Phase 1 trial of denosumab safety, pharmacokinetics, and pharmacodynamics in Japanese women with breast cancer–related bone metastases

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Denosumab, a fully human monoclonal antibody to receptor activator of nuclear factor–kappa B ligand (RANKL), suppresses bone resorption. This open-label, multicenter, phase 1 study evaluated the safety, pharmacodynamics, and pharmacokinetics of denosumab in Japanese women with breast cancer–related bone metastases. Patients (n = 18; median age, 57 years) received a single subcutaneous injection of denosumab 60 mg or 180 mg or three doses of denosumab 180 mg on days 1, 29, and 57 (every 4 weeks) and were followed for ≥ 141 days. No major safety concerns related to denosumab were noted in any cohort. All patients experienced at least 1 adverse event (AE); most were mild (grade ≤ 2). One patient reported grade 4 myositis and grade 3 anemia, malaise, and dysphagia that the investigator deemed treatment-related; other treatment-related AE were grade ≤ 2. No antidenosumab antibodies or clinically significant changes in laboratory findings, vital signs, or electrocardiograms were observed. Pharmacokinetics were approximately dose-linear. Denosumab caused rapid, substantial, and sustained suppression of urinary N-telopeptide corrected for creatinine (uNTx/Cr) across all doses; at day 85, the median change from baseline uNTx/Cr ranged from −61.9% to −90.8%. No dose-limiting toxicity was observed at any dosage. Coupled with pharmacokinetic and pharmacodynamic data, these results were consistent with those observed in non-Japanese populations. (*Cancer Sci* 2008; 99: 1237–1242)

Bone is a common site of metastasis in breast cancer. An estimated 75% of women with advanced breast cancer will develop bone metastases,[1–3] which are characterized by pain, fracturing, and spinal cord compression that cause morbidity for many patients. Receptor activator of nuclear factor–kappa B ligand (RANKL) is a key mediator of the 'vicious cycle' of bone destruction in metastatic cancer. RANKL is a critical mediator of osteoclast differentiation, function, and survival.[4–6] Within the bone microenvironment, tumor cells secrete factors that stimulate stromal cells and osteoblasts to express and secrete RANKL, which binds to its cognate receptor RANK on the surface of precursor and mature osteoclasts. Osteoclast-mediated bone resorption releases growth factors that further stimulate tumor growth, resulting in a propagation of bone destruction and tumor cell proliferation.[7] RANKL has recently been shown to promote migration of RANK-expressing tumor cells to bone.[8]

Patients with bone metastases often have increased bone turnover that can be measured using biochemical markers of bone resorption and formation, such as urinary N-telopeptide (uNTx) and bone-specific alkaline phosphatase (BSAP). Elevated levels of bone turnover markers are correlated with an increased risk of skeletal complications, disease progression, and death.[9–12] A key objective in the management of bone metastases is to minimize skeletal morbidity by re-establishing the homeostasis of bone metabolism. If excessive osteolysis is inhibited, skeletal complications caused by bone metastases may be prevented or delayed.

Denosumab is a fully human monoclonal antibody that binds and inhibits RANKL, thus inhibiting osteoclast-mediated bone destruction. Results from clinical trials in non-Japanese women with breast cancer-related bone metastases showed that denosumab suppressed bone turnover, and the incidence of adverse events was similar in the denosumab and control groups.[2,13] The objectives of this trial were to evaluate the safety, pharmacokinetics, and pharmacodynamics of denosumab in Japanese women with bone metastases associated with breast cancer and to compare the results of this trial with those from a similar study in an analogous population of non-Japanese women (NCT00091832, Clinical Trials.gov).[2,13,14]

Materials and Methods

This study was conducted according to the principles of the Japanese Ministry of Health, Labour, and Welfare, and the International Conference on Harmonisation regulations and guidelines. Institutional Review Boards at each clinical site approved the protocol and all amendments. An Efficacy and Safety Evaluation Committee monitored patient safety during the study as needed. Patients provided appropriate written informed consent.

Study design. In this phase 1 open-label, multicenter, dose-ascending single, and multiple dose study, patients were sequentially enrolled in one of three cohorts. Patients in the first cohort received a single 60-mg subcutaneous injection of denosumab. If no safety signals were observed in the first cohort after 8–10 days, patients were enrolled in the second cohort and received a single 180-mg subcutaneous injection of denosumab. After an 8- to 10-day period for observation of safety of the second dose, patients were enrolled in the third cohort and received three 180-mg subcutaneous injections of denosumab at 4-week intervals (Q4W) on days 1, 29, and 57. Doses were chosen to be comparable with those administered in a study in non-Japanese women with breast cancer and bone metastases.[2] Although no formal stopping rules were specified in the protocol, safety signals that were considered when making dose escalation decisions included adverse events (AE), vital signs, and serum chemistry and hematology values.

Endpoints. The primary endpoint of the study was the subject incidence of AE, including physical findings, changes in laboratory values, vital signs, and 12-lead electrocardiogram

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(ECG) data. Adverse events were classified using the Common Terminology Criteria for Adverse Events version 3.0. Secondary endpoints included serum denosumab levels (pharmacokinetics); the maximum observed serum concentration (C_{max}), time at which C_{max} was reached (T_{max}); the area under the concentration-time curve (AUC_{0-t}); the accumulation ratio (AR, for cohort 3 only), the beta-phase half-life ($t_{1/2,\beta}$, for cohorts 1 and 2 only) after administration of the first dose (pharmacokinetics); the presence of serum antidenosumab antibodies; and the percent change in the bone turnover marker urinary N-telopeptide corrected for creatinine (uNTx/Cr) from baseline to study day 85 (pharmacodynamics). Exploratory endpoints included pharmacokinetic parameter estimates following the last dose; the percent change in uNTx at study day 141 in cohort 3; the percent change in additional bone turnover markers (serum type I collagen cross-link C telopeptide [sCTx], bone-specific alkaline phosphatase, and osteocalcin) from baseline at study day 85 (all cohorts) and day 141 (cohort 3 only); and the proportion of patients experiencing skeletal-related events (SRE), defined as bone fracture, surgery or radiation therapy to bone, and spinal cord compression.

Patient eligibility. Patients were non-pregnant Japanese women with histologically or cytologically confirmed breast cancer and radiographic evidence of at least one bone metastasis, enrolled at three centers in Japan. Eligible patients were 20–74 years of age, with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2 and adequate organ function. Concurrent chemotherapy or hormonal therapy was allowed as long as the regimen did not change within 13 days before or after administration of the denosumab dose. Patients with prior SRE were eligible to participate in the study except for those who had evidence of an impending fracture in weight-bearing bones; major surgery to bone within 4 weeks before the first dose of denosumab; radiation therapy to bone within 2 weeks before the first dose of denosumab; or treatment with radioisotopes directed to bone within 8 weeks before the first dose of denosumab. Other exclusion criteria included cytotoxic chemotherapy within 13 days before denosumab administration; unresolved toxicities > grade 2 from previous chemotherapy regimens; central nervous system metastasis that was symptomatic or required treatment; or prior administration of osteoprotegerin or denosumab or administration of calcitonin, parathyroid hormone-related peptides, mithramycin, gallium nitrate, or strontium ranelate within 6 months; or systemic corticosteroid treatment during the study. Patients were also excluded if they reported or had evidence of disorders that could affect bone metabolism; prior malignancies (excluding the targeted breast cancer, basal cell carcinoma, or cervical cancer *in situ*) within 3 years; uncontrolled systemic disease; major surgery or traumatic injury within 4 weeks; HIV infection; bisphosphonate use within 4 weeks of the first dose of denosumab; or an organic or psychiatric disorder that might prevent the patient from completing the study.

Study procedures. Patients in the 60 mg and 180 mg single-dose cohorts received a single subcutaneous injection of denosumab in the anterior abdominal wall on study day 1. These patients were scheduled to have a total of nine study visits (study days 1, 2, 4, 8, 15, 22, 29, 57, and 85). Patients in the 180 mg Q4W cohort received a subcutaneous injection in the anterior abdominal wall on study days 1, 28, and 57. Patients in this cohort were scheduled to have a total of 12 study visits (study days 1, 8, 15, 22, 29, 57, 64, 71, 78, 85, 113, and 141). Screening assessments included medical and medication histories, physical examination including height and weight measurements, assessment of ECOG PS, hematology, serum chemistry, urinalysis, pregnancy tests, and spinal X-ray imaging. Existing SRE were noted. Throughout the study, physical examinations, monitoring of vital signs, weight measurements, electrocardiograms, and collection of urine and blood were performed periodically. Adverse events, laboratory values, and concomitant medications were recorded and assessed at all study visits. Patients did not receive calcium or vitamin D supplementation in this study.

Statistics and data analysis. The study planned to enroll six patients in each of three treatment cohorts for a total sample size of 18 patients. The safety analysis subset included all patients who received at least 1 dose of denosumab. The pharmacokinetic (PK) analysis subset included all patients in the safety subset who had an evaluable serum denosumab concentration-time profile. Pharmacodynamic evaluations were conducted among patients in the safety subset. Data were reviewed for safety before each dose escalation. Demographics and other baseline characteristics (values obtained 1 week before the first dose administration) were summarized using descriptive statistics. For continuous data, descriptive statistics included mean, median, standard deviation or standard error; and number of subjects, minimum, and maximum. Frequencies and percentages were presented for nominal categorical variables, including the number and percent of subjects.

Results

Patients. The first patient was enrolled on 22 November 2004 and the last patient visit occurred on 26 October 2005. A total of 19 patients were enrolled, including six in each cohort; a seventh patient in the 180 mg single-dose cohort withdrew at the physician's discretion before receiving denosumab because of disease progression; this patient was not included in any results. All patients were Japanese women. The overall median age was 57 years (range, 28–67 years) (Table 1). Patients in the 60 mg and 180 mg Q4W cohorts were of similar ages; patients in the 180 mg single-dose cohort had a median age of 47 years (range, 28–61 years). All women had an ECOG PS of 0 or 1. The median time since the original diagnosis was 6.2 years (range, 0.1–19.1), and the median time since the diagnosis of bone metastasis was 0.31 years (range, 0–5.6). Prior to the study, eight patients (44%) had never experienced an SRE, six patients (33%) had experienced only one SRE, and four patients (22%) had experienced two or more SRE (Table 1).

Safety

Adverse events. No deaths occurred during the study, and no patients withdrew from the study because of AE. All 18 patients who received at least one dose of denosumab experienced at least one AE during the study, most of which were mild. The most common AE were fatigue, anorexia, headache, malaise, and nausea (Table 2).

Two patients, both in the 180 mg single-dose group, reported serious AE (Table 2). One of these patients reported grade 4 myositis and grade 3 anemia, dysphagia, and malaise that were deemed by the investigator to be treatment-related; she also experienced grade 4 metastatic brain cancer and depression and grade 3 herpes zoster infection and liver disorder that were not treatment-related. The other patient experienced febrile neutropenia (absolute neutrophil count $< 1.0 \times 10^9$/L, temperature ≥ 38.5°C), which was not deemed treatment-related and was resolved with outpatient treatment. Two patients experienced mild, asymptomatic hypocalcemia (grades 1 and 2) that was deemed treatment-related. Nine patients reported other grade 1 or 2 AE that were deemed by investigators to be treatment-related (blurred vision, nausea, chest pain, fatigue, decreased white blood cell count, hyperkalemia, arthropathy, muscle spasms, pain in extremity, hypoesthesia, seborrheic dermatitis, and hot flush). No SRE occurred during the study.

Laboratory findings, vital signs, and ECG results. No clinically significant changes were observed in laboratory findings or vital signs except for the anemia, neutropenia, and liver disorder described above. Abnormal findings in ECGs were observed in four patients in the 60 mg cohort after dosing with denosumab.

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Table 1. Baseline patient demographics and disease characteristics

	60 mg SC (single dose) (n = 6)	180 mg SC (single dose) (n = 6)	180 mg Q4W (3 doses) (n = 6)	Total (n = 18)
Sex – (%)				
Female	6 (100)	6 (100)	6 (100)	18 (100)
Race – n (%)				
Japanese	6 (100)	6 (100)	6 (100)	18 (100)
Age – years				
Median (min, max)	58 (52, 66)	47 (28, 61)	60 (47, 67)	57 (28, 67)
ECOG PS – n (%)				
0	3 (50)	3 (50)	4 (67)	10 (56)
1	3 (50)	3 (50)	2 (33)	8 (44)
Hormone receptor status – n (%)[†]				
Negative	4 (67)	0 (0)	0 (0)	4 (22)
Positive	2 (33)	5 (83)	6 (100)	13 (72)
Unknown	0 (0)	1 (17)	0 (0)	1 (6)
Time since original diagnosis – years				
Median (min, max)	6.4 (0.9, 10.4)	3.3 (0.1, 12.8)	7.4 (1.6, 19.0)	6.2 (0.1, 19.0)
Time since bone metastases – years				
Median (min, max)	0.38 (0.0, 3.8)	0.32 (0.1, 2.5)	0.25 (0.1, 5.6)	0.31 (0.0, 5.6)
Total number of previous SRE – n (%)				
0	1 (17)	4 (67)	3 (50)	8 (44)
1	3 (50)	1 (17)	2 (33)	6 (33)
2	1 (17)	1 (17)	0 (0)	2 (11)
3	1 (17)	0 (0)	0 (0)	1 (6)
>3	0 (0)	0 (0)	1 (17)	1 (6)

ECOG, Eastern Cooperative Oncology Group; PS, performance status; Q4W, every 4 weeks; SC, subcutaneous; SRE, skeletal-related events.
[†]Tumors were screened for expression of the estrogen receptor (ER) or progesterone receptor (PR).

Table 2. Adverse events summary

Event – n (%) (patients)	60 mg SC (single dose) (n = 6)	180 mg SC (single dose) (n = 6)	180 mg Q4W (3 doses) (n = 6)	Total (n = 18)
All AE	6 (100)	6 (100)	6 (100)	6 (100)
Serious AE	0 (0)	2 (33)[†]	0 (0)	2 (11)
Treatment-related AE	3 (50)	3 (50)	3 (50)	9 (50)
Serious treatment-related AE	0 (0)	1 (16.7)[‡]	0 (0)	1 (5.6)
Deaths on study	0 (0)	0 (0)	0 (0)	0 (0)
Adverse events occurring in 2 or more patients				
Fatigue	2 (33)	1 (17)	2 (33)	5 (28)
Anorexia	2 (33)	1 (17)	1 (17)	4 (22)
Headache	2 (33)	0 (0)	2 (33)	4 (22)
Malaise	1 (17)	3 (50)	0 (0)	4 (22)
Nausea	1 (17)	2 (33)	1 (17)	4 (22)
Arthralgia	2 (33)	0 (0)	1 (17)	3 (17)
Constipation	1 (17)	1 (17)	1 (17)	3 (17)
Diarrhea	2 (33)	0 (0)	1 (17)	3 (17)
Metastases to bone	1 (17)	1 (17)	1 (17)	3 (17)
Edema	1 (17)	1 (17)	1 (17)	3 (17)
Shoulder pain	1 (17)	1 (17)	1 (17)	3 (17)
Stomatitis	2 (33)	1 (17)	0 (0)	3 (17)
Alopecia	2 (33)	0 (0)	0 (0)	2 (11)
Chest pain	1 (17)	1 (17)	0 (0)	2 (11)
Hot flush	0 (0)	1 (17)	1 (17)	2 (11)
Hypoesthesia	0 (0)	0 (0)	2 (33)	2 (11)
Hypocalcemia	1 (17)	1 (17)	0 (0)	2 (11)
Insomnia	0 (0)	2 (33)	0 (0)	2 (11)
Metastases to liver	0 (0)	1 (17)	1 (17)	2 (11)
Nasopharyngitis	1 (17)	1 (17)	0 (0)	2 (11)
Neutrophil count decreased	2 (33)	0 (0)	0 (0)	2 (11)
Pain in extremity	1 (17)	1 (17)	0 (0)	2 (11)
White blood cell count decreased	2 (33)	0 (0)	0 (0)	2 (11)

[†]Includes 1 patient with grade 4 myositis and 1 patient with grade 3 febrile neutropenia.
[‡]Includes 1 patient with grade 4 myositis.
AE, adverse events; SC, subcutaneous.

Table 3. Pharmacokinetic parameters following denosumab administration

Dose	Number of doses	C_{max} mean (SD) (μg/L)	T_{max} median (range) (day)	AUC_{0-t} mean (SD) (μgday/L)	$t_{1/2,\beta}$ mean (SD) (day)
60 mg	Single	7.73 (3.13)	8.0 (7.0–28)	351.0 (144.0)	24.7 (2.44)
180 mg	Single	31.10 (14.9)	10 (4.0–28)	1320.0 (640.0)	29.1 (7.15)
180 mg Q4W	1	24.10 (5.13)	18 (7.0–28)	545.0 (123.0)	NA
	3	48.0 (9.34)	14 (7.0–21)	1210.0 (240.0)	NA

C_{max} = Maximum observed serum concentration.
T_{max} = Time at which C_{max} was observed.
AUC_{0-t} = Area under the concentration-time curve from time zero to the time of the last observation (which corresponds to AUC_{0-84} for cohorts 1 and 2, and AUC_{0-28} for cohort 3).
$t_{1/2,\beta}$ = Beta-phase half-life.
NA, not applicable.

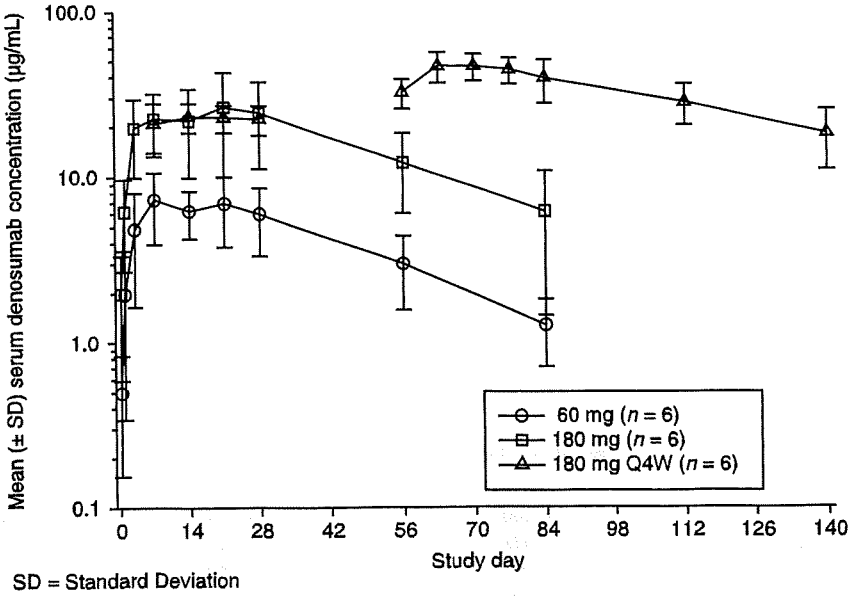

Fig. 1. Denosumab demonstrated approximately dose-linear pharmacokinetics. Serum concentration-time profiles were biphasic, with an absorption phase and a beta phase that immediately followed peak concentrations. Mean half-life values associated with the beta phase ($t_{1/2,\beta}$) were comparable between the two single-dose cohorts (25–29 days).

The findings included transient sinus tachycardia, supraventricular extrasystole, transient ST elevation; one patient with previously documented atrioventricular (AV) block experienced transient first-degree AV block. None of these were considered as AE although the AV block was considered clinically significant by the investigator. No antidenosumab antibodies were observed.

Pharmacokinetics. Denosumab demonstrated approximately dose-linear pharmacokinetics over the dose range investigated. Absorption of denosumab appeared to be rapid, with maximal exposures observed in median times of 8–10 days after a single dose and 14–18 days after Q4W dosing. In the 180 mg Q4W cohort, an approximate 2.2-fold accumulation was observed from the first dose to the third dose. The serum denosumab concentration increased in an approximately dose-proportional manner, with a four-fold increase in mean C_{max} and 3.8-fold increase in AUC_{0-t}-values (Table 3). Serum concentration-time profiles were biphasic, with an absorption phase and a beta phase that immediately followed peak concentrations. Mean half-life values associated with the beta phase ($t_{1/2,\beta}$) were comparable between the two single-dose cohorts (25–29 days) (Fig. 1).

Pharmacodynamics. The suppression of uNTx/Cr was rapid, substantial, and sustained (Table 4). At day 85, the median percent changes from uNTx/Cr values at baseline were –91% (range, –23% to –93%) in the 60 mg single-dose group, –62% (range, –74% to +54%) in the 180 mg single-dose group, and –85% (range, –69% to –98%) in the 180 mg Q4W group. These values exclude five patients (two in the 180 mg single-dose group, three in the 180 mg Q4W group) whose baseline values were below quantifiable limits (BQL). By day 2, the median change in uNTx/Cr was –70% (range, –85% to +10%) in the 60 mg group and –70% (range, –80% to –65%) in the 180 mg single-dose group. In the 180 mg Q4W group, by week 2 (the first visit after administration of denosumab), uNTx/Cr changed a median of –64% (range, –10% to –96%). At day 141, the median percent change in uNTx/Cr in the 180 mg Q4W group (three patients) was –63% (range, –60% to –96%).

At day 85, the median percent change from baseline sCTx values was –89% (range, –68% to –97%) in the 60 mg group, –76% (range, –84% to +206%) in the 180 mg single-dose group, and –80% (range, –90% to +53%) in the 180 mg Q4W group (Table 4). These results reflect the exclusion of two patients in the 180 mg Q4W group because of missing data and a baseline BQL value. At day 141, the median percent change in sCTx in the 180 mg Q4W group was –80% (range, –62% to –93%). Effects of denosumab therapy on bone turnover markers are summarized in Table 4.

Comparison with results in non-Japanese women. In a randomised, phase 2, dose-ranging study in women with breast cancer (study NCT00091832, Clinical Trials.gov),[2] five denosumab dosing regimens were evaluated in 212 non-Japanese women with breast cancer and bone metastases who were bisphosphonate-

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Table 4. Denosumab effects: Changes from baseline in bone turnover markers

Bone turnover marker	60 mg SC (single dose)	180 mg SC (single dose)	180 mg Q4W (3 doses)	Total
	n = 6	*n* = 4	*n* = 3	*n* = 13
uNTx/Cr (nmol/mmol) – median percentage change (min, max)†				
Baseline	109 (19, 233)	55 (21, 60)	29 (20, 389)	60 (19, 389)
Day 2	–70 (–85, 9)	–70 (–80, –65)	–	–69 (–85, 9)
Week 2	–82 (–94, –40)	–75 (–90, –9)	–64 (–96, –10)	–77 (–96, –9)
Day 85	–91 (–93, –23)	–62 (–74, 54)	–85 (–98, –69)	–85 (–98, 54)
Day 141	–	–	–63 (–96, –60)	–63 (–96, –60)
sCTx (ng/mL) – median percentage change (min, max)†	*n* = 6	*n* = 6	*n* = 4	*n* = 16
Baseline	0.5 (0.2, 1.8)	0.3 (0.1, 0.9)	0.2 (0.2, 1.7)	0.3 (0.1, 1.8)
Day 2	–69 (–83, –54)	–63 (–75, –8)	–	–65 (–83, –8.1)
Week 2	–81 (–93, –69)	–77 (–85, –31)	–77 (–92, –50)	–80 (–93, –31)
Day 85	–89 (–97, –68)	–76 (–84, 206)	–80 (–90, 53)	–82 (–97, 206)
Day 141	–	–	–80 (–93, –62)	–80 (–93, –62)
BSAP (U/L) – median percentage change (min, max)	*n* = 6	*n* = 6	*n* = 6	*n* = 18
Baseline	30.4 (25.0, 44.3)	29.8 (17.9, 63.7)	26.9 (15.3, 114.4)	29.0 (15.3, 114.4)
Day 2	–0.8 (–7.8, 14.7)	1.9 (–11.8, 9.5)	–	–0.2 (–11.8, 14.7)
Week 2	0.4 (–25.4, 12.1)	3.3 (–23.9, 10.3)	0.7 (–18.4, 16.5)	1.7 (–25.4, 16.5)
Day 85	–48.3 (–60.0, 35.6)	–42.9 (–78.5, –9.5)	–34.3 (–63.2, 23.5)	–45.6 (–78.5, 35.6)
Day 141	–	–	–53.4 (–68.5, –12.5)	–53.4 (–68.5, –12.5)
Osteocalcin (ug/L) – median percentage change (min, max)	*n* = 6	*n* = 6	*n* = 6	*n* = 18
Baseline	13.8 (7.2, 17.6)	9.6 (4.2, 13.3)	10.9 (4.1, 30.5)	12.2 (4.1, 30.5)
Day 2	13.6 (–5.3, 21.6)	–3.2 (–40.2, 50.0)	–	1.4 (–40.2, 50.0)
Week 2	21.1 (9.5, 34.1)	12.5 (–4.0, 84.4)	22.9 (–15.9, 79.0)	18.2 (–15.9, 84.4)
Day 85	–28.0 (–50.3, –21.7)	–31.6 (–57.1, –18.8)	–7.7 (–57.9, 63.9)	–29.1 (–57.9, 63.9)
Day 141	–	–	– 41.7 (–63.5, –1.5)	–41.7 (–63.5, –1.5)

BSAP, bone-specific alkaline phosphatase; SC, subcutaneous; sCTx, serum type I collagen cross-link C-telopeptide; uNTx/Cr, urinary N-telopeptide corrected for creatinine.
†Patients whose baseline values were below quantifiable limits were excluded from this analysis.

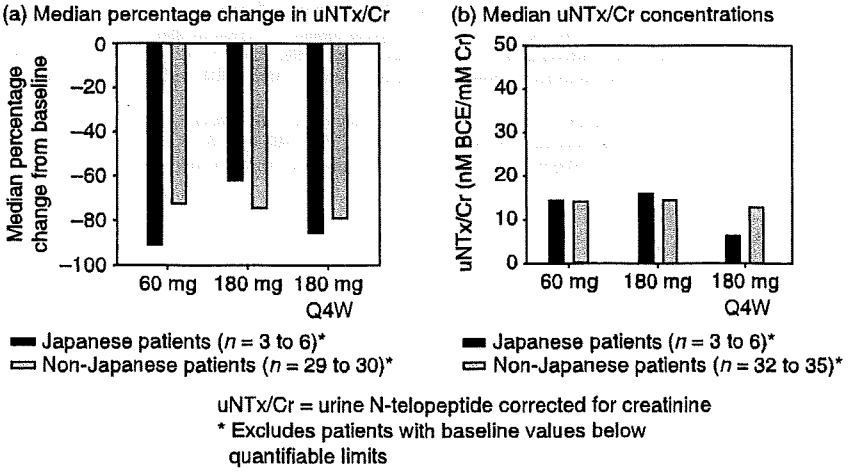

Fig. 2. At comparable dosing levels, results of Japanese patients were similar to those of non-Japanese patients for the median percent change from the baseline in (a) uNTx/Cr and (b) median uNTx/Cr concentrations. Q4W, every 4 weeks; uNTx/Cr, urine N-telopeptide corrected for creatinine.

naive. At comparable dosing levels after excluding patients with baseline BQL levels of uNTx/Cr, results in the current study were similar to those in the non-Japanese study for the median percent change from baseline for uNTx/Cr (Fig. 2) and the median uNTx/Cr concentrations (Fig. 2b). No marked differences were seen in safety profiles or in serum concentrations or other PK parameters between Japanese and non-Japanese patients.

Discussion

As expected, the adverse event profile in this study was similar to that observed in advanced cancer patients undergoing systemic therapy, with no dose-limiting toxicities observed. In a phase 2 study of non-Japanese women with metastatic breast cancer, denosumab treatment was not associated with any severe or serious treatment-related adverse events or with any dose-dependent increase in adverse events.[2] The only grade 4 AE that was deemed treatment-related by the investigator was myositis, although the investigator considered paraneoplastic syndrome to be the primary possible etiology of the myositis. This patient, who had active metastatic disease, exhibited substantially elevated levels of creatine phosphokinase (CPK) at baseline. She developed a further elevation in CPK levels and proximal muscular weakness with myalgia in the extremities on day 29. The histological findings indicated non-specific myositis with no apparent evidence of neurogenic change, collagen disorder, or viral infection. The patient was taking three concomitant medications (goserelin acetate, tamoxifen citrate,

and loxoprofen sodium) that the investigator considered to be potentially suspect. Because of these factors, it is difficult to establish the role of denosumab in the development of myositis. This event was resolved after treatment with steroids.

The pharmacokinetics of denosumab with respect to dose and time were consistent in this population with results observed in analogous non-Japanese populations. The range of baseline uNTx/Cr values observed in this study (19–389 nmol/mmol) was within the range observed in the phase 2 study of non-Japanese patients with breast cancer (5 nmol/mmol to 942 nmol/mmol),[2] and baseline variability among patients was similar in the two studies. Median percent changes in uNTx/Cr were also similar in both Japanese and non-Japanese populations regardless of the median baseline uNTx/Cr levels. No relationship was observed between baseline uNTx/Cr levels and the median percent change in uNTx/Cr.[2] In this study, the suppression of the bone turnover marker uNTx/Cr was rapid (occurring within 24 h), substantial (≥60%), and sustained (up to 12 weeks). These results are comparable to those seen in non-Japanese patients, in which median reductions were ≥70%.[2,13,14] The safety, pharmacokinetic, and pharmacodynamic profiles in denosumab-treated Japanese women were not markedly different from those in non-Japanese populations.[2,13,14] These results demonstrate that the 120 mg Q4W regimen identified in a phase 2 study of non-Japanese patients[14] is appropriate for Japanese as well as non-Japanese patients, a conclusion supported by the Japanese investigators in the current study. This study supports further investigation of denosumab for treatment of bone metastases and the prevention and treatment of SREs in Japanese patients with breast cancer. Multiple global phase 3 trials of denosumab are in progress for patients with advanced cancer and bone metastases; underlying malignancies include breast cancer (including Japanese patients), prostate cancer and other solid tumors, and multiple myeloma.

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Phase I and II pharmacokinetic and pharmacodynamic study of the proteasome inhibitor bortezomib in Japanese patients with relapsed or refractory multiple myeloma

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The purpose of this phase I and II study was to evaluate the safety, pharmacokinetics, pharmacodynamics, and efficacy of bortezomib in Japanese patients with relapsed or refractory multiple myeloma. This was a dose-escalation study designed to determine the recommended dose for Japanese patients (phase I) and to investigate the antitumor activity and safety (phase II) of bortezomib administered on days 1, 4, 8, and 11 every 21 days. Thirty-four patients were enrolled. A dose-limiting toxicity was febrile neutropenia, which occurred in one of six patients in the highest-dose cohort in phase I and led to the selection of 1.3 mg/m² as the recommended dose. Adverse events ≥ grade 3 were rare except for hematological toxicities, although there was one fatal case of interstitial lung disease. The overall response rate was 30% (95% confidence interval, 16–49%). Pharmacokinetic evaluation showed a biexponential decline, characterized by a rapid distribution followed by a longer elimination, after dose administration, whereas the area under the concentration–time curve increased proportionately with the dose. Bortezomib was effective in Japanese patients with relapsed or refractory multiple myeloma. A favorable tolerability profile was also seen, although the potential for pulmonary toxicity should be monitored closely. The pharmacokinetic and pharmacodynamic profiles of bortezomib in the present study warrant further investigations, including more relevant administration schedules. (*Cancer Sci* 2008; 99: 140–144)

Multiple myeloma, one of the B-cell lymphatic tumors, is a malignant hematopoietic tumor with poor prognosis for which a cure cannot ever be expected. The peak age of onset is high at 65–70 years, and its onset in patients younger than 40 years is rare. The median survival of patients with multiple myeloma is approximately 6–12 months if untreated, but it is prolonged to approximately 3 years with the administration of chemotherapy; the 5-year survival rate has been reported to be approximately 25% and the 10-year survival rate is <5%.[1,2] As initial therapy for multiple myeloma, melphalan + prednisolone therapy and vincristine + doxorubicin + dexamethasone therapy have been used as global standards.[3,4] High-dose chemotherapy combined with autologous hematopoietic stem-cell transplantation is reported to be significantly superior to multiagent chemotherapy in terms of response rate and progression-free survival,[5] and is considered to be a standard therapy primarily for patients who are 65 years old or younger. However, no consensus has been reached on the standard therapy for relapsed or chemotherapy-refractory multiple myeloma patients.[6–8] Multiple myeloma is an intractable disease with poor prognosis that continues to relapse, and the duration to relapse becomes shorter in patients who repeatedly receive treatment. There are no available treatment options in which durable efficacy can be expected after relapse, and therefore effective therapeutic choices with new mechanisms of action have been long awaited.

Bortezomib is a novel small molecule that is a potent selective and reversible inhibitor of the proteasome, and has been approved for the treatment of recurrent or refractory multiple myeloma in the USA and Europe. The pharmacokinetics (PK) of bortezomib were reported in a phase I study in which it was administered in combination with gemcitabine twice weekly for 2 weeks followed by a 10-day rest period,[9] and in another phase I study in which it was administered once weekly for 4 weeks followed by a 13-day rest period.[10] Both studies were conducted in patients with advanced solid tumors and not patients with multiple myeloma. Therefore, the present phase I and II study was designed to assess the PK and pharmacodynamic (PD) effects of bortezomib in multiple myeloma patients, particularly in a Japanese population. In addition, efficacy and safety were evaluated to determine the recommended dose (RD).

Patients and Methods

Eligibility. The main eligibility criteria were: confirmed multiple myeloma according to the South-west Oncology Group diagnostic criteria;[11] had received at least previous standard front-line therapy (including melphalan and predonisone, vincristine, doxorubicin, and dexamethasone chemotherapy, and high-dose chemotherapy with autologous stem cell transplantation); had documentation of relapse or refractoriness to the last line of therapy and required therapy because of progressive disease at enrolment. Progressive disease was defined as at least one of the following: more than 25% increase in monoclonal immunoglobulin in the serum or urine; development of new osteolytic lesions or soft tissue tumors, or worsening of existing lesions; hypercalcemia (corrected serum calcium value of >11.5 mg/dL); relapse from complete response (CR); the presence of measurable disease lesions; Karnofsky performance status ≥ 60; 20–74 years of age; adequate bone marrow function (absolute neutrophil count ≥ 1000/mm³, platelets ≥ 75 000/mm³, and hemoglobin ≥ 8 g/dL),

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hepatic function (aspartate aminotransferase and alanine aminotransferase levels ≤2.5 times the upper limit of institutional normal range, total bilirubin ≤1.5 times the upper limit of institutional normal range), renal function (creatinine clearance ≥ 30 mL/min), and cardiac function (left ventricular ejection fraction ≥ 55% by echocardiography without New York Heart Association class III to IV congestive heart failure) in the previous 2 weeks; and had received no systemic chemotherapy or radiotherapy in the previous 4 weeks. This study was approved by the Institutional Review Board of each participating hospital. All patients gave written informed consent and the study was conducted in accordance with Good Clinical Practice for Trials of Drugs and the Declaration of Helsinki.

Study design. The RD was determined based on the occurrence of dose-limiting toxicity (DLT) in Japanese patients and in the dose-escalating phase I of the study. The safety and efficacy of bortezomib at the RD were assessed in phase II. In phase I, three patients were enrolled in the 0.7 mg/m^2-dose group, and six patients each in the 1.0 and 1.3 mg/m^2-dose groups. DLT was defined as ≥grade 3 non-hematological toxicity or grade 4 hematological toxicity for which the relation to bortezomib could not be ruled out. The RD was defined as a dose level with a DLT incidence closest to but lower than the estimated (expected) value of 30%. Bortezomib was administered for up to six cycles.

Drug administration. Bortezomib, supplied by Janssen Pharmaceutical (Tokyo, Japan) in vials containing 3.5 mg, was administered by intravenous push over 3–5 s on days 1, 4, 8, and 11, followed by a 10-day rest period, with this 3-week period comprising one cycle. There was an interval of at least 72 h between doses.

Response and safety assessments. Patients were monitored for response after every two treatment cycles by quantitation of serum immunoglobulins, serum protein electrophoresis and immunofixation (IF), and collection of a 24-h urine specimen for total protein, electrophoresis, and IF. Response was evaluated using the European Group for Blood and Marrow Transplantation criteria,[12] after cycles 2, 4, and 6.

Adverse events were assessed and graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 from the first dose until 28 days after the last dose of bortezomib.

Pharmacokinetic and pharmacodynamic analysis. Plasma bortezomib concentrations and blood 20S proteasome activity were measured in phase I. Blood samples were collected before each dose, at 5, 15, and 30 min, and 1, 2, 4, 6, 8, 12, 24, and 48 h after treatment on days 1 and 11. The measurement of plasma bortezomib concentration was conducted at Advion BioSciences (Ithaca, NY, USA) using liquid chromatography/tandem mass spectrometry (LC/MS/MS).[13] The measurement of blood 20S proteasome activity was conducted at Millennium Pharmaceuticals (Cambridge, MA, USA) using the synthetic fluorescence substrate method validated for the chymotrypsin-like activity/trypsin-like activity ratio.[14]

Results

Patients and dose escalation. The study was conducted from May 2004 to January 2006, and 34 patients were enrolled. Patient characteristics are shown in Table 1. All patients had secretory-type myeloma, and the breakdown was 20 patients (59%) with IgG type, eight patients (24%) with IgA type, three patients (9%) with light-chain type, and three patients (9%) with IgA and light-chain type. Most patients had received prior therapy with steroids, alkylating agents, and/or vinca alkaloids. Ten patients (29%) had received stem cell transplantation including high-dose therapy. The median number of lines of prior therapy was two (range: one to eight). Osteolytic lesions were observed in 30 patients (88%) and soft-tissue tumors were observed in seven (21%). The median number of treatment cycles was four (range: one to six), and the median duration of treatment was 79 days (range: 1–152 days). Ten patients (29%) completed all six cycles. The reasons for discontinuation of therapy in 25 patients were progressive disease in 11 patients, patient's own request in six patients, serious adverse events in four patients, DLT in two patients, and others in three patients. Three patients were enrolled in the 0.7 mg/m^2 group and six in the 1.0 mg/m^2 group, and no DLT were observed at any dose level. In the 1.3 mg/m^2 group, DLT (grade 3 febrile neutropenia) occurred in one of the six patients. Therefore, 1.3 mg/m^2 was determined to be the RD in subsequent phase II, in which 18 patients were enrolled.

Table 1. Patient characteristics

Patient characteristic	*n*	%
Patients	34	
Sex		
Female	12	35
Male	22	65
Age (years)		
Median	60	
Range	34–72	
Durie-Salmon stage		
I	0	
II	15	44
III	19	56
Time since diagnosis (years)		
Median	3.4	
Range	1.0–13.7	
Karnofsky performance status		
100	15	44
90–80	18	53
70–60	1	3
Serum interleukin-6 (pg/mL)		
Mean	4.2	
Range	0.5–30.2	
Cytogenetics		
Karyotype abnormal	4	12
del(13)(q14)	7	21
t(11; 14)	4	12
Prior therapy		
Chemotherapy	34	100
Steroids	34	100
Alkylating agents	33	97
Vinca alkaloids	27	79
Anthracyclines	22	65
Thalidomide	8	24
Interferon	7	21
Radiation therapy	6	18
Autologous hematopoietic stem cell transplantation	10	29

Adverse events. The safety analysis dataset consisted of all patients who received at least one dose of bortezomib (34 patients). Adverse events observed in ≥20% of patients are shown in Table 2. The events observed at a high frequency (≥50%) were lymphopenia, neutropenia, leukopenia, thrombocytopenia, anemia, asthenia, diarrhea, constipation, nausea, anorexia, and pyrexia. At least one ≥grade 3 adverse event was observed in 88% of the patients. Major ≥grade 3 adverse events were hematological toxicities including lymphopenia, neutropenia, leukopenia, thrombocytopenia, and anemia. Grade 4 hematological toxicities included neutropenia in six patients (18%), three of which experienced this adverse event during cycle 1. At least grade 3 non-hematological toxicities occurred in fewer than 10%, and no DLT during cycle 1 were observed. Grade 4 non-hematological toxicities included hematuria, blood amylase

Table 2. All adverse events occurring in at least 20% of patients ($n = 34$)

Dose (mg/m²)	0.7		1.0		1.3		All			
No. of Patients	($n = 3$)		($n = 6$)		($n = 25$)		($n = 34$)		Total	%
NCI-CTC grade	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4		
Adverse event										
Hematologic										
Lymphopenia	3	0	4	2	8	17	15	19	34	100
Neutropenia	1	1	2	4	7	16	10	21	31	91
Leukopenia	2	0	6	0	11	12	19	12	31	91
Thrombocytopenia	1	0	4	0	12	11	17	11	28	82
Anemia	2	0	2	3	10	8	14	11	25	74
Nonhematological										
Asthenia†	3	0	3	0	15	0	21	0	21	62
Diarrhea	1	0	2	0	15	1	18	1	19	56
Constipation	2	0	3	0	14	0	19	0	19	56
Nausea	2	0	2	0	14	0	18	0	18	53
Anorexia	3	0	2	0	14	0	18	0	18	53
Pyrexia	0	0	4	0	14	0	18	0	18	53
Peripheral neuropathy‡	0	0	3	0	12	1	15	1	16	47
AST increased	1	0	1	0	11	2	13	2	15	44
LDH increased	1	0	1	0	12	1	14	1	15	44
Vomiting	1	0	0	0	9	1	10	1	11	32
Rash	0	0	1	0	10	0	11	0	11	32
ALP increased	0	0	2	0	8	0	10	0	10	29
Headache	0	0	1	0	8	0	9	0	9	27
ALT increased	1	0	1	0	7	0	9	0	9	27
Hyperglycaemia	0	0	2	0	5	0	7	0	7	21
Hyponatremia	1	0	0	1	5	0	6	1	7	21
Renal impairment	1	0	1	0	5	0	7	0	7	21
CRP increased	0	0	1	0	6	0	7	0	7	21
Weight decreased	0	0	0	0	7	0	7	0	7	21

†Including fatigue and malaise. ‡Including peripheral sensory neuropathy, peripheral motor neuropathy, and hypoesthesia. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; LDH, lactate dehydrogenase; NCI-CTC, National Cancer Institute Common Toxicity Criteria.

increase, and blood uric acid increase in one patient (3%) each. Hematuria was attributed to prostate cancer and judged as not related to bortezomib. The underlying disease was considered to be involved in the blood uric acid increase; this event was judged unlikely to be related to bortezomib. At the occurrence of grade 4 blood amylase increase, blood amylase isozymes were pancreatic-type in 86% and salivary-type in 14%. There were no gastrointestinal symptoms, such as abdominal pain, associated with amylase increase. Abdominal echography revealed no finding suggesting pancreatitis or pancreatolithiasis, and the relevant events recovered 5 days after the onset. The causality of the grade 4 blood amylase increase with bortezomib was evaluated as 'probable', and therefore treatment was continued at a reduced dose from 1.3 to 1.0 mg/m².

One case of interstitial lung disease (ILD) that resulted in a fatal outcome was observed in phase II. The patient with grade 5 ILD had developed the event on day 10 in cycle 2 after receiving seven doses of bortezomib in total. Pyrexia, non-productive cough, hypoxia, and dyspnea were observed as early symptoms, and antibiotics, antimicrobials, steroid pulse therapy, and oxygen inhalation were initiated to treat it. However, respiratory failure worsened, so the patient was put on a ventilator, and the study was discontinued. After the onset of ILD, bronchoalveolar lavage was conducted, but the causative pathogen could not be identified. The available examinations for β-D-glucan, cytomegalovirus antigenemia, influenza virus, and urinary antigen of *Legionella* were found to be negative. The diagnosis from the pathological findings was diffuse alveolar damage. A retrospective analysis of the pretreatment computed tomography (CT) images indicated that the patient had subtle interstitial shadows in the basal region of both lungs. In response, the protocol was amended to exclude patients with abnormal pretreatment bilateral interstitial shadows on CT. No cases of fatal pulmonary toxicity were observed thereafter.

Efficacy. Thirty-three patients were evaluable for efficacy, excluding one ineligible patient who had another malignancy (prostate cancer). Objective responses were observed in 10 of 33 patients (30%; 95% confidence interval 16–49%), including five IF-positive complete responses (CR^{IF+}) and five partial responses. Of the 10 responders, five patients had one line of prior therapy, two patients had three lines of prior therapy, and three patients had four or more lines of prior therapy. It is noteworthy that one patient who had received eight lines of prior therapy, including high-dose chemotherapy with autologous stem-cell transplantation, showed CR^{IF+}. Of the 10 patients who had received prior autologous hematopoietic stem cell transplantation, two patients showed CR^{IF+}, and three patients showed PR. With respect to osteolytic lesions, which is one of the efficacy endpoints, partial regression in five patients, partial disappearance in one patient, and regression of soft-tissue tumors in two patients were observed.

Pharmacokinetics and pharmacodynamics. The mean plasma bortezomib concentration–time profiles on days 1 and 11 obtained from 16 patients enrolled in phase I are shown in Fig. 1a. PK parameters obtained using non-compartmental analysis are shown in Table 3. The plasma bortezomib concentration–time

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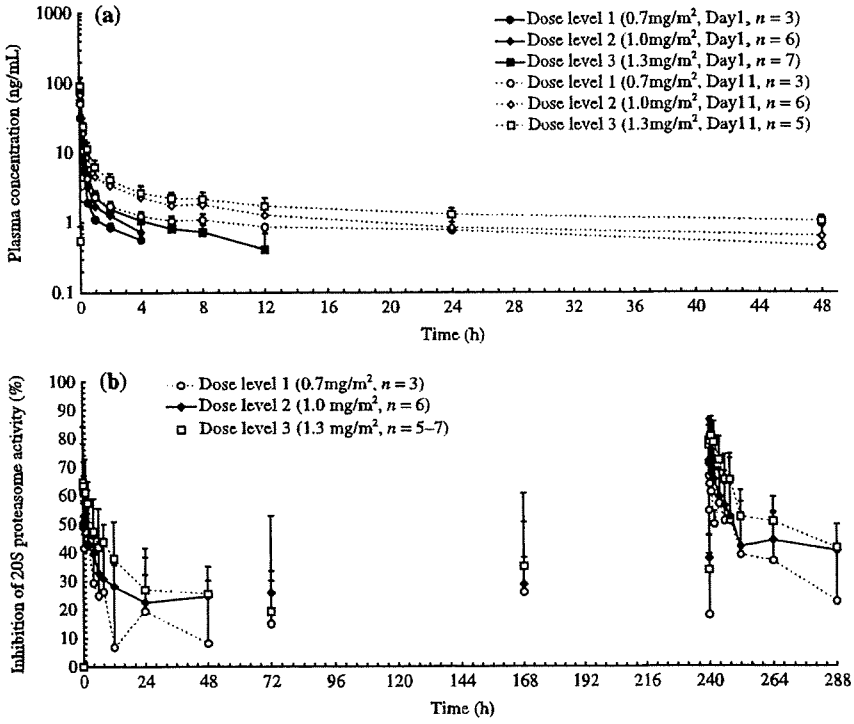

Fig. 1. (a) Plasma bortezomib concentrations (mean + SD). (b) Inhibition of blood 20S proteasome activity (mean + SD).

Table 3. Pharmacokinetic parameters (non-compartmental analysis)

Parameter	Day	Dose (mg/m²) 0.7 ($n = 3$)	1.0 ($n = 6$)	1.3 ($n = 5$–7)†
C_0 (ng/mL)	1	73.75 ± 7.89	144.92 ± 179.31	185.84 ± 57.65
	11	130.68 ± 71.97	147.19 ± 72.33	187.03 ± 54.31
AUC	1	14.04 ± 0.70	28.58 ± 24.86	46.50 ± 19.89
(ng · h/mL)	11	112.01 ± 47.74	108.39 ± 52.32	186.60 ± 49.79
Half life (h)	1	3.31 ± 0.88	6.81 ± 8.81	16.11 ± 20.75
	11	64.59 ± 30.29	32.46 ± 12.91	57.39 ± 24.92
Clearance	1	83.35 ± 10.52	105.41 ± 75.66	51.97 ± 18.99
(L/h)	11	11.77 ± 4.67	19.63 ± 14.50	12.10 ± 3.73
V_z (L)	1	406.92 ± 154.03	520.08 ± 349.87	894.41 ± 682.35
	11	978.51 ± 263.13	731.69 ± 242.35	957.81 ± 350.40
V_{ss} (L)	1	186.46 ± 85.02	288.90 ± 260.74	507.75 ± 558.30
	11	812.60 ± 202.03	540.03 ± 218.72	763.81 ± 271.64
C_0 ratio	11/1	1.789 ± 0.973	1.848 ± 1.133	1.103 ± 0.249
AUC ratio	11/1	7.940 ± 3.247	5.363 ± 2.970	5.142 ± 0.543

†Day 1, $n = 7$; day 11, $n = 5$. Values are mean ± SD. AUC, area under the concentration–time curve from time zero to infinity; AUC ratio, AUC on day 11/AUC on day 1; C_0, plasma concentration at the end of administration; C_0 ratio, C_0 on day 11/C_0 on day 1; V_z, the apparent volume of distribution during the terminal phase; V_{ss}, the apparent volume of distribution at steady state.

profiles showed a biphasic elimination profile, characterized by rapid distribution followed by a longer elimination at all dose levels. At any dose level, the elimination half-life ($t_{1/2}$) on day 11 was prolonged, and systemic clearance (CL) was lower compared with day 1. Therefore, delayed elimination of bortezomib from plasma associated with repeated administrations was observed, and the plasma bortezomib concentration after administration (C_0, estimated value) and area under the plasma concentration–time curve (AUC) showed higher values on day 11 compared with day 1. AUC showed dose dependency, whereas C_0 did not.

The inhibition of blood 20S proteasome activity is shown in Fig. 1b. The 20S proteasome inhibition recovered over time at all dose levels, but was prolonged compared with the temporal decrease in plasma bortezomib concentration, and the inhibition was still observed before treatment on days 4, 8, and 11.

Discussion

In the present study, bortezomib was generally well tolerated in the 25 Japanese patients whose treatments were started at the RD of 1.3 mg/m². Hematological toxicities, gastrointestinal toxicities, and peripheral neuropathies observed in our patients were similar to those reported for patients in clinical studies from the USA and Europe.[15,16] Most could be managed without interventions or with the usual symptomatic therapy. Grade 4 neutropenia was observed in 18% of patients, but treatment could be continued with dose reduction. The response rate obtained in the present study was comparable to that reported by Richardson *et al.* in a pivotal phase III study.[16] In addition, patients who had received heavy prior therapy also showed a consistent response. Therefore, 1.3 mg/m² is considered appropriate as an initial dose of bortezomib in Japanese patients. There was a fatal pulmonary disorder event (ILD) in one patient treated with the 1.3 mg/m² dose in which a causal relationship with bortezomib could not be ruled out. Hence, special care should be taken prior to initiating treatment with bortezomib to evaluate patients (e.g. chest X-ray or chest CT scan) and during and after bortezomib treatment if they develop subjective symptoms such as dyspnea, cough, and fever.

The assessment of PK and PD in multiple myeloma patients treated with bortezomib twice weekly for 2 weeks was conducted for the first time in Japanese patients. A decrease in CL associated with increased exposures and subsequently longer $t_{1/2}$ values were observed after repeated administration and dose escalation. The relatively large volume of distribution suggests that bortezomib may be distributed extensively into the extravascular tissues. It can be postulated that CL values on day 1 are apparent values observed due to rapid tissue distribution, whereas

saturation of proteasome binding sites and tissue distribution occur after multiple dosing, and the CL value on day 11 may be a better representation of the true value.

It was also found that the blood 20S proteasome inhibition at each dose level recovered over time, but was prolonged compared with the temporal decrease in plasma bortezomib concentration. Similarly to CL, this could be due to the large distribution volume of bortezomib and its slow return from tissues to plasma.

Delayed elimination and enhanced proteasome inhibition were observed with repeated administration and dose increase, but no clear tendency in the incidence or degree of adverse reactions was observed. However, the PD results of the present study in Japanese patients demonstrate that the inhibition of 20S proteasome activity does not recover even after 72 h, which is specified as a minimum interval for bortezomib dosing. Accordingly, when bortezomib is used in clinical practice, it is important to determine the optimal dosage and determine whether it is appropriate to administer bortezomib while considering the balance between safety and efficacy.

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ORIGINAL REPORT

Dofequidar Fumarate (MS-209) in Combination With Cyclophosphamide, Doxorubicin, and Fluorouracil for Patients With Advanced or Recurrent Breast Cancer

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ABSTRACT

Purpose

To evaluate the efficacy and tolerability of dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) therapy in comparison with CAF alone, in patients with advanced or recurrent breast cancer. Dofequidar is a novel, orally active quinoline derivative that reverses multidrug resistance.

Patients and Methods

In this randomized, double-blind, placebo-controlled trial, patients were treated with six cycles of CAF therapy: 28 days/cycle, with doxorubicin (25 mg/m^2) and fluorouracil (500 mg/m^2) administered on days 1 and 8 and cyclophosphamide (100 mg orally [PO]) administered on day 1 through 14. Patients received dofequidar (900 mg PO) 30 minutes before each dose of doxorubicin. Primary end point was overall response rate (ORR; partial or complete response). In total, 221 patients were assessable.

Results

ORR was 42.6% for CAF compared with 53.1% for dofequidar + CAF, a 24.6% relative improvement and 10.5% absolute increase (P = .077). There was a trend for prolonged progression-free survival (PFS; median 241 days for CAF *v* 366 days for dofequidar + CAF; P = .145). In retrospectively defined subgroups, significant improvement in PFS in favor of dofequidar was observed in patients who were premenopausal, had no prior therapy, and were stage IV at diagnosis with an intact primary tumor. Except for neutropenia and leukopenia, there was no statistically significant excess of grade 3/4 adverse events compared with CAF. Treatment with dofequidar did not affect the plasma concentration of doxorubicin.

Conclusion

Dofequidar + CAF was well tolerated and is suggested to have efficacy in patients who had not received prior therapy.

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INTRODUCTION

Despite the advances in chemotherapeutic intervention, many cancers are either inherently resistant or develop resistance to chemotherapy.[1,2] Consequently, multidrug resistance (MDR) remains a major obstacle to the successful treatment of cancer.[1,3,4] One mechanism by which MDR operates is via the increased cellular efflux of cytotoxic compounds due to increased expression of membrane transport proteins such as P-glycoprotein (P-gp) and MDR-associated protein (MRP).[1,4,5] MDR affects many structurally and functionally unrelated agents including cytotoxic drugs that are hydrophobic, natural products, such as taxanes, vinca alkaloids, anthracyclines, epipodophyllotoxins, topotecan, dactinomycin, and mitomycin.[1,6,7] These represent some of the most commonly used chemotherapeutic agents.

In tumors with low levels of P-gp expression at baseline or diagnosis, P-gp expression increases after exposure to chemotherapy agents, thus leading to the development of MDR. In breast cancer patients who had received prior chemotherapy, P-gp expression has been shown to increase from 11% in untreated patients to 30% after chemotherapy.[8] Furthermore, compared with P-gp–negative tumors, a significant increase in resistance to paclitaxel and doxorubicin was reported in P-gp positive breast cancer tissue, irrespective of prior therapy.

The degree of P-gp expression also strongly correlated with the degree of drug resistance observed.[8]

Chemotherapy remains the treatment of choice for women with hormone receptor–negative and hormone-refractory breast cancer disease.[9-11] However, many tumors that are initially responsive to chemotherapy frequently relapse and develop resistance to the broad spectrum of cytotoxic drugs currently employed.[8,12,13] Consequently, MDR remains a major reason for treatment failure in patients with metastatic breast cancer and highlights the urgent need for MDR modifiers in breast cancer chemotherapy.

Since the discovery of verapamil as an MDR-reversing agent,[14] many compounds have been investigated as MDR inhibitors.[14-16] Dofequidar fumarate (Fig 1), is a novel, orally active, quinoline-derived inhibitor of MDR.[17] In preclinical studies, dofequidar reversed MDR in P-gp– and MRP-1–expressing cancer cells in vitro (1 to 3 μmol/L), as well as enhancing the antitumor effects of doxorubicin in MDR tumor–bearing mice.[17-19] A phase I trial in healthy volunteers showed dofequidar to be well tolerated (10 to 1,200 mg) with no dose-limiting toxicities and an effective plasma concentration was maintained for 8 hours at 900 mg (data on file, Schering AG, Berlin, Germany). In a phase II combination trial in patients with recurrent breast cancer, dofequidar potentiated the antitumor effects of CAF (cyclophosphamide, doxorubicin, and fluorouracil) therapy; patients who had not responded to treatment with three cycles of CAF responded to subsequent treatment with dofequidar plus CAF. The numbers of patients with an objective response were two of seven at 600 mg and two of six at 900 mg dofequidar, though dose escalation was stopped at 1,200 mg due to increased hematologic toxicity (data on file, Schering AG). On the basis of this result, this phase III study was conducted to compare the efficacy and safety of dofequidar plus CAF with placebo plus CAF in patients with advanced or recurrent breast cancer.

PATIENTS AND METHODS

Study Design

This was a randomized, multicenter, double-blind, placebo-controlled trial conducted at 46 centers across Japan, comparing the efficacy and safety of dofequidar plus CAF with placebo plus CAF. Female patients (age 20 to 70 years) with advanced (stage IV at diagnosis with an intact primary tumor) or recurrent breast cancer were enrolled onto the study. Other inclusion criteria included a histologically defined, measurable or assessable primary lesion; two or fewer regimens of prior chemotherapy in both neo/adjuvant and metastatic settings, (excluding prior endocrine or single-agent fluorouracil therapy); 180 mg/m^2 anthracyclines (doxorubicin equivalent) or less previously; a performance status of 0 to 2; and adequate bone marrow, renal, hepatic and cardiac functions. Patients who progressed or had a recurrence in less than 6 months with anthracycline-containing chemotherapy, and those who had a history of major cardiac disease, uncontrolled hypertension, symptomatic brain metastasis, or simultaneous malignancy were excluded. The trial was approved by the institutional review board and was conducted in accordance with the Declaration of Helsinki (1996). All patients provided written informed consent before study entry.

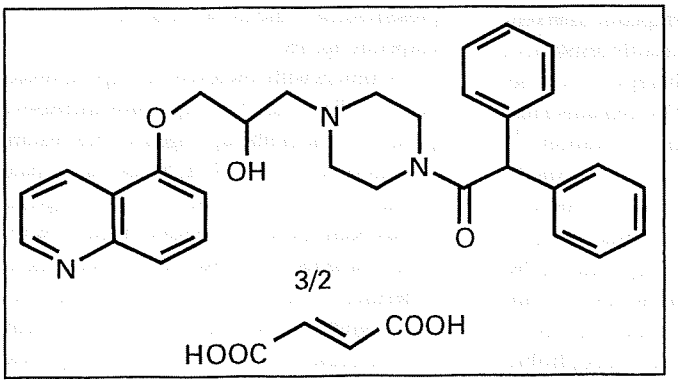

Fig 1. Structure of dofequidar (MS-209).

Dosing and Dose Modification for Toxicity

Patients were treated with six cycles of CAF therapy with dofequidar or placebo, and each treatment cycle lasted for 28 days; drugs were administered as follows: days 1 and 8, doxorubicin (25 mg/m^2) and fluorouracil (500 mg/m^2), each infused over 15 minutes; days 1 through 14, cyclophosphamide (100 mg orally [PO]); dofequidar (900 mg/d; 3 × 300 mg tablets) or placebo administered 30 minutes before each doxorubicin dose to ensure adequate blood concentration of dofequidar. The doses of doxorubicin and fluorouracil were reduced to 20 mg/m^2 and 400 mg/m^2, respectively, if any of the following criteria were met: grade 3 nonhematologic toxicity (except nausea and vomiting); grade 3 or worse neutropenia (< 1,000/mm^3) maintained for at least 5 days with an episode of fever of 38.5°C or higher; grade 3 or worse thrombocytopenia (< 50,000/mm^3); and grade 4 neutropenia (< 500/mm^3). The next cycle was postponed for 3 weeks unless the patient had a WBC count of at least 4,000/mm^3, or a neutrophil count of at least 2,000/mm^3 and a platelet count of at least 100,000/mm^3. Patients were followed up for 3 months after completion or discontinuation of treatment.

Treatment Assignment

Patients were randomly assigned to their treatment by the Trial Register Center. Treatment assignment was securely stored and coded until completion of the study. Investigators were also blinded to the assigned treatment. Patients were stratified by the number of prior chemotherapy regimens, including adjuvant chemotherapy, by a history of prior use of anthracyclines, and by the presence of liver metastases.

Efficacy

The primary study end point was the overall response rate (ORR) in the full analysis set (FAS; all patients who received treatment at least once and met all inclusion/exclusion criteria). Efficacy assessment by lesion and ORR assessment were made at each treatment cycle (every 4 weeks) and at treatment completion. Objective responses were assessed through blinded reading of radiographs by an independent expert panel. The secondary study end points included complete response rate (CR), time to treatment failure (TTF), time to progression (TTP), and progression-free survival (PFS).

Subgroup analyses were conducted to assess PFS within specific patient subpopulations, including premenopausal women, patients who had no prior therapy, and patients who had advanced primary breast cancer.

Safety and Tolerability

Adverse events (AEs) were recorded at the end of each treatment cycle and at the end of the study period using data from the safety population (all patients who received treatment at least once in the study). AEs were categorized according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 2. The incidence of significant decreases in left ventricular ejection fraction (LVEF) and serious AEs were recorded. The CBC was evaluated weekly. Serum chemistries and urinalysis were evaluated every 2 weeks. The minimum hematology values and LVEF in each treatment cycle were also recorded and analyzed in the per-protocol set (PPS; all patients who received treatment at least once and had no protocol deviations).

Pharmacokinetics

To assess the effect of concomitant dofequidar use on the pharmacokinetics of doxorubicin, the plasma doxorubicin concentration on day 1 of cycle 1 was compared between treatment groups. Blood samples were taken at baseline and at 15 minutes, 30 minutes, and 1, 2, 4, and 6 hours after the start of doxorubicin administration. Plasma doxorubicin concentrations were determined by reversed-phase high-performance liquid chromatography. Area

under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule.

Statistical Analyses

The primary end point was analyzed using the Fisher's exact test at a significance level of 2.5% in a one-sided test. A difference in response rates of 20% between the two treatment groups was used as the basis for a statistically significant difference. CR, TTF, TTP and PFS were analyzed by the log-rank test at a significance level of 5% in a two-sided test. The CR, TTF, TTP and PFS were analyzed in the FAS, and the pharmacokinetic data analyzed in the PPS.

RESULTS

Patient Characteristics

A total of 227 patients were recruited onto the study (Fig A1, online only), of which 225 patients were included in the safety analysis (n = 113 for the dofequidar group; n = 112 for the placebo group); two patients did not receive the study treatment and were thus excluded. Four patients did not meet the inclusion/exclusion criteria; therefore, the FAS consisted of 221 patients (n = 113 for the dofequidar group; n = 108 for the placebo group). The PPS consisted of 199 patients (n = 100 for the dofequidar group; n = 99 for the placebo group). There were 22 patients excluded from the PPS analysis due to protocol deviations. Baseline patient characteristics were well balanced between the two treatment arms (Table 1). Most patients had predominantly recurrent disease and had received prior chemotherapy plus endocrine therapy. Also, many patients who had advanced primary breast cancer had received no prior therapy.

Table 1. Patient Demographics (full analysis set)

Characteristic	Dofequidar + CAF (n = 113) No.	%	Placebo + CAF (n = 108) No.	%
Age, years				
Mean	54.4		52.4	
SD	7.69		8.97	
Medical history known	65	57.5	60	55.6
Weight, kg				
Mean	56.2		54.1	
SD	7.52		7.73	
Height, cm				
Mean	154.7		154.7	
SD	5.71		5.61	
Body surface area, m^2				
Mean	1.5		1.5	
SD	0.11		0.11	
Disease state				
Recurrent	81	71.7	80	74.1
Advanced	32	28.3	28	25.9
Prior therapy				
Radiotherapy + chemotherapy + endocrine therapy	32	22.1	32	29.6
Chemotherapy + endocrine therapy	55	48.7	54	50.0
Radiotherapy	1	0.9	1	0.9
No prior therapy	25	22.1	21	19.4
Menopausal status				
Premenopausal	24	21.2	26	24.1
Postmenopausal	88	77.9	79	73.1

Abbreviations: CAF, cyclophosphamide, doxorubicin, and fluorouracil; SD, standard deviation.

Efficacy

The ORR, rated as CR or partial response rate, was 42.6% for CAF plus placebo versus 53.1% for dofequidar plus CAF (Table 2). Although this represents a 24.6% relative improvement and a 10.5% absolute increase in response rate for patients receiving dofequidar plus CAF compared with those receiving CAF plus placebo, this response was not statistically significant ($P = .077$). A higher value was observed in the dofequidar treatment group for all secondary end points compared with placebo, though these results were not statistically significant. Among them, Figure 2 shows a trend for prolonged PFS (median, 241 days for CAF plus placebo *v* 366 days for dofequidar plus CAF; $P = .145$).

Dofequidar plus CAF significantly improved PFS in several patient subgroups, including patients who were premenopausal ($P = .046$; Fig 3A), patients who had not received prior therapy ($P = .0007$; Fig 3B), and patients who had advanced primary breast cancer ($P = .017$; Fig 3C). An extended follow-up showed that dofequidar plus CAF also significantly improved overall survival ($P = .0034$; Fig 3D) in patients who had no prior therapy.

Safety and Tolerability

A similar number of patients completed six treatment cycles in both groups (n = 53 for the dofequidar group; n = 51 for the placebo group). The mean number of treatment cycles was 4.5 in the dofequidar group and 4.3 in the placebo group. More than half of patients in both groups included in each cycle from cycle 2 onward had a delay in treatment, mostly due to prolonged hematologic toxicities.

Dofequidar plus CAF was well tolerated throughout the study. No statistically significant excess of grade 3/4 AEs, except for neutropenia ($P = .006$) and leukopenia ($P = .005$), was found in the dofequidar group compared with placebo (Table A1, online only). Importantly, there was no marked difference in the incidence of neutropenia-related morbidity, such as febrile neutropenia or infection, between the two treatment groups. No significant differences in the incidence of cardiac AEs were found between the two treatment groups. In addition, dose intensities of chemotherapeutic agents were similar in both treatment arms. No significant difference in the incidence of serious AEs (SAEs) was observed between either group. However, there was a trend for a higher incidence of SAEs from leukopenia in the dofequidar group than in the placebo group ($P = .060$; Fisher's exact test); five leukopenia cases were reported for dofequidar, whereas no such case was reported for placebo.

A total of 124 patients discontinued the study (n = 61 for the dofequidar group; n = 63 for the placebo group). The major reasons for discontinuation were progressive disease (n = 23 for the dofequidar group; n = 28 for the placebo group), grade 4 hematologic toxicity (n = 20 for the dofequidar group; n = 6 for the placebo group), failure to meet treatment continuation criteria (n = 6 for the dofequidar group; n = 8 for the placebo group), and consent withdrawal (n = 6 for the dofequidar group; n = 12 for the placebo group). Of the 225 patients who received treatment in the study, 14 patients died during the treatment period (n = 3), the follow-up period (n = 2), or the follow-up period after study termination (n = 9). There were 49 other serious AEs in 32 patients during the study and follow-up period.

Pharmacokinetics

The mean plasma concentrations of doxorubicin in the dofequidar- and placebo-treatment groups at 15 minutes postadministration reached 0.997 μg/mL and 1.259 μg/mL, respectively, followed by biphasic elimination in both treatment groups. Mean plasma concentrations in

Table 2. Response Rates for Patients Treated With Dofequidar Plus CAF (n = 113) or Placebo Plus CAF (n = 108)

Treatment Group	Parameter (No. of patients)					Overall Response Rate (%)	95% CI
	Complete Response	Partial Response	No Change (stable disease)	Progressive Disease	Not Assessable		
Dofequidar	5	55	40	10	3	53.1	43.5 to 62.5
Placebo	4	42	41	14	7	42.6	33.1 to 52.5

NOTE. Odds ratio = 1.53 (range, 0.87-2.69); $P = .077$ for dofequidar *v* placebo.
Abbreviation: CAF, cyclophosphamide, doxorubicin, and fluorouracil.

the dofequidar and placebo groups remained similar at 1, 2, 4, and 6 hours after the start of doxorubicin administration. Thus the elimination pattern for the first 6 hours after the start of administration was similar in both groups. The plasma concentrations of doxorubicin in the terminal phase (4 and 6 hours postadministration) were slightly higher in the dofequidar group compared with placebo (1.2- to 1.3-fold). However, AUC (0 to 6 hours) values showed no statistically significant difference between the dofequidar and placebo groups (mean, 0.480 $\mu g \cdot h/mL$; standard deviation [SD], 0.324; range, 0.237-1.692; and mean, 0.407 $\mu g \cdot h/mL$; SD, 0.062; and range, 0.289-0.500, respectively). Therefore, treatment with dofequidar did not affect the plasma concentrations of doxorubicin in patients (Fig 4).

DISCUSSION

Chemotherapy remains the preferred adjuvant treatment for patients with hormone receptor–negative disease and for patients with more aggressive, hormone receptor–positive tumors.[11,20] However, despite the use of conventional adjuvant chemotherapy regimens, a significant proportion of patients with breast cancer still experience disease recurrence because of inherent or acquired drug resistance.[12] In this randomized phase III trial, the efficacy and safety of the multidrug resistance inhibitor dofequidar plus CAF was compared with CAF plus placebo in patients with recurrent or advanced breast cancer. Although, there was an observed relative improvement and absolute increase in response rate for patients who received dofequidar plus CAF, these results did not reach statistical significance. This improvement in response rate may have been reflected in the observation that there was a trend for prolonged PFS, which favored patients in the dofequidar plus CAF group.

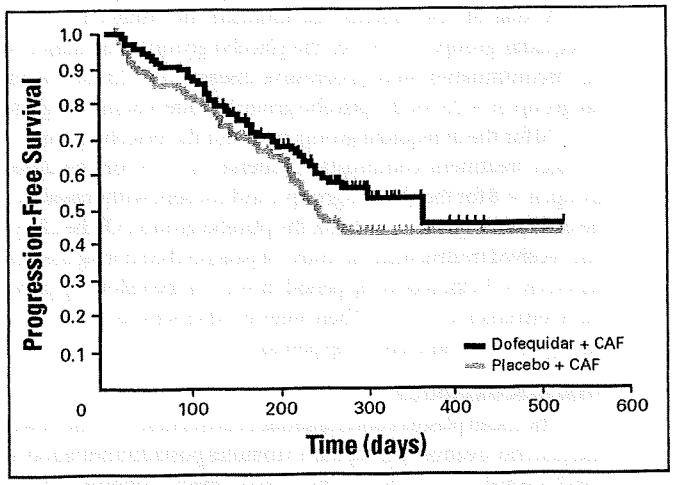

Fig 2. Progression-free survival in patients treated with dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) and placebo plus CAF ($P = .145$).

To date, only two randomized trials have examined the efficacy of a P-gp inhibitor in combination with chemotherapy in breast cancer patients. Wishart et al[21] examined quinidine combined with epirubicin in patients with advanced breast cancer, but failed to show any significant difference in overall survival or PFS compared with placebo. In a more recent prospective study of patients with anthracycline-resistant metastatic breast cancer (n = 99), verapamil combined with vindesine and fluorouracil resulted in a significantly longer overall survival and a higher response rate compared with patients who did not receive the P-gp inhibitor (median survival, 323 *v* 209 days; $P = .036$, respectively; ORR, 27% *v* 11%; $P = .04$, respectively).[22]

In the subgroup analyses, dofequidar in combination with CAF displayed a significantly increased PFS in patients who had not received prior therapy, who had advanced primary breast cancer or who were premenopausal. In addition, dofequidar also significantly improved overall survival in the patient group who had no prior therapy. Although the patient numbers in these analyses were small, the results remain important within these clinically significant patient populations. Both preclinical and clinical data have indicated that newer-generation MDR modulators can prevent the development of resistance.[23,24] A phase I/II trial in patients with acute myeloid leukemia showed that dosing with cyclosporine before and in combination with daunorubicin prevented chemotherapy resistance, while also resulting in a decrease in MDR-1 RNA expression.[24] Our results may highlight one potential treatment approach to MDR tumors that has not yet been fully exploited in the clinical environment, specifically the prevention of the emergence of resistance through the early use of P-gp inhibitors.[1-3] It seems reasonable that agents such as dofequidar may be useful in the adjuvant or even neoadjuvant setting with the goal of preventing or delaying the induction of MDR associated with chemotherapy.

The potential clinical significance of P-gp and MRP expression in breast cancer is supported by the results from a number of studies. For example in a study of primary breast cancer patients (n = 259), MRP expression was associated with an increased risk of treatment failure in patients with small tumors (T1) and node-positive patients who received adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy but not in node-negative patients.[25] Burger et al[12] reported that the expression of MDR1 mRNA in primary breast tumors was inversely correlated with the efficacy of first-line chemotherapy. Additionally, the high level of MDR1 expression was suggested to be a significant predictor of poor prognosis in patients

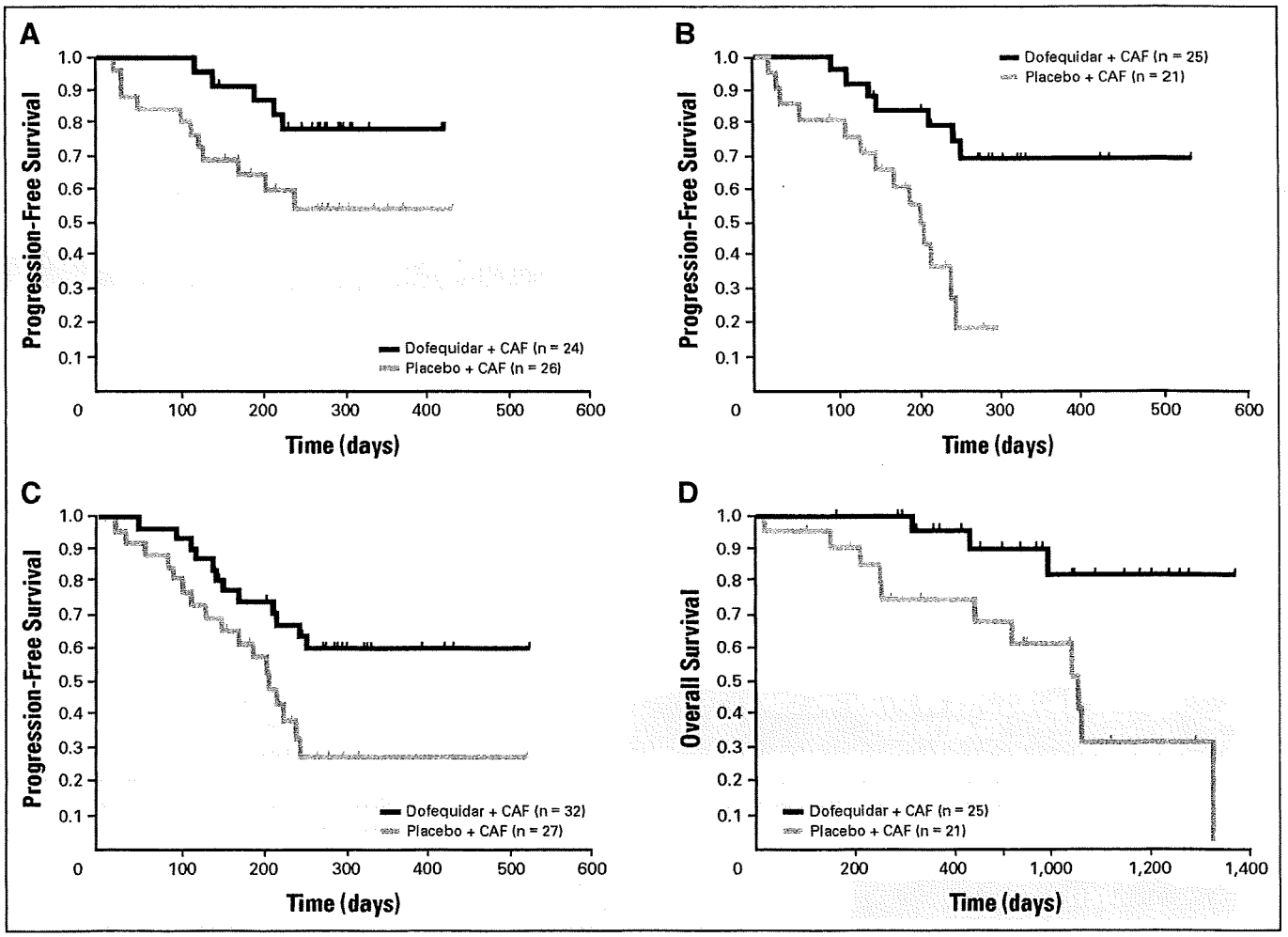

Fig 3. Subgroup analyses. (A) Progression-free survival in premenopausal patients (P = .046); (B) progression-free survival in patients who had no prior therapy (P = .0007); (C) progression-free survival in patients who were stage IV at diagnosis with an intact primary tumor (P = .017); and (D) overall survival in patients who had no prior therapy (P = .0034).

with advanced disease.[12] Significantly increased expression of P-gp and MRP-1 has also been reported in an immunohistochemical study of patients treated with preoperative chemotherapy, whereas pretreatment expression of MRP-1 was associated with significantly shorter PFS in patients.[26] In a more recent study, MRP-1 expression was shown to be an independent predictor for shorter relapse-free survival and overall survival, after adjuvant CMF treatment, in premenopausal, hormone receptor–positive patients.[27] However, MRP-1 expression did not affect patients' response to adjuvant tamoxifen plus goserelin treatment.[27]

These findings and our results support the view of Leonard et al,[3] who indicate that future patients will need to be carefully selected for the identification and development of effective drug-resistance modulators. Patient populations who may derive maximal benefit from MDR inhibition, for example, the no-prior-therapy, advanced-disease, or premenopausal patient group in the present study, could quite easily be overlooked or lost within a large, heterogeneous trial population.[3] Furthermore, by refining future clinical trials to incorporate specific disease and patient characteristics, a clearer picture of drug resistance in cancer will be obtained and the most effective MDR inhibitor/chemotherapeutic agent(s) selected.

Many MDR inhibitors have required high serum concentrations for MDR reversal, which resulted in unacceptable toxicity, thereby limiting their clinical impact.[7,28-32] Although more recent agents have shown improved tolerability profiles, this has been countered by unpredictable pharmacokinetic interactions with other transporter molecules (eg, cytochrome P450–mediated drug metabolism and excretion, necessitating dose reductions in chemotherapy agents and leading to inconsistent chemotherapy dosing among patients).[1,5] Similarly, the addition of the MDR-modulating agent valspodar (PSC 833) to chemotherapy agents did not improve treatment outcome.[33,34] Toxicity was increased in the valspodar-treated group compared with chemotherapy agents alone, despite the reduction of chemotherapy doses in the valspodar-containing regimen. In our study, dofequidar was well tolerated, with no indication of the unacceptable toxicity associated with early MDR inhibitors. Importantly, dofequidar did not affect the plasma concentrations of doxorubicin in patients during the study and displayed an acceptable pharmacokinetic profile.

In conclusion, this study suggests that treatment with dofequidar resulted in possible clinical benefit for patients who had not received prior therapy, who were premenopausal, or who were stage IV at diagnosis with an intact primary tumor. Dofequidar was also well

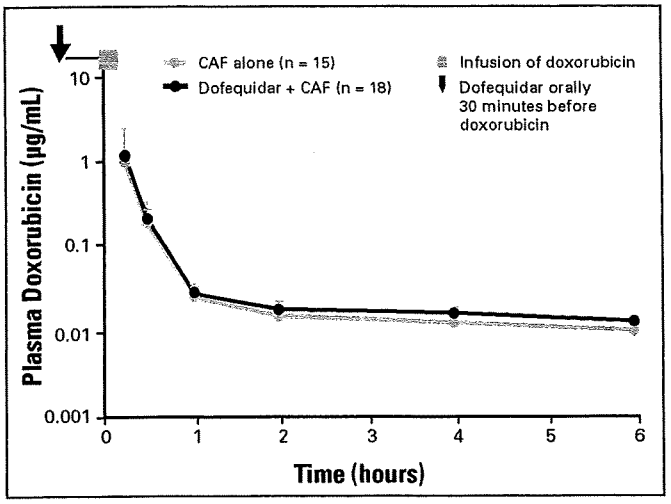

Fig 4. Plasma levels of doxorubicin in patients receiving dofequidar or placebo. CAF, cyclophosphamide, doxorubicin, and fluorouracil.

tolerated in the clinical setting and had no impact on doxorubicin pharmacokinetics. Further studies are merited to assess the effect of dofequidar in specific patient populations with breast cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author or immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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Prospective Study of Positron Emission Tomography for Evaluation of the Activity of Lapatinib, a Dual Inhibitor of the ErbB1 and ErbB2 Tyrosine Kinases, in Patients with Advanced Tumors

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Background: To evaluate the role of FDG-PET in assessing anti-tumor efficacy of molecular targeted drugs, we prospectively performed FDG-PET and CT for response evaluation in patients treated with lapatinib, a dual inhibitor of ErbB1 and ErbB2 tyrosine kinases.
Methods: Lapatinib was given orally once a day at doses ranging from 1200 to 1800 mg in a phase I study. CT and FDG-PET were performed before treatment, and at 1, 2 and 3 months after the initiation of the treatment and every 2 months thereafter.
Results: A total of 29 FDG-PET examinations were performed in eight patients with various solid tumors and the metabolic activity in the tumor was evaluated as SUVmax. The best responses, as assessed by CT, were as follows; one partial response, four stable disease and three disease progression. The partial response was observed in a patient with trastuzumab-resistant breast cancer, whose SUVmax was decreased by 60% from baseline. In all of the four patients whose best response was stable disease, the SUVmax was decreased by 6–42% one month after the start of treatment. Prolonged stable disease (10 months) was observed in a patient with colon cancer, whose SUVmax was decreased by 42%. In the patient group with disease progression, SUVmax was increased in two out of three patients.
Conclusions: FDG-PET detected decreases in the metabolic activity of the tumors in patients who experienced clinical benefits on treatment with lapatinib. Thus, FDG-PET may be useful for the evaluation of molecular targeted drugs, such as lapatinib.

Key words: FDG-PET – lapatinib – phase I – pharmacodynamics – biomarker

INTRODUCTION

Recently, many molecular targeted drugs that act as cytostatic, rather than cytotoxic, agents have been developed. It is expected that they may slow or stop the growth of tumors, without causing existing tumors to shrink. Furthermore, their toxicities are expected to be mild. Therefore, toxicity and decrease in tumor size may not be used as endpoints in phase I and phase II studies, respectively and new endpoints are necessary for the clinical trial of molecular targeted drugs.

Positron emission tomography with the glucose analog fluorine-18 fluorodeoxyglucose (FDG-PET) allows the noninvasive serial measurements of glucose metabolism in tumors. In oncology, FDG-PET was first used for the diagnosis and staging of tumors. FDG uptake is closely related to the number and proliferative capacity of viable tumor cells (1,2). Therefore, treatment-induced changes resulting in tumor cell death or growth arrest leads to a reduction in FDG uptake. It has been reported that FDG-PET may be useful for the evaluation of anti-cancer treatments using cytotoxic chemotherapy (3–7). These data suggest that FDG-PET may offer a surrogate marker for clinical benefit in traditional chemotherapy. If molecular targeted drugs also inhibit the proliferation of cancer cells, reduction of FDG uptake by tumors should also occur after treatment with molecular targeted drugs. Therefore, FDG-PET can be expected to be a surrogate marker for the action of cytostatic molecular targeted drugs as well.

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Lapatinib is a new drug that inhibits the epidermal growth factor receptor tyrosine kinases ErbB1 and ErbB2. By inhibiting signals from these receptors, lapatinib blocks several downstream pathways involved in cell proliferation, invasion and apoptosis, such as ERK-1/2 and AKT, respectively. Phase I trials have been conducted in the USA (8), but the maximum tolerated dose was not determined because lapatinib was generally well tolerated. Biological activities, including partial responses in patients with trastuzumab-resistant breast cancer and disease stabilization of a variety of carcinomas, were reported. A phase I clinical trial has been conducted in patients with solid tumors in Japan (9). To evaluate the efficacy of lapatinib by FDG-PET and to correlate the results of FDG-PET with response evaluation by CT, FDG-PET was prospectively performed in a subsidiary study. This communication is therefore a report of the FDG-PET study conducted in association with a phase I study of lapatinib, details of which will be reported separately.

PATIENTS AND METHODS

Patients and Treatment

This phase I study was conducted in two institutions, Kinki University Hospital and the National Cancer Center Hospital East. Lapatinib was administered orally once daily until patients had disease progression or unacceptable toxicities. Six patients per dose were treated at 900, 1200, 1600 or 1800 mg/day. At the National Cancer Center Hospital East, three patients per group received 1200, 1600 or 1800 mg/day. Of these nine patients, eight were enrolled in the FDG-PET study. The protocols of the phase I study and the FDG-PET study were approved by the Institutional Review Board of the National Cancer Center. Written informed consent was obtained from each patient.

Evaluation of Tumor Responses

At study entry, up to three representative lesions were selected for tumor evaluation. The sum of the largest diameter of each of these lesions was assessed with CT and the maximum standardised uptake values (SUVmax), which is the maximum pixel value within the region of interest, of the same lesions were recorded with FDG-PET. CT and FDG-PET were performed before treatment, and at 1, 2 and 3 months after the initiation of the treatment and every 2 months thereafter. Responses by CT were classified according to the response evaluation criteria in solid tumors (RECIST). Treatment discontinuation owing to disease progression was based on CT findings according to the protocol of the phase I study. The results of the PET analysis were not taken into account for this purpose.

Time to progression was defined as the time from first dose of lapatinib to the earliest documentation of progression, death from any cause, or withdrawal from the trial for any reason.

FDG-PET was performed using a GE-Advanced scanner (General Electric Medical Systems, Milwaukee, WI, USA) with an axial field of view of 15 cm and a slice thickness of 4.75 mm. The SUVmax is known to be affected by several factors, including plasma glucose levels, time from FDG injection to measurement and body weight. Therefore, all patients fasted for at least 6 h before FDG-PET scanning and plasma glucose level was measured just before the FDG injection. Exactly 60 min after intravenous injection of 259–310 MBq FDG, an attenuation-corrected whole body scan was acquired in seven bed positions (5-min emission and 1-min transmission).

RESULTS

A total of 29 FDG-PET examinations were performed in eight patients whose characteristics are listed in Table 1. All patients had prior chemotherapy and four patients with non-small cell lung cancer had failed gefitinib treatment.

Time from FDG injection to the start of PET scanning was exactly 60 min in 28 examinations and 59 min on one occasion. Plasma glucose levels were always below 120 mg/dl (median, 97 mg/dl; range, 77–116). Little change in body weight of individual patients was observed during the course of the study.

The median number of days between pretreatment FDG-PET and treatment initiation was 4 (range, 1–12) days. In 93% of examinations, CT and FDG-PET were performed within 7 days; the median number of days between CT and FDG-PET examination was 1 (range, 0–21 days).

The best responses, as assessed by CT, were as follows; one partial response, four stable disease and three disease progression. Figure 1 shows the time course of the SUVmax and tumor responses in each patient.

The single partial response was observed in a patient with trastuzumab-resistant breast cancer (Her2, 3+; ER/PgR, negative) receiving 1600 mg/day, whose SUVmax was decreased by 60% from baseline one month after the start of treatment when a partial response was documented by CT; thereafter, SUVmax began to increase again 2 months before progression was documented by CT (Fig. 2A, B).

In all four patients whose best response was stable disease, the SUVmax was decreased by 6–42% 1 month after the start of treatment. In a patient with colon cancer with prolonged stable disease (10 months), the SUVmax was decreased by 42% at the first post-treatment evaluation and maintained thereafter for 9 months. The SUVmax also began to increase 1 month earlier than the documentation of disease progression by CT (Fig. 3A, B).

In the patient group with disease progression, the best SUVmax response ranged from +5% to −42%. SUVmax was increased by 4–5% in two out of three patients with CT-assessed progressive disease (Fig. 1). In a patient whose