, respectively, for febrile neutropenia with Grade 4 leucopenia or neutropenia, Grade 4 thrombocytopenia and Grade 3 or 4 non-hematological toxicity.

Criteria for starting radiotherapy with weekly CPT-11 administration were the same as those mentioned above as the entry criteria of this study. Patients who developed Grade 4 diarrhea during induction chemotherapy with CPT-11 and CDDP were off-treatment. If the same criteria were not fulfilled at 6 weeks after initiation of the second course of chemotherapy, CPT-11 administration was terminated. In that case, only radiotherapy was given.

Four weeks after the start of the second course of CPT-11 plus CDDP, weekly CPT-11 with concomitant TRT was started. The initial opposing anterior–posterior treatment fields encompassed the primary tumor, the bilateral mediastinal lymph nodes and the ipsilateral hilar nodes. The supraclavicular nodes were included within the field when there was clinical evidence of their involvement. A 1.5-cm tumor-free margin was required. The fraction size delivered was 2.0 Gy, 5 days per week. Thus, the total radiation dose was 60 Gy in 30 fractions over 6 weeks. The methods for spinal block and boost after the first 40 Gy deliveries were left to the discretion of the treating radiation oncologist. The patients also received intravenous CPT-11 weekly up to six times with TRT concurrently. CPT-11 was dosed to 30 mg/m^2/week and administered intravenously for 60–90 min immediately before the chest irradiation.

During chemoradiotherapy, if the WBC count fell to $<$2000/μl or the neutrophil count fell to $<$1000/μl, radiotherapy was suspended. After neutropenia improved to Grade 1 or 0, radiotherapy could be restarted. If the platelet count fell to $<$75 000/μl, radiotherapy was suspended until recovery from thrombocytopenia. If patients had a fever of 38°C or higher, chemoradiotherapy was suspended until they were afebrile. If Grade 3 or 4 radiation-related esophagitis was seen, chemoradiotherapy was suspended but could be started again when this toxicity improved to Grade 1 or 0. If the PaO_2 level decreased by 10 torr or more compared to the baseline value, chemoradiotherapy was suspended, and if it returned to baseline, treatment could be restarted carefully. CPT-11 was not administered if the WBC count fell to $<$3000/μl, the platelet count fell to $<$100 000/μl, or Grade 2 or more diarrhea developed on the treatment day.

Treatment Evaluation

Tumor response was evaluated according to the World Health Organization response criteria (13). Response was confirmed by extramural review in this study. Toxicity was evaluated once a week according to the Japan Clinical Oncology Group (JCOG) toxicity criteria (14). The complete blood cell count was checked twice a week. Routine blood chemistry, arterial blood gas and chest radiographs were checked at least once a week until the patient had apparently recovered from all acute toxic effects after the completion of the treatment. After completion of treatment, response was evaluated every 6 months until disease progression or for 2 years, by chest CT, brain MRI or CT, abdominal CT or ultrasound.

Study Design and Statistical Methods

This trial was designed as a multicentre, prospective, single-arm, phase II study and the study protocol was approved by the Clinical Trial Review Committee of JCOG before study activation and the institutional review board of each participating institution before patient enrollment. After pre-treatment staging and eligibility evaluation, patients were registered at the JCOG Data Center by facsimile or by telephone. The study was performed by the JCOG Lung Cancer Study Group and all study data were managed by the JCOG Data Center.

The primary endpoint of this study was the %2 year survival in all eligible patients. The overall survival (OS) was measured from the date of patient registration to the date of death due to any cause. If a patient was alive at the final follow-up, OS was censored at the last contact date. The OS was estimated using the Kaplan–Meier method and the confidence interval (CI) of survival proportion was calculated by Greenwood's formula. The expected and threshold levels of the %2 year OS were set to be 33.0 and 18.9%, which were derived from the expected and threshold value of median survival 15 and 10 months with an assumption of exponential distribution, respectively. With one-sided alpha of 0.10 and beta of 0.10, the planned total sample size for eligible patients was 62. Considering ineligible patients, 65 patients were to be registered with a 1.5 year entry period and a 2 year follow-up period. The analyses were carried out using the SAS release 8.1 (Carry NC.).

RESULTS

Patients' Characteristics

A total of 68 patients from 14 institutions were enrolled in this study between February 1998 and January 1999. The patients' characteristics are listed in Table 1. The patients included 52 men and 16 women, with a median age of 63 (range, 46–70) years. The histological classifications included adenocarcinoma in 34 patients and squamous cell carcinoma in 33. ECOG PS was 0 in 22 patients and 1 in 46. Twelve patients with more than 5% weight loss over 6 months were included. Twenty-eight patients were in Stage IIIA and 40 were in Stage IIIB. All 68 patients were eligible and evaluable for both efficacy and safety.

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Table 1. Patients' characteristics

	Number (%)
Enrolled	68
Eligible	68
Age (years)	
Median	63
Range	46–70
Sex	
Male	52 (76.5)
Female	16
PS (ECOG)	
0	22
1	46
Weight loss	
≥5%	12 (17.6)
<5%	50
Unknown	6
Smoking	
Never	10 (14.7)
Ever	58
Histology	
Adenocarcinoma	34
Squamous cell carcinoma	33
Large cell carcinoma	1
Clinical stage	
IIIA	28
IIIB	40
T-stage	
T1	9
T2	23
T3	11
T4	25
N-stage	
N0	2
N1	6
N2	46
N3	14

ECOG, Eastern Cooperative Oncology Group.

Treatment Delivery and Protocol Compliance

Of the 68 patients enrolled in the study, 38 (55.9%) completed both the scheduled chemotherapy and the radiotherapy. In 18 patients, induction chemotherapy was terminated due to disease progression (4 patients) and unacceptable toxicity (14 patients). Four patients developed disease progression during induction chemotherapy with CDDP plus CPT-11. One patient had stable disease; however, the predicted area of the radiation field exceeded half of one lung. In seven patients, Grade 3 or 4 diarrhea and Grade 4 ileus persisted, whereas in another patient, an allergic skin reaction developed and resulted in termination of induction chemotherapy. Six patients had prolonged toxicity during the induction chemotherapy and could not receive radiotherapy.

Forty-nine patients received TRT according to the protocol and 38 patients received the complete 60 Gy of radiation with weekly CPT-11. Radiotherapy could not be completed for 11 patients; the median dose was 48 (range, 12–56) Gy. The reason for not receiving radiotherapy was disease progression in two patients and toxicity in nine patients.

Toxicity

Toxicity was assessed throughout induction chemotherapy, during CPT-11 concurrent with TRT treatment, and during the follow-up period until 2 years after the date of enrollment. Table 2 presents the toxicity during the induction chemotherapy with CPT-11 plus CDDP and Table 3 shows the acute toxicity for the chemoradiotherapy.

Grade 3 or 4 neutropenia and diarrhea, which were common toxicities during the CPT-11-containing chemotherapy regimen, occurred in 73.5 and 20.6% of the patients, respectively, during the induction chemotherapy. Eleven patients had Grade 2/3 esophagitis during chemoradiotherapy, and it caused termination of the therapy in only one patient. Pulmonary toxicity, which is listed as decreasing PaO_2 or dyspnea in Table 3, developed during the chemoradiotherapy. In nine patients, radiotherapy was terminated due to development of any grades of radiation pneumonitis. There was one treatment-related death due to radiation pneumonitis. This patient had completed the induction chemotherapy of CDDP plus CPT-11 without serious toxicity and moved to the chemoradiotherapy phase. After a radiation dose of 32 Gy, hypoxia and dyspnea developed. The protocol treatment was terminated and the patient was treated with appropriate medication and supportive care. This patient died due to this toxicity at 39 days after the last treatment day.

Response, Survival and Recurrence Pattern

Of the 68 patients, 6 achieved complete responses and 38 achieved partial responses, giving a response rate of 64.7% (95% CI, 52.2–75.9%).

Figure 2 shows the OS curve of all patients enrolled in this study. After follow-up for 26 months after the last enrollment, the median survival time (MST) was 16.5 (95% CI, 12.6–19.8) months. The 1- and 2 year survival rates in the 68 patients were 65.8% (95% CI, 54.4–77.1%) and 32.9% (95% CI, 21.6–44.1%), respectively. For the primary endpoint, the lower limit of 80% CI, which corresponds to one-sided alpha 0.1, of %2 year survival was 25.5% (greater than the prespecified threshold value 18.9%). The overall progression-free survival rate was 26.5% (95% CI,

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Table 2. Toxicity during the induction chemotherapy phase

Item	*N*	JCOG grade					%3–4
		0	1	2	3	4	
Leukopenia	68	4	17	23	21	3	35.3
Neutropenia	68	3	0	15	29	21	73.5
Anemia	68	8	21	24	15	–	22.1
Thrombocytopenia	68	56	8	2	2	0	2.9
Bilirubin	67	53	–	12	2	0	3.0
GOT	68	47	17	3	1	0	1.5
GPT	68	37	22	7	2	0	2.9
ALP	67	42	23	1	1	0	1.5
Creatinine	68	55	13	0	0	0	0
PaO_2	53	29	22	2	0	0	0
Hypercalcemia	67	63	4	0	0	0	0
Hypocalcemia	67	64	2	1	0	0	0
Hyponatremia	68	21	30	10	6	1	10.3
Hypokalemia	68	36	15	12	5	0	7.4
Nausea/vomiting	68	6	22	34	6	–	8.8
Diarrhea	68	13	24	17	10	4	20.6
Stomatitis	68	65	3	0	0	0	0
Esophagitis	68	67	0	1	0	0	0
Infection	68	50	8	7	2	1	4.4
Dyspnea	68	63	1	2	2	0	2.9
Fever	68	45	9	13	0	1	1.5
Neuropathy	68	67	1	0	0	–	0
Constipation	68	44	21	3	0	0	0
Eruption	68	61	2	4	1	0	1.5
Alopecia	68	11	48	9	–	–	–

JCOG, Japan Clinical Oncology Group; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase.

Table 3. Acute toxicity during the chemoradiotherapy phase

Item	*N*	JCOG grade					%3–4
		0	1	2	3	4	
Leukopenia	49	4	10	23	12	0	24.5
Neutropenia	49	12	13	15	6	3	18.4
Anemia	49	1	13	25	10	–	20.4
Thrombocytopenia	49	47	2	0	0	0	0
Bilirubin	49	46	–	3	0	0	0
GOT	49	37	11	1	0	0	0
GPT	49	32	14	2	1	0	2.0
ALP	49	37	11	1	0	0	0
Creatinine	49	47	2	0	0	0	0
PaO_2	46	14	21	8	2	1	6.5
Hypercalcemia	48	45	3	0	0	0	0
Hyponatremia	49	32	12	5	0	0	0
Hypokalemia	49	36	11	2	0	0	0
Nausea/vomiting	49	22	20	7	0	–	0
Diarrhea	49	33	13	3	0	0	0
Stomatitis	49	46	3	0	0	0	0
Esophagitis	49	7	31	9	2	0	4.1
Infection	49	42	5	2	0	0	0
Dyspnea	49	47	1	1	0	0	0
Fever	49	28	5	16	0	0	0
Neuropathy	49	48	1	0	0	–	0
Paralysis	49	48	0	0	1	0	2.0
Constipation	49	38	9	2	0	0	0
Eruption	49	45	3	1	0	0	0
Alopecia	49	11	30	8	–	–	–

JCOG, Japan Clinical Oncology Group; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase.

16.0–37.0%) at 1 year and 13.2% (95% CI, 5.2–21.3%) at 2 years, with a median of 8.8 months (95% CI, 6.8–10.4 months).

The sites of initial failure are shown in Table 4. The primary tumor inside the radiation field was the site of initial failure in 20 patients, while distant metastasis was the cause of failure in 34 patients. In 10 patients, including all three patients who achieved a complete response, there was no evidence of recurrent disease.

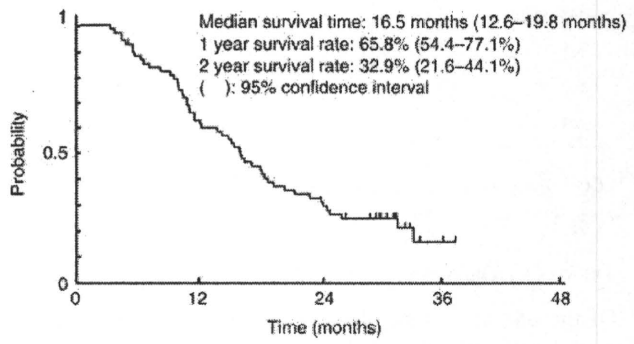

Figure 2. Kaplan–Meier survival curve including all 68 eligible patients.

DISCUSSION

This multi-center, phase II study demonstrated that two cycles of induction chemotherapy with CDDP plus CPT-11, followed by concomitant TRT with weekly low dose CPT-11, was effective with acceptable toxicity in patients with locally advanced NSCLC. The MST was 16.5 months (95% CI, 12.6–19.8 months) and the 1- and 2 year survival

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Table 4. Pattern of failure

Site of initial failure	Patients (*n*)	%
Inside radiation field	20	29.4
Outside radiation field	34	50.0
Overlap above	4	5.9
Response continued	10	14.7
Unknown	0	0

rates in the 68 patients were 65.8% (95% CI, 54.4–77.1%) and 32.9% (95% CI, 21.6–44.1%), respectively. The primary endpoint, the %2 year survival, was met with the prespecified decision criteria in this study.

Although some investigators have reported that concurrent administration of full-dose chemotherapy and TRT is possible, it is considered difficult for many regimens, especially with third-generation agents, such as CPT-11, which are difficult to use at their full doses for concurrent chemoradiotherapy because of the high incidence of toxicity. Therefore, for concurrent chemotherapy with TRT, these chemotherapeutic agents have been used at reduced doses in several reported clinical studies (15–18). In the Japanese trial of Furuse et al. (19), the concurrent combination of TRT with full-dose mitomycin, vindesine and CDDP (MVP), which was an old chemotherapy regimen, was considered to have the best efficacy for locally advanced NSCLC. We conducted a randomized, phase III trial comparing third-generation chemotherapeutic agents of paclitaxel or CPT-11 plus carboplatin with the MVP regimen, with early concurrent TRT in patients with Stage III NSCLC (20). When we selected the investigational arms, the strategy of early concurrent TRT with reduced low-dose chemotherapeutic agents followed by consolidation chemotherapy was considered to be better without obvious clinical evidence. Although the present results are promising, we did not decide to select this strategy of induction chemotherapy followed by concomitant TRT with CPT-11.

Recently, some articles have shown that addition of induction chemotherapy before concurrent chemoradiotherapy increases toxicity and provided no survival benefit (17,18). In the present study, only 49 among 68 patients could receive TRT according to the protocol, although the radiotherapy should be a key treatment option for patients with locally advanced NSCLC. Furthermore, of the 49 patients, 38 (56%) completed 60 Gy of TRT with weekly CPT-11. This lower treatment delivery due to early disease progression and unacceptable toxicity was thought to be critical disadvantages of the strategy for induction chemotherapy followed by chemoradiotherapy. Further studies to investigate the role of induction chemotherapy followed by chemoradiotherapy may not be necessary until more active anticancer drugs appear.

In view of toxicity management, neutropenia and diarrhea were considered to be common toxicities requiring careful management during combination chemotherapy using CPT-11. Pneumonitis and esophagitis induced by CPT-11 and concomitant TRT have been considered serious, but they were easily manageable in the present study. Under the investigator's careful observation, the concurrent TRT with CPT-11 was sometimes terminated due to radiation pneumonitis. In the present study, one patient died due to radiation pneumonitis; this incidence of fatal radiation pneumonitis was considered acceptable. Esophagitis was very mild and less toxic in Japanese patients than in Western patients.

In conclusion, induction chemotherapy with CPT-11 plus CDDP, followed by concomitant TRT with weekly low-dose CPT-11, was feasible and effective with acceptable toxicity according to the prespecified decision criteria in this study. However, lower TRT delivery in this treatment strategy was a critical disadvantage in treatment for patients with locally advanced NSCLC. We did not decide to select this regimen for further investigations.

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Conflict of interest statement

None declared.

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PHASE I STUDIES

Feasibility study of two schedules of sunitinib in combination with pemetrexed in patients with advanced solid tumors

Isamu Okamoto · Toshio Shimizu · Masaki Miyazaki · Junji Tsurutani · Yasuko Ichikawa · Masaki Terashima · Masayuki Takeda · Soichi Fumita · Emiko Ohki · Nobuyuki Kimura · Junichi Hashimoto · Kazuhiko Nakagawa

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Summary *Background* Sunitinib is an oral multitargeted tyrosine kinase inhibitor of vascular endothelial growth factor and platelet-derived growth factor receptors, as well as of other receptor types. We have performed a feasibility study to investigate the safety of sunitinib in combination with pemetrexed for treatment of advanced refractory solid tumors. *Methods* Sunitinib was administered once daily on a continuous daily dosing (CDD) schedule (37.5 mg/day) or a 2-weeks-on, 1-week-off treatment schedule (50 mg/day, Schedule 2/1) in combination with pemetrexed at 500 mg/m^2 on day 1 of repeated 21-day cycles. *Results* Twelve patients were enrolled in the study: six on the CDD schedule and six on Schedule 2/1. None of the treated patients experienced a dose-limiting toxicity. Toxicities were manageable and similar in type to those observed in monotherapy studies of sunitinib and pemetrexed. Pharmacokinetic analysis did not reveal any substantial drug–drug interaction. One patient with squamous cell lung cancer showed a partial response and five patients had stable disease. *Conclusions* Combination therapy with sunitinib administered on Schedule 2/1 (50 mg/day) or a CDD schedule (37.5 mg/day) together with standard-dose pemetrexed (500 mg/m^2) was well tolerated in previously treated patients with advanced solid tumors.

Keywords Sunitinib · Pemetrexed · Feasibility study · Solid tumors

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Introduction

Progress in the molecular biology of solid tumors has established the important role of tumor angiogenesis and the multiple signaling pathways underlying this process in tumor development [1]. Moreover, antiangiogenic therapy that targets signaling by the vascular endothelial growth factor (VEGF) pathway represents a key advance in clinical oncology [2, 3]. Sunitinib (SUTENT®) is an oral multitargeted tyrosine kinase inhibitor of VEGF receptors (VEGFR1 to VEGFR3), platelet-derived growth factor receptors (PDGFRα and PDGFRβ), and other receptor tyrosine kinases [4–6]. It has shown single-agent activity and acceptable tolerability in phase I/II studies of patients with a variety of advanced refractory solid tumors [4]. The clinical benefits observed with sunitinib have resulted in multinational approval for its use in the treatment of patients with advanced renal cell carcinoma or imatinib-resistant or -intolerant gastrointestinal stromal tumors [7, 8].

As targeted agents such as sunitinib enter into clinical practice, there is interest in assessment of the efficacy and safety of these agents administered in combination with chemotherapy in cancer patients, including those with treatment-refractory tumors. Preclinical studies indicate that the combination of sunitinib with chemotherapeutic agents results in increased antitumor activity [9]. One chemotherapeutic agent tested, pemetrexed, is an antimetabolite that suppresses cell replication by inhibiting multiple enzymes in

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the folate pathway and which shows clinical activity against a broad range of solid tumors, including non-small cell lung cancer (NSCLC) and mesothelioma [10–12]. The adverse effects of sunitinib are largely nonoverlapping with those of pemetrexed, making the latter an appropriate agent, in terms of safety, for combination with sunitinib. We have now performed a feasibility study to assess the safety and tolerability of two dosing schedules of sunitinib (continuous daily dosing [CDD] schedule and 2 weeks on treatment followed by 1 week off treatment [Schedule 2/1]) in combination with fixed-dose (500 mg/m^2) pemetrexed.

Patients and methods

Study population

Patients with histologically proven advanced solid tumors and who were 20 years of age or older were enrolled in the study. Other key inclusion criteria included: prior treatment with one or more chemotherapy regimens; an Eastern Cooperative Oncology Group performance status of ≤1; resolution of acute toxicities resulting from prior therapy; adequate organ function; and a life expectancy of ≥3 months. Key exclusion criteria included: prior treatment with pemetrexed or sunitinib or irradiation of≥25% of bone marrow; hemoptysis (≥5 mL per episode or ≥10 mL/day) occurring ≤4 weeks before the onset of study treatment; chemotherapy, surgery, or radiation therapy instituted <4 weeks before the start of the study (with the exception of palliative radiotherapy for nontarget lesions); symptomatic or uncontrolled brain metastases, spinal cord compression, carcinomatous meningitis, or leptomeningeal disease; a history of cardiac disease, cerebrovascular events, or pulmonary embolism within the 12 months prior to the onset of study treatment; ongoing cardiac dysrhythmias of National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) grade≥2, atrial fibrillation (any grade), or a prolonged QTc interval; hemorrhage of CTCAE grade 3 within the 4 weeks before the start of the study treatment; or hypertension that could not be controlled with standard antihypertensive agents.

Study design and treatment

The study was a randomized, open-label study (NCT00732992) of sunitinib in combination with pemetrexed in patients with advanced solid tumors. The primary objective was assessment of overall safety, including dose-limiting toxicities (DLTs), for two treatment regimens of sunitinib plus pemetrexed. Secondary endpoints included plasma pharmacokinetic evaluations and preliminary antitumor activity.

Sunitinib was administered orally, once daily, according to either the CDD schedule or Schedule 2/1. Pemetrexed (500 mg/m^2) was administered as a 10-min infusion on day 1 of a 21-day cycle. Patients were instructed to take 500 μg of folate daily, beginning 1 week before day 1 of cycle 1 until study discontinuation. Vitamin B_{12} (1 g) was injected intramuscularly 1 week before day 1 of cycle 1 and again every 9 weeks until study discontinuation. A phase I dose-escalation trial of sunitinib in combination with pemetrexed conducted outside of Japan had demonstrated tolerability of the combination of sunitinib at 37.5 mg (CDD schedule) or 50 mg (Schedule 2/1) with pemetrexed at 500 mg/m^2 [13]. On the basis of these results, we selected the starting doses of sunitinib in the present study as 37.5 mg for the CDD schedule cohort and 50 mg for the Schedule 2/1 cohort. Doses were interrupted or reduced if adverse events of grade 3 or 4 were observed. Doses were delayed if a patient did not meet the following criteria on the first day of each subsequent cycle: absolute neutrophil count of ≥2,000 cells/μL, platelet count of ≥100,000 cells/μL, and calculated creatinine clearance of ≥45 mL/min. Patients were allowed to undergo a maximum of two dose reductions of either drug; the minimum dose for pemetrexed was 250 mg/m^2 and that for sunitinib was 25 mg/day. Treatment was repeated in a 21-day (3-week) cycle until disease progression, unacceptable toxicity, or withdrawal of patient consent occurred. DLTs were assessed during the first treatment cycle and were used to determine whether the dose or schedule was feasible. They were defined as drug-related toxicities of grade 3 or 4, including neutropenia (grade 3 with infection, grade 4 for ≥7 days, or febrile for >24 h), thrombocytopenia (grade≥3 with bleeding or grade 4 for ≥7 days), lymphopenia accompanied by an opportunistic infection, or any nonhematologic toxicity of grade 3 or 4 for ≥7 days. Initially, six patients were randomized to each dosing schedule (three patients each). If no more than one of the three patients experienced a DLT by day 21 of cycle 1, then an additional six patients (three patients each) were randomized for treatment at the same dose. If ≥2/3 or ≥2/6 patients on a schedule experienced a DLT, the dose was reduced and three additional patients were enrolled.

All patients provided written informed consent. The study was approved by the institutional review board of Kinki University Hospital and was performed in accordance with the International Conference on Harmonization of Good Clinical Practice guidelines, as well as with applicable local laws and regulatory requirements.

Study assessments

Safety was assessed according to CTCAE version 3.0. In patients with measurable disease, objective response was

determined according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 [14].

Pharmacokinetic evaluations

For patients randomized to the CDD schedule, blood samples were collected on day 1 of cycle 2 (sunitinib, before as well as 2, 4, 6, 8, 10, and 24 h after dosing; pemetrexed, before as well as 10 min and 1, 2, 4, 6, 8, 10, and 24 h after dosing) to evaluate pharmacokinetic parameters. For patients randomized to Schedule 2/1, blood samples were collected on day 14 of cycle 1 to determine the trough level of sunitinib. The plasma concentrations of sunitinib, its active metabolite (SU12662), and pemetrexed were measured by validated high-performance liquid chromatography and tandem mass spectrometry, with the lower limits of detection being 0.1 ng/mL for sunitinib and SU12662 and 0.1 μg/mL for pemetrexed. Standard plasma pharmacokinetic parameters were estimated by noncompartmental methods. They included the maximum plasma concentration (C_{max}), plasma predose concentration (C_{trough}), time to C_{max} (T_{max}), area under the plasma concentration-time profile from time zero to 24 h after dosing (AUC_{0-24}), area under the plasma concentration-time profile from time zero to infinity ($AUC_{0-\infty}$), elimination half-life ($t_{1/2}$), oral clearance (CL/F), clearance (CL), and volume of distribution at steady state (V_{ss}).

Statistical analysis

Given the exploratory nature of the study, all analyses were descriptive, with no formal statistical test performed on the data.

Results

Patient characteristics

Twelve patients were enrolled in the study from August to November 2008: six patients for the CDD schedule and six for Schedule 2/1. The most common malignancy in the 12 treated patients was NSCLC (n=9, 75%). All patients received at least one dose of the study treatment. Patient demographic and baseline characteristics are summarized in Table 1.

Treatment delivery

A total of 66 cycles of treatment with sunitinib plus pemetrexed was completed, with a median number of cycles per patient of four for the CDD schedule and five for Schedule 2/1. All 12 patients were ultimately withdrawn from the study, the most common reason for which was disease progression (three patients on the CDD schedule and five patients on Schedule 2/1). Treatment was withdrawn because of adverse events in one patient on each schedule (hemoglobin decrease for the CDD schedule and febrile neutropenia for Schedule 2/1). Seven dose reductions each for sunitinib and pemetrexed were instituted (three for the CDD schedule and four for Schedule 2/1), mainly as a result of myelosuppression.

Safety

All 12 patients were evaluable for safety analysis. None of the patients treated on the CDD schedule or Schedule 2/1 experienced a DLT, whereas all individuals experienced at least one adverse event during the study. The major adverse events during the entire treatment period are shown in

Table 1 Patient characteristics according to dosing schedule

	CDD schedule (n=6)	Schedule 2/1 (n=6)
Median (range) age (years)	55.5 (48–69)	66.0 (57–69)
Male/female (n)	6/0	4/2
ECOG performance status 0/1 (n)	2/4	4/2
Primary malignancy (n)		
NSCLC	6	3
Pancreatic cancer	0	1
Pancreatic neuroendocrine tumor	0	1
Uterine sarcoma	0	1
Previous therapy (n)		
Surgery	2	2
Chemotherapy	6	6
Number of prior regimens (n)		
1	3	5
2	2	1
≥3	1	0

CDD schedule continuous daily dosing of sunitinib (37.5 mg) plus pemetrexed (500 mg/m^2) once every 3 weeks, *Schedule 2/1* 2-weeks-on and 1-week-off dosing of sunitinib (50 mg) plus pemetrexed (500 mg/m^2) once every 3 weeks, *ECOG* Eastern Cooperative Oncology Group, *NSCLC* non-small cell lung cancer

Table 2. The most common nonhematologic toxicities (any grade) across both schedules were fatigue (n=11), taste alteration (n=9), skin discoloration (n=8), anorexia (n=8), and fever (n=8). Nonhematologic toxicities of grade 3 included diarrhea (n=2) as well as fatigue, proteinuria, and dehydration (n=1 each) on the CDD schedule, and an increase in alanine aminotransferase and hypertension (n=1 each) on Schedule 2/1. No nonhematologic toxicities of grade 4 were observed for either schedule. The most common hematologic toxicity of grade 3 or 4 was a decrease in neutrophil number, with six patients (CDD schedule, n=4; Schedule 2/1, n=2) experiencing this

Table 2 Treatment-emergent (all-causality) adverse events (NCI CTCAE version 3.0) occurring with an incidence of ≥2 cases (or of special interest) in patients on either the CDD schedule or Schedule 2/1

Adverse event	CDD schedule (n=6)				Schedule 2/1 (n=6)				Total (n=12)
	Grade				Grade				All grades[a]
	1	2	3	4	1	2	3	4	
Nonhematologic									
Fatigue	3	1	1	0	6	0	0	0	11
Taste alteration	1	2	0	0	6	0	0	0	9
Skin discoloration	5	0	0	0	3	0	0	0	8
Anorexia	3	1	0	0	3	1	0	0	8
Fever	3	1	0	0	3	1	0	0	8
AST increased	4	0	0	0	1	1	0	0	6
Diarrhea	1	0	2	0	2	0	0	0	5
Stomatitis	1	1	0	0	2	1	0	0	5
ALT increased	2	1	0	0	1	0	1	0	5
Hypoalbuminemia	0	2	0	0	1	2	0	0	5
Hand–foot syndrome	2	1	0	0	2	0	0	0	5
Vomiting	2	0	0	0	2	0	0	0	4
Cough	0	3	0	0	1	0	0	0	4
Rash	2	1	0	0	1	0	0	0	4
Eyelid edema	1	0	0	0	2	0	0	0	3
Cheilitis	0	0	0	0	3	0	0	0	3
Nausea	1	0	0	0	1	1	0	0	3
Nasopharyngitis	1	0	0	0	2	0	0	0	3
Proteinuria	0	0	1	0	1	1	0	0	3
Constipation	1	0	0	0	1	0	0	0	2
Edema	2	0	0	0	0	0	0	0	2
Infection	0	0	0	0	0	2	0	0	2
Dehydration	0	1	1	0	0	0	0	0	2
Pain-joint	1	0	0	0	1	0	0	0	2
Headache	2	0	0	0	0	0	0	0	2
Neuropathy	0	0	0	0	2	0	0	0	2
Hypertension	0	1	0	0	0	0	1	0	2
Hypothyroidism[b]	0	0	0	0	0	1	0	0	1
TSH increased[b]	0	1	0	0	0	0	0	0	1
Epistaxis[b]	1	0	0	0	0	0	0	0	1
Hemorrhage[b]	0	1	0	0	0	0	0	0	1
Hematologic									
Platelets decreased	3	1	2	0	3	0	1	0	10
Leukocytes decreased	0	2	3	0	0	4	1	0	10
Neutrophils decreased	0	0	4	1	0	1	2	1	9
Hemoglobin decreased	0	2	1	0	1	1	0	0	5
Lymphopenia	0	0	1	0	0	0	1	0	2
Febrile neutropenia[b]	0	0	0	0	0	0	1	0	1

NCI CTCAE National Cancer Institute Common Terminology Criteria for Adverse Events, *CDD* continuous daily dosing, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *TSH* thyroid-stimulating hormone

[a] No adverse events of grade 5 occurred

[b] Adverse events of special interest occurring with an incidence of <2 on either the CDD schedule or Schedule 2/1

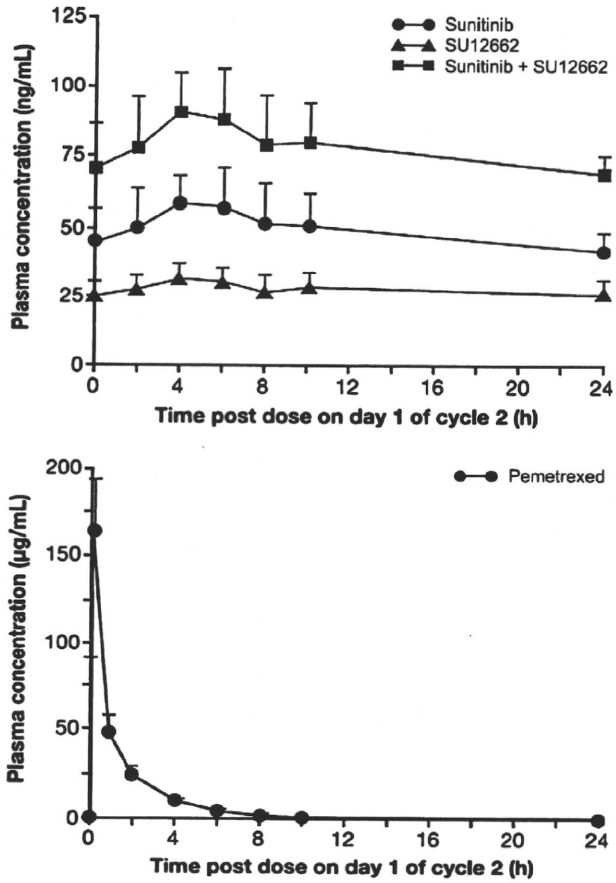

Fig. 1 Plasma concentration-time profiles for sunitinib, SU12662, total drug (sunitinib + SU12662), and pemetrexed on day 1 of cycle 2 for the CDD schedule. Data are means±standard deviation (SD) for three patients

adverse event at grade 3 and two patients (n=1 for each schedule) at grade 4. Other hematologic toxicities of grade 3 or 4 included a decrease in leukocytes of grade 3 in four patients (CDD schedule, n=3; Schedule 2/1, n=1), a decrease in platelets of grade 3 in three patients (CDD schedule, n=2; Schedule 2/1, n=1), and a decrease in hemoglobin level of grade 3 in one patient (CDD schedule).

Adverse events considered to be serious occurred in three patients (CDD schedule, n=2; Schedule 2/1, n=1): one patient on the CDD schedule had dehydration (grade 2), one patient on the CDD schedule had infectious enteritis and dehydration (both of grade 3), and one patient on Schedule 2/1 had pyrexia (grade 2), pneumothorax (grade 1), pleural effusion (grade 1), and febrile neutropenia (grade 3). There were no deaths during the study.

Pharmacokinetics

The mean plasma concentration-time profiles and pharmacokinetic parameters for sunitinib, its active metabolite (SU12662), total drug (sunitinib + SU12662), and pemetrexed for three patients who received the planned treatment on the CDD schedule are shown in Fig. 1 and Tables 3 and 4. The mean C_{trough} for day 1 of cycle 2 was 45.6 ng/mL for sunitinib, 25.1 ng/mL for SU12662, and 70.6 ng/mL for total drug, and each of the corresponding mean plasma concentration-time profiles showed relatively slow absorption and elimination, consistent with previous observations [15]. The pharmacokinetic parameters obtained for sunitinib (37.5 mg) on the CDD schedule with pemetrexed (500 mg/m^2) in the present study did not appear to differ substantially from the dose-normalized parameters previously obtained for single dosing of sunitinib at 25 or 50 mg [15, 16]. The plasma concentration of pemetrexed during sunitinib continuous dosing declined with a fast elimination rate (mean $t_{1/2}$ was 2.75 h), and the $t_{1/2}$, CL, and V_{ss} values were similar to those previously obtained for single dosing of pemetrexed at 500 mg/m^2 [17]. For Schedule 2/1, the mean C_{trough} for day 14 of cycle 1 in six patients who received the planned treatment was 78.5 ng/mL

Table 3 Pharmacokinetic parameters of sunitinib, SU12662, and total drug (sunitinib + SU12662) for the CDD schedule

Parameter	Sunitinib	SU12662	Total drug
C_{trough} (ng/mL)	45.6±11.7 (26)	25.1±5.08 (20)	70.6±13.9 (20)
	[41.3]	[28.0]	[69.3]
T_{max} (h)	4 (4–6)	4 (4–4)	4 (4–6)
C_{max} (ng/mL)	59.9±10.9 (18)	31.6±5.49 (17)	91.56±14.2 (15)
	[59.6]	[34.7]	[94.3]
AUC_{0-24} (ng·h/mL)	1,190±247 (21)	675±107 (16)	1,866±269 (14)
	[1,161]	[665]	[1,951]
CL/F (L/h)	32.4±6.65 (20) [32.3]	ND	ND

CDD continuous daily dosing, *ND* no data, *SD* standard deviation

Data are arithmetic means±SD (coefficient of variation, (%) [median], with the exception of those for T_{max}, which are medians (range). Sampling was performed on day 1 of cycle 2

Table 4 Pharmacokinetic parameters of pemetrexed for the CDD schedule

Parameter	Value
T_{max} (h)	0.167 (0.167–0.167)
C_{max} (μg/mL)	1636±30.7 (19) [167]
$AUC_{0-\infty}$ (μg·h/mL)	1916±36.3 (19) [202]
$t_{1/2}$ (h)	2.7546±0.531 (19) [2.558]
CL (L/h)	4.976±1.38 (28) [4.30]
V_{ss} (L)	10.46±3.13 (30) [10.9]

CDD continuous daily dosing, *SD* standard deviation

Data are arithmetic means ± SD (coefficient of variation, (%) [median], with the exception of those for T_{max}, which are medians (range). Sampling was performed on day 1 of cycle 2

for sunitinib, 38.2 ng/mL for SU12662, and 117.0 ng/mL for total drug. The plasma concentration of sunitinib observed for both schedules was considered to have achieved a steady state on the basis of previous results [15]. The C_{trough} values of sunitinib, SU12662, and total drug observed for both the CDD schedule (sunitinib, 37.5 mg/day) and Schedule 2/1 (sunitinib, 50 mg/day) suggested that the plasma concentrations increased in a dose-dependent manner.

Tumor response

Eight of the 12 patients were evaluable for response by RECIST. A partial response was observed in one patient with NSCLC on the CDD schedule, whereas five patients (two on the CDD schedule and three on Schedule 2/1) had stable disease (Fig. 2a). Most patients showed a decrease in the size of the target lesion while on the study treatment.

Discussion

Our feasibility study investigated the overall safety of sunitinib administered on the CDD schedule or Schedule 2/1 in combination with pemetrexed for the treatment of subjects with advanced refractory solid tumors. Phase I studies of sunitinib monotherapy have been performed according to various schedules, including a 3-week cycle consisting of treatment for 2 weeks followed by a 1-week rest period (Schedule 2/1), a 4-week cycle comprising treatment for 2 weeks followed by 2 weeks off treatment (Schedule 2/2), or a 6-week cycle of treatment for 4 weeks followed by 2 weeks off treatment (Schedule 4/2) [18, 19]. Daily dosing with sunitinib at 50 mg resulted in a target

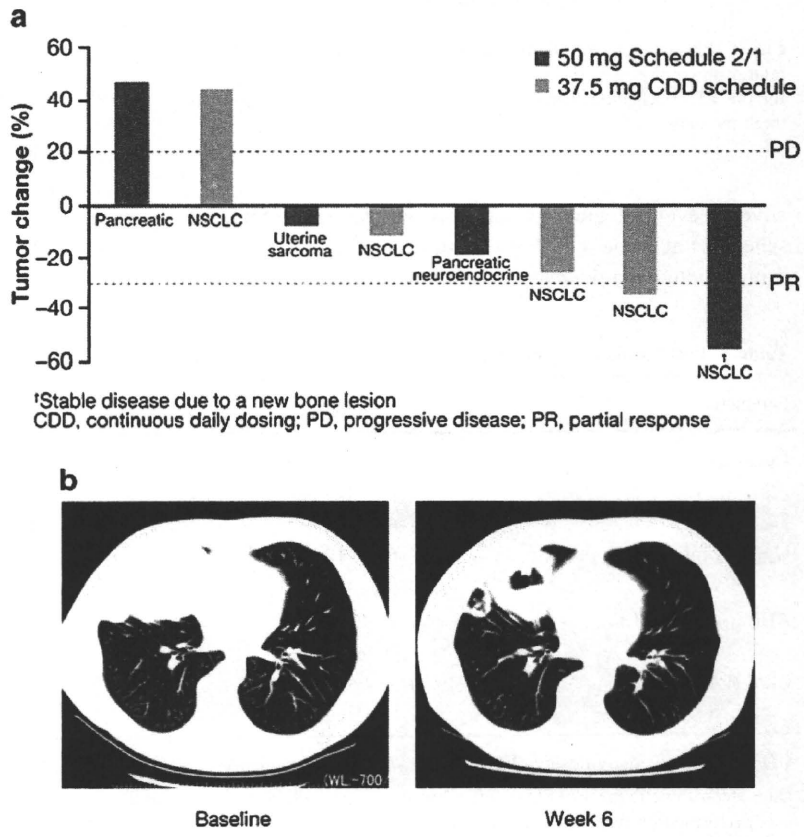

Fig. 2 Tumor response. **a** maximum percentage change in the size of the target lesion in the eight evaluable patients. *PD* progressive disease, *PR* partial response, †stable disease due to a new bone lesion. **b** computed tomography of a solid tumor in the right lung of a patient indicated by † in part **a** at baseline (left panel) and on day 14 of cycle 2 for the CDD schedule. The tumor showed marked central cavitation after treatment

plasma concentration greater than the 50 ng/mL required to inhibit PDGFR and VEGFR, and DLTs of fatigue, asthenia, and thrombocytopenia occurred at a dose of 75 mg on all schedules; a recommended dose of 50 mg was thus established for Schedules 2/1, 2/2, and 4/2 [4]. Preclinical and clinical studies showing tumor regrowth during the off-dosing period suggested that better tumor control might be achieved with sunitinib on a CDD schedule [20, 21]. Subsequent clinical trials demonstrated that CDD of sunitinib at 37.5 mg was well tolerated and showed clinical activity largely similar to that observed for administration on intermittent schedules, providing flexibility in dosing schedule [22–24].

Phase I studies have shown that myelosuppression is the predominant DLT of pemetrexed [25]. We previously found that the maximum tolerated dose of pemetrexed supplemented with folic acid and vitamin B_{12} was 1,200 mg/m^2, which was twice the previously determined such dose (600 mg/m^2) for administration without vitamin supplementation [17, 26]. The results of randomized trials comparing pemetrexed at 500 mg/m^2 versus 900 mg/m^2 or 1,000 mg/m^2 in patients with recurrent NSCLC showed that the higher doses did not exhibit a greater clinical efficacy than the lower dose, thereby establishing the clinically recommended dose of 500 mg/m^2 for pemetrexed supplemented with folic acid and vitamin B_{12} [27, 28].

Given the differences in metabolism and elimination between sunitinib and pemetrexed, we assessed the safety of the combination of recommended doses of these drugs. We initiated treatment with sunitinib at 50 mg/day on Schedule 2/1 or at 37.5 mg on the CDD schedule together with pemetrexed at 500 mg/m^2. There were no DLTs in the 12 patients of the present study who received both drugs at the recommended single-agent doses. Most toxicities were mild or moderate in extent, and similar in type to those observed in the monotherapy studies of sunitinib and pemetrexed. All toxicities of grade 3 or 4 were reversible and manageable with symptomatic treatment and dose reduction or interruption. Hypertension is often associated with treatment with angiogenesis inhibitors, including sunitinib, but this condition developed in only two patients in the present study and, in both cases, blood pressure was controlled with standard antihypertensive therapy. No patients experienced cardiac abnormalities, including electrocardiogram (ECG) changes or a decline in left ventricular ejection fraction to below the lower limit (50%).

In the present study, the full pharmacokinetic profile was evaluated at steady state only for the CDD schedule, given that pharmacokinetic interaction is generally assessed with high drug exposure. The concomitant administration of pemetrexed and sunitinib showed no marked effect on the pharmacokinetics of either drug, compared with previous single-dosing results. These findings suggest that there was no substantial pharmacokinetic interaction between sunitinib and pemetrexed, consistent with the differences in the pathways of metabolism and elimination for these drugs. Sunitinib is primarily metabolized by cytochrome P450-3A4 (CYP3A4) in hepatic microsomes, whereas pemetrexed is not metabolized to an appreciable extent, but is primarily eliminated renally [4, 29]. It is not likely that sunitinib or its metabolites inhibit the renal elimination of pemetrexed. In addition, *in vitro* studies with human liver microsomes suggested that pemetrexed administration is not likely to result in clinically relevant inhibition of the metabolic clearance of drugs metabolized by CYP3A [30]. The trough plasma concentrations for total drug (sunitinib + SU12662) in both treatment arms of the present study suggest that sufficient exposure was achieved with regard to target inhibition, according to the required inhibitory concentration values.

Although tumor evaluation was not the primary objective of the present study, and the small sample size precludes any definitive conclusions regarding treatment efficacy, antitumor activity data were suggestive of a potential clinical benefit. It is possible that further pemetrexed studies might be restricted to patients with non-squamous NSCLC because of the pemetrexed label indications [31]. However, the one partial response in the present study was observed in a patient with squamous NSCLC; the tumor cavitation apparent in this patient after study treatment (Fig. 2b) is characteristic of the antitumor effect of antiangiogenic therapy. Given that sunitinib has shown promising single-agent activity in patients with recurrent NSCLC [22, 32], further research is warranted to determine whether sunitinib might improve the effect of pemetrexed, not only in nonsquamous NSCLC, but also in squamous NSCLC.

In conclusion, combination therapy with sunitinib administered according to Schedule 2/1 (50 mg/day), or a CDD schedule (37.5 mg/day) together with standard-dose pemetrexed (500 mg/m^2), was well tolerated in previously treated patients with advanced solid tumors. In both dosing schedules, sunitinib exposure remained above the target plasma concentration in the presence of pemetrexed. Given that both sunitinib and pemetrexed have shown antitumor activity as single agents for various types of solid tumors including NSCLC, sunitinib in combination with pemetrexed is a viable therapeutic regimen that warrants future investigation.

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ORIGINAL ARTICLE

Phase I and pharmacologic study of BNP7787, a novel chemoprotector in patients with advanced non-small cell lung cancer

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Abstract

Purpose We conducted a phase I trial of BNP7787 (disodium 2,2′-dithio-bis-ethane sulfonate, Tavocept™), a novel chemoprotective and antitumor enhancing agent administered in combination with paclitaxel and cisplatin. The primary aim was to determine a safe and potentially efficacious BNP7787 dose for preventing and mitigating paclitaxel- and cisplatin-induced toxicities and to evaluate for preliminary evidence of efficacy of treatment.

Patients and methods Twenty-two patients with stage IIIB/IV non-small cell lung cancer (NSCLC) received BNP7787 alone 1 week before co-administration of BNP7787 with paclitaxel followed by cisplatin. Twenty-one patients were treated with BNP7787 in escalating doses of 4.1–41.0 g/m^2 concurrently with paclitaxel 175 mg/m^2 and cisplatin 75 mg/m^2 every 3 weeks.

Results The appropriate dose was determined to be 18.4 g/m^2 of BNP7787 although no dose-limiting toxicity was observed up to 41.0 g/m^2. Mild intravenous site discomfort, thirst, and nausea were the most common toxicities. One patient developed grade 2 skin rash, which was severe enough to preclude further study treatment. The $AUC_{0\text{-inf}}$ of the metabolite mesna was approximately 6.3% of the $AUC_{0\text{-inf}}$ of BNP7787. Co-administration of paclitaxel and cisplatin did not appear to influence the pharmacokinetics of BNP7787 and mesna. The overall response rate was encouraging; 43% including 11 patients with prior chemotherapy.

Conclusions The recommended dose for phase II/III studies is 18.4 mg/m^2 of BNP7787 in combination with paclitaxel and cisplatin. Further studies are warranted to assess whether BNP7787 prevents and mitigates common and

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serious paclitaxel- and cisplatin-related side effects and enhances the efficacy of paclitaxel and cisplatin in advanced NSCLC patients.

Keywords Chemoprotector · BNP7787 · Mesna · Phase I study · Lung cancer

Introduction

Lung cancer is the leading cause of cancer-related deaths in most industrialized countries. Platinum-containing doublets have emerged as the standard of care for the first-line treatment of advanced non-small cell lung cancer (NSCLC) [15]. However, many medical oncologists have not routinely used cisplatin-based chemotherapy to treat patients with advanced NSCLC because of serious side effects, such as cisplatin nephrotoxicity and other toxicities. Chemotherapy-induced neuropathy is also a major dose-limiting side effect of many commonly used chemotherapeutic agents, including platinum drugs and taxanes [10, 23]. Evidence from randomized controlled trials support that cisplatin-based doublets result in superior survival advantages over carboplatin-based treatment in advanced NSCLC [5, 16].

Disodium 2,2′-dithio-bis-ethane sulfonate (BNP7787, Tavocept™, dimesna) is an investigational new agent that is being developed as a chemoprotector against cisplatin- and taxane-induced toxicities and to enhance the antitumor effects of chemotherapy [2, 7–9]. BNP7787 is stable and chemically inert in plasma due to the high PO_2 in plasma, presence of a disulfide linkage, and the absence of enzymatic metabolism of disulfides in plasma [7–9, 20]. In nonclinical studies, BNP7787 has been observed to distribute to the kidney, gastrointestinal tract, bone marrow, and neuronal cells, and it is further postulated that a substantial proportion of BNP7787 undergoes non-enzymatic conversion to mesna, one of its metabolites, as well as mixed disulfides which are also pharmacologically active [7–9, 11, 20]. In preclinical studies, a high concentration of BNP7787 and active disulfide metabolites as well as mesna that are generated in the kidney which can provide significant nephroprotection mediated by pharmacologically active mixed disulfides that inhibit gamma-glutamyl transpeptidase toxification, which is postulated to be an underlying major mechanism of cisplatin renal toxicity [11, 20]. In addition, BNP7787 administration can provide nephroprotection by locally inactivating mono-hydrated cisplatin by the formation of non-toxic thioplatinum complexes [11]. Similar cytoprotective effects by BNP7787 have been observed for toxicities associated with paclitaxel and carboplatin [4, 8, 9].

Based on the promising preclinical results of BNP7787, a phase I trial was carried out in patients with advanced NSCLC. The objectives of this phase I study were (*a*) to identify a safe dose of BNP7787 alone and in combination with cisplatin and paclitaxel; (*b*) to describe and quantify the clinical toxicities of BNP7787; (*c*) to determine the pharmacokinetics of BNP7787 and one of its active metabolites, mesna; and (*d*) to obtain preliminary evidence of therapeutic activity in patients with advanced NSCLC.

Patients and methods

GCP compliance

This phase I clinical trial in Japan was conducted in accordance with (1) World Medical Association Declaration of Helsinki: Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects (1996), (2) the Japanese Pharmaceutical Affairs Law and Guidelines for Implementation of Clinical Studies for Drugs (Japanese GCP).

Patient selection

Patients were enrolled in this study if they met all of the following criteria: histologic or cytologic diagnosis of NSCLC; no surgery within 2 weeks, no radiotherapy or chemotherapy within 3 weeks prior to study entry (within 6 weeks for nitrosourea or mitomycin C); a measurable or evaluable lesion; a performance status of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) scale; adequate bone marrow function (leukocyte count, 4,000–120,000/μL; neutrophil count $\geq$1,500/μL; platelet count $\geq$100,000/μL), normal hepatic function (bilirubin $<1.5\times$ the upper limit of normal; aspartate amino-transferase (AST) and alanine amino-transferase (ALT) levels $<2.5\times$ the upper limit of normal), and renal function (creatinine $\leq$ the upper limit of normal and creatinine clearance $\geq$60 mL/min); between 20 and 74 years of age; written informed consent to the study. Patients were ineligible if they had serious infectious diseases or other severe complications (interstitial pneumonia, uncontrollable diabetes or hypertension); atrial fibrillation, serious arrhythmias, or uncontrollable heart failure in previous 6 months; massive pleural or pericardial effusion; symptomatic brain metastases; active concurrent malignancies; lactating or pregnant women, or those attempting to become pregnant; men not willing to use contraception; hearing impairment or neuropathy; had a history of a drug allergy (alcohol, cremophor, etc.); had other medical problems severe enough to prevent compliance with the protocol. The study was approved in advance by the Institutional Review Board and by the Hospital Ethics Committee.

Dosage, dose escalation procedure and drug administration

BNP7787 was supplied from BioNumerik Pharmaceuticals, Inc., San Antonio, TX as a lyophilized powder containing 2 or 10 g of the formulated drug. The starting dose of BNP7787 was 4.1 g/m^2. This starting dose was based on the analysis and calculation of a BNP7787:cisplatin = 50:1 molar ratio derived from the demonstration of partial cisplatin nephroprotection in rats and elucidating this important relationship to formulate and justify a safe starting dose in humans [7], as well as prior safety data from phase I clinical trials. Dose escalation steps of BNP7787 in cohorts of at least three patients each according to the protocol were 8.2, 12.3, 18.4, 27.6, and 41.0 g/m^2. The highest dose tested was justified based on prior non-clinical GLP safety studies, and phase I clinical trials. In the event of dose-limiting toxicity in one patient at any dose level, a maximum of three additional patients would be treated. BNP7787 was given as a single agent by a 30-min intravenous (iv) infusion 1 week prior to chemotherapy so that pharmacokinetic data on BNP7787 alone could be collected and compared and again on day 1. On treatment day 1, paclitaxel dissolved in 500 mL saline was administered intravenously over 3 h at a dose of 175 mg/m^2. Immediately following paclitaxel administration, the calculated dosage of BNP7787 for the given dose level (in a minimum volume of 100 mL) was administered intravenously over 30–45 min, which was immediately followed by intravenous cisplatin 75 mg/m^2 in 500 mL of normal saline administered over 1 h. Study treatment was administered every 3 weeks (one cycle). Prophylactic medications to prevent hypersensitivity reactions to paclitaxel were administered, including iv dexamethasone 20 mg at 12 and 6 h prior to paclitaxel infusion, and diphenhydramine 50 mg po and ranitidine 50 mg iv 30 min before paclitaxel administration for paclitaxel hypersensitivity prophylaxis. Patients received 1.6 L of normal saline and/or electrolyte maintenance fluid prior to BNP7787 administration. Cisplatin was dissolved and infused along a program of forced diuresis that included at least 1.0 L of normal saline and/or electrolyte maintenance fluid, 300 mL of 20% mannitol and furosemide 20 mg iv. Prophylactic antiemetics during and after cisplatin administration consisted of granisetron and dexamethasone.

Patients with evidence of disease progression or those who experienced intolerable toxicity were removed from the study treatment.

Pharmacokinetics

Sample collection

A volume of 5 mL sample of blood was obtained immediately preceding the BNP7787 treatment, 20 min before the end of the BNP7787 infusion, 10 min before the end of the BNP7787 infusion, at the end of the infusion, and at 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, and 24 h after infusion 1 week prior to chemotherapy and day 1 of chemotherapy. The blood samples were transported to the laboratory on ice. Ethylenediaminetetraacetic acid blood samples were centrifuged at room temperature for 1 min at 10,000 rpm. Plasma was separated and immediately deproteinized by adding methanol. All samples were stored frozen at −20°C until analysis.

LC/MS/MS assay

The samples for pharmacokinetic were shipped on dry ice to keep the specimens frozen during shipment to the laboratory. The total amount of BNP7787 and mesna was determined by a validated method using liquid chromatographic–tandem mass spectrometry (LC/MS/MS) at Nemoto Science Co., (Tokyo, Japan; a Finnigan TSQ7000 mass spectrometer (Thermo Electron Co., USA) equipped with an electrospray ionization (ESI) source was used for all measurements. The HPLC separations were performed with a Waters 2690 Separation module (Waters, Milford, MA, USA), using a Mightysil RP-18 GP (3.0 × 150 mm; Kanto Chemical Co., Tokyo, Japan). The system was operated using LCQUAN software (version 1.2; Thermo Electron). The mobile phase was 10 mmol/L ammonium acetate (pH 6.0) at a flow rate of 0.3 mL/min. The ion transfer capillary temperature was 25°C, the nitrogen sheath gas flow rate 80 psi, and the auxiliary gas flow rate setting 15 units. The mass spectra were obtained in a selective reaction monitoring (SRM) mode via negative ESI interface within a range of m/z 141–281 (BNP7787), 81–141 (mesna), respectively. The lower limit of quantification (LLQ) for BNP7787 and mesna was 1.00 and 0.10 μg/mL, respectively. The intra-assay precision and accuracy were satisfactory. Precision was less than 7.1 and 10.0%, accuracy was ranged from −7.0 to 3.7% (−16% at LLQ) and −4.2 to 2.0% for BNP7787 and mesna, respectively. Inter-assay precision was less than 7.9 and 10.0%, accuracy ranged from −10.0 to 7.4% and −6.7 to 2.6% for BNP7787 and mesna, respectively. The contribution ratio (r^2), calculated using 1/X weighed regression analysis were higher than 0.99 for both compounds.

Pharmacokinetic analyses

The following pharmacokinetic parameters of BNP7787 and mesna were estimated using WinNonlin Version 2.1 (Pharsight Corporation, Mountain View, CA). C_{max} was the maximum drug concentration, and T_{max} was the elapsed time to reach C_{max} after the intravenous infusion. $AUC_{0\text{-inf}}$ was area under the concentration–time curve calculated by

Table 1 Patient characteristics

Total no. of patients	22
Sex	
Male	15
Female	7
Age: median (range)	59.5 years (38–74)
Performance status (ECOG)	
0	6
1	16
Previous treatment	
Chemotherapy	9
Chemotherapy + radiotherapy	2
Surgery	3
Surgery + radiotherapy	1
None	7
No. of prior chemotherapy regimens	
Median	1
Range	0–2
Stage	
IIIB	4
IV	18
Histology	
Adenocarcinoma	15
Squamous cell carcinoma	4
Large cell carcinoma	2
Adenosquamous cell carcinoma	1

global summation of the linear trapezoidal rule from time zero up to the last measurable data point at 24 h post administration with extrapolation to infinity. The elimination rate constant (λz) was determined by log-linear regression analysis of the terminal phase of the plasma concentration–time curves. The terminal half-life ($T_{1/2}$) was calculated by the equation: $T_{1/2} = \ln 2/\lambda z$. The mean residence time (MRT) was calculated by the equation of AUMC (area under the first moment – time curve)/AUC – [infusion time]/2. Clearance (CL) was calculated by dividing the actual dose received by the AUC. The volume of distribution at steady state (Vd_{ss}) was calculated by the equation of CL × MRT.

Evaluation

Tumors were staged based on a complete medical history and physical examination, routine chest radiography, bone scintiscanning, computed tomography (CT) of the chest and abdomen, whole-brain magnetic resonance imaging (MRI) or CT scan, and fiberoptic bronchoscopy. Staging was performed according to previous TNM criteria [14]. Prior to the first course of treatment, a complete blood count (including a differential white cell count and platelet count), biochemistry tests (including renal function, hepatic function, and electrolytes), electrocardiogram, and urinalysis were performed. Complete blood count and biochemistry tests were repeated at least twice a week after this initial evaluation, while the other investigations were repeated at least every 6 weeks to evaluate the target lesions. A complete blood count was repeated every day until recovery, when ANC <500/μl, leukocyte count <1,000/μl, or platelet count <10,000/μl was observed during the first cycle of treatment. Adverse events were recorded and graded using the National Cancer Institute Common Toxicity Criteria, Version 2.0. Tumor response was classified in accordance with WHO criteria [13].

Results

Between April 2000 and December 2001, 22 patients participated in this trial. Demographics of the study patient population are shown in Table 1. Seven patients were women and fifteen were men, and the median age was 59.5 years (range: 38–74 years) with a median performance status of 1. Dosing information is shown in Table 2. In this study, a total of 74 cycles of BNP7787 were administered. The number of treatment cycles administered per patient ranged from 1 to 4 (1 cycle in 1 patient, 2 in 4 patients, 3 in 3 patients, and 4 in 14 patients). One patient developed grade 2 skin rash after

Table 2 Dose escalation scheme and treatment administered to patients

Dose level	No. of patients	BNP7787		Chemotherapy		
		Dose (g/m^2)	Total no. of cycles	Paclitaxel (mg/m^2)	Cisplatin (mg/m^2)	Total no. of cycles
1	3	4.1	10	175	75	7
2	6	8.2	18	175	75	12
3	4	12.3	13	175	75	9
4	3	18.4	12	175	75	9
5	3	27.6	9	175	75	6
6	3	41.0	12	175	75	9

Table 3 Worst toxicities due to single agent BNP7787 administration at all dose levels

Side effects	Dose of BNP7787 (g/m^2)																							
	4.1 ($n = 3$)				8.2 ($n = 6$)				12.3 ($n = 4$)				18.4 $n = (3)$				27.6 ($n = 3$)				41.0 ($n = 3$)			
	Grade				Grade				Grade				Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Local iv site discomfort (injection site pain and reaction)	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	3	0	0	0	3	0	0	0
Thirst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Facial flush (feeling of warmth, flushing, hot flush)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0
Skin rash	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Numbness	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Nausea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Vomiting	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Palpitation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Nasal obstruction (nasal obstruction, rhinitis)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0

Table 4 Worst toxicities observed at different dose levels of BNP7787 coadministered with paclitaxel and cisplatin

Side effects	Dose of BNP7787 (g/m^2)																							
	4.1 ($n = 3$)				8.2 ($n = 5$)				12.3 ($n = 4$)				18.4 $n = (3)$				27.6 ($n = 3$)				41.0 ($n = 3$)			
	Grade				Grade				Grade				Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Neutropenia	0	0	0	2	0	0	0	5	0	0	1	3	0	0	1	2	0	0	1	2	0	0	0	3
Leukopenia	0	1	1	0	0	2	3	0	2	0	2	0	1	0	1	0	1	1	1	0	0	2	1	0
Lymphopenia	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0
Anemia (Hb decreased)	0	1	0	0	2	0	0	0	0	1	1	0	1	1	0	0	0	1	0	0	3	0	0	0
Thrombocytopenia	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neutropenic fever	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0
Nausea	2	0	0	0	2	2	1	0	0	1	2	0	2	0	1	0	1	2	0	0	1	0	1	0
Vomiting	0	1	0	0	0	3	1	0	1	0	2	0	0	0	1	0	0	1	0	0	0	0	0	0
Anorexia	2	0	1	0	1	2	0	0	0	2	1	0	2	0	1	0	1	2	0	0	0	2	1	0
Constipation	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	0
General fatigue	1	0	1	0	2	1	0	0	1	1	0	0	2	1	0	0	2	0	1	0	2	1	0	0
Myalgia	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	2	0	0	0	1	0	0	0
Arthralgia	0	1	0	0	2	2	0	0	1	1	1	0	2	1	0	0	2	0	1	0	1	1	0	0
Alopecia	2	1	0	0	1	2	0	0	2	2	0	0	0	2	0	0	1	2	0	0	0	3	0	0
Peripheral neuropathy	3	0	0	0	4	0	0	0	1	1	0	0	2	0	0	0	3	0	0	0	2	0	0	0
Increase in creatinine	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

the first course of BNP7787 alone at the level 2; this condition precluded further protocol treatment. A total of 21 patients and 52 cycles of paclitaxel plus cisplatin were administered (1 cycle in 4 patients, 2 in 3 patients, and 3 in 14 patients). All treated patients were assessed for BNP7787 toxicity, and 21 patients were assessed for toxicity and response to BNP7787 co-administered with paclitaxel plus cisplatin.

Toxicities

Side effects from BNP7787

Generally, toxicities related to BNP7787 administration were very mild. No grade 2 or worse toxicity was observed at all dose levels of BNP7787, except for one instance of grade 2 skin rash observed at dose level 2,

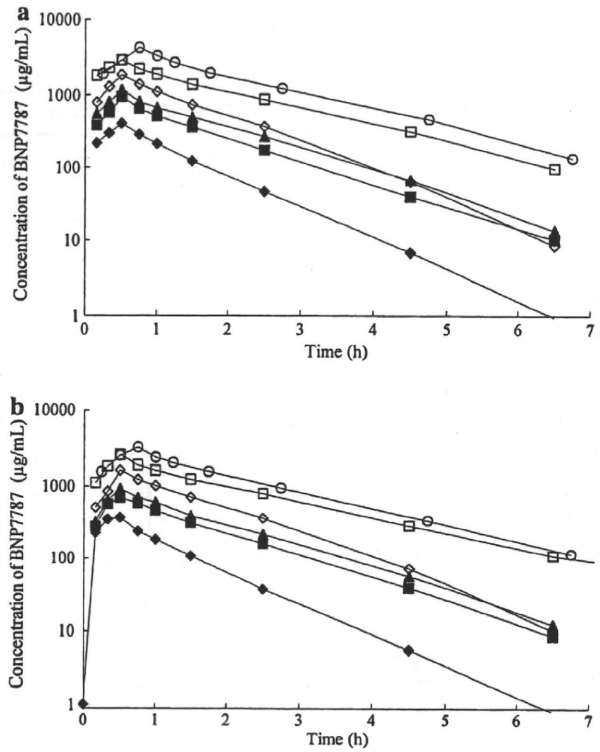

Fig. 1 Plasma concentration–time curves of BNP7787 in patients receiving BNP7787 treatment at dose of 4.1 g/m^2 (*filled diamond*), 8.2 g/m^2 (*filled square*), 12.3 g/m^2 (*filled triangle*), 18.4 g/m^2 (*open diamond*), 27.6 g/m^2 (*open square*) and 41.0 g/m^2 (*open circle*) in alone (**a**) and in combination with paclitaxel and cisplatin (**b**). Each point represents the mean of patients. Infusion time was 30 min except for two patients at a dose level of 27.6 g/m^2 and all three patients at dose level of 41.0 g/m^2

which resulted in cessation of protocol treatment in one patient (Table 3). The only adverse event encountered at dose levels 1 through 4 was grade 1 local iv site discomfort (injection site pain and reaction) in four of 16 (25.0%) patients. At dose levels 5 and 6, which represented BNP7787 dose levels of 27.6 and 41.0 g/m^2, all patients experienced transient, reversible grade 1 local iv site discomfort. At the dose level 5, extension of the infusion time from 30 to 45 min lessened the injection site discomfort. At the highest dose level (41.0 g/m^2), all patients received BNP7787 as a 45-min infusion to lessen the duration, intensity and frequency of injection site pain, based on prior phase I observations [3, 23]. Despite prolongation of the infusion time, all patients complained of discomfort of grade 1 at the local iv infusion site. The local iv site discomfort subsided promptly after completion of the BNP7787 infusion. Other frequently observed BNP7787-related events comprised facial flush (4), thirst (3), nasal obstruction (2), nausea and vomiting (1), and palpitation (1). All these toxicities were grade 1 and disappeared promptly after the end of the infusion.

Side effects from a combination of cisplatin and paclitaxel

Clinically important grade 3/4 non-hematologic toxicities experienced over all courses of treatment included gastrointestinal events of nausea (24% of patients), anorexia (19%), vomiting (19%), and constipation (14%; Table 4). General fatigue was noted in 10% of patients. Joint or muscle pain was reported in 10 or 5%, respectively, of patients. Grade 2 peripheral neuropathy was reported in 5% of patients. No grade 2 or worse renal toxicity was observed in this trial.

The principal grade 3/4 hematologic toxicities were neutropenia (95% of patients), leukopenia (43%) and lymphopenia (5%; Table 4). Grade 3 anemia (5%) was observed. No grade 3/4 thrombocytopenia was observed in this trial. Grade 3 neutropenic fever was noted in 19% of patients. There were no treatment-related deaths during the trial. There were no significant influences of the BNP7787 dose on the occurrence of these adverse events when compared to historically similar patients.

Pharmacokinetics

Plasma samples for BNP7787 were obtained from 22 patients who received BNP7787 alone, and from 21 patients who received BNP7787 in combination with paclitaxel and cisplatin. The plasma concentration–time curves for the different doses of BNP7787 are shown in Fig. 1a, b, and the pharmacokinetic parameters derived from the plotted data are listed in Tables 5 and 6. The plasma concentrations of BNP7787 reached the C_{max} at the end of 30–45 min infusion, and it increased proportionally by the dose. Plasma elimination curves for BNP7787 alone and in combination with chemotherapy were fitted using a one-compartment model with terminal half-life of 0.62–2.33 h and 0.66–1.67 h, with or without chemotherapy, respectively. Formation of the metabolite mesna was rapidly observed following administration of the parent compound, and the mesna C_{max} was reached between 0.5 and 1 h after the end of the BNP7787 infusion. The plasma concentration of the mesna metabolite decreased more slowly than that of BNP7787 with the half-life of 0.88–6.45 h and 0.90–6.28 h, respectively (Fig. 2a, b). No significant differences were observed in pharmacokinetic parameters between BNP7787 alone and in combination with chemotherapy. The ratio of C_{max} of the metabolite mesna to that of BNP7787 ranged from 1.4 to 4.2%; this observation is consistent with prior observations and predicted by the free thiol hypothesis [9]. The mean $AUC_{0\text{-inf}}$ of mesna was approximately 6.2% of the $AUC_{0\text{-inf}}$ of BNP7787. The observed BNP7787 plasma half-life, Vd_{ss}, CL, and MRT also remained relatively constant over the dose ranges studied, regardless of chemotherapy administration. The