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ORIGINAL ARTICLE

A phase I and pharmacologic study of sequences of the proteasome inhibitor, bortezomib (PS-341, Velcade™), in combination with paclitaxel and carboplatin in patients with advanced malignancies

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Abstract
Purpose Bortezomib, a selective inhibitor of the 20S proteasome with activity in a variety of cancers, exhibits sequence-dependent synergistic cytotoxicity with taxanes and platinum agents. Two different treatment schedules of bortezomib in combination with paclitaxel and carboplatin were tested in this phase I study to evaluate the effects of scheduling on toxicities, pharmacodynamics and clinical activity.
Methods Patients with advanced malignancies were alternately assigned to receive (schedule A) paclitaxel and carboplatin (IV d1) followed by bortezomib (IV d2, d5, d8) or (schedule B) bortezomib (IV d1, d4, d8) followed by paclitaxel and carboplatin (IV d2) on a 21-day cycle.
Results Fifty-three patients (A 25, B 28) were treated with a median of 3 cycles (range 1–8) for schedule A and 3.5 cycles (range 1–10) for schedule B. Grade 3 or higher treatment related hematologic adverse events in all cycles of treatment included neutropenia (A 52%, B 50%), anemia (A 12%, B 7.1%) and thrombocytopenia (A 16%, B 17.9%). Non-hematologic treatment related adverse events were fairly mild (primarily grades 1 and 2). The maximum tolerated dose and the recommended doses for future phase II trials are bortezomib 1.2 mg/m^2, paclitaxel 135 mg/m^2 and carboplatin AUC = 6 for schedule A and bortezomib 1.2 mg/m^2, paclitaxel 175 mg/m^2 and carboplatin AUC = 6 for schedule B. Six (21.4%) partial responses (PR) were seen with schedule B. In contrast, only 1 (4%) PR was achieved with schedule A. Similar proteasome inhibition was achieved at MTD for both schedules.
Conclusion Administration of sequential bortezomib followed by chemotherapy (schedule B) was well tolerated and associated with an encouraging number of objective responses in this small group of patients. Further studies with this administration schedule are warranted.

Keywords PS-341 · Ubiquitin–proteasome system · Chemotherapy

Introduction

The ubiquitin–proteasome pathway is essential for the precise control of intracellular protein turnover and plays a critical role in regulating cell cycle, neoplastic growth and metastasis [1, 2]. Inhibitors of the proteasome arrest tumor growth and spread through multiple mechanisms, including down regulation of NF-kappaB, MAPK, bcl-2, and modulation of the tumor suppressor p53, cyclins, cyclin dependent kinase inhibitors p21 and p27, Bax and cell adhesion molecules [2].

Bortezomib (PS-341, Velcade™) is a boronic acid dipeptide derivative which is a potent proteasome inhibitor binding specifically and selectively to the enzyme's chymotrypsin-like active site. It is the first of

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its class to undergo clinical testing and has demonstrated anti-tumor activity in a variety of malignancies including non-small cell lung cancer [3], prostate cancer [4], multiple myeloma [5], mantel cell lymphoma [6] and indolent non-hodgkin's lymphoma [7, 8]. Bortezomib is well tolerated with manageable toxicities in phase I and phase II clinical trials both as a single agent and in combination with other agents. Dose limiting toxicities included diarrhea and peripheral neuropathy [2, 9]. Bortezomib has recently been approved by Food and Drug Administration for the treatment of refractory multiple myeloma who have received at least 1 prior therapy [10].

Bortezomib demonstrated the greatest activity when combined with standard chemotherapeutic agents in preclinical models of various tumor types, including breast, lung, colon, pancreatic and ovarian [9, 11–16]. Additive/synergistic effect of bortezomib was observed with a number of chemotherapeutic agents including cyclophosphamide, cisplatin, 5-flurouracil, paclitaxel, docetaxel, doxorubicin, CPT-11 and gemcitabine in these studies [9, 11–15]. It is postulated that bortezomib overcomes resistance to conventional chemotherapy by blocking chemotherapy-induced NF-kappaB activation as well as increasing cell cycle regulatory proteins, p21, p27, transcription factor p53 and decreasing the expression of bcl-2 [9, 12–14]. Sequence of administration has been shown to be important when combining bortezomib with chemotherapeutic agents in preclinical studies, however, conflicting data exists in regards to the optimum sequencing of administration [17–20]. While bortezomib was found to improve the efficacy of gemcitabine in MIA-PaCa-2 human pancreatic cancer cells [18] and the combination of carboplatin/gemcitabine in A549 non-small-cell cancer cell line when administered after chemotherapy [17], the administration of docetaxel prior to bortezomib produced a greater cell kill compared with the reverse sequence [20]. Therefore, sequence of administration has become an important issue to address when combining bortezomib with chemotherapeutic agents.

The combination of paclitaxel and carboplatin is widely employed for therapy of a variety of tumor types including advanced non-small cell lung, ovarian, and bladder cancers. In addition, it has been shown that bortezomib prolongs tumor growth delay and decreases lung metastasis when used in combination with either cisplatin or paclitaxel in mice bearing the Lewis lung carcinoma [11]. Bortezomib also increases the tumor cell killing of cisplatin in the EMT-6 murine mammary carcinoma tumor model [11]. It is thus rational to test the combination of bortezomib with paclitaxel and a platinum compound. The cytotoxic species generated from both cisplatin and carboplatin are identical [21]. Carboplatin was chosen as the platinum agent in the present study because of a more convenient dosing schedule and less severe non-hematologic toxicities than cisplatin.

The primary goal of this phase I study was to determine the maximum tolerated doses (MTDs) of the combination of bortezomib plus paclitaxel plus carboplatin in patients with advanced malignancies. To evaluate the importance of sequencing of administration, two different treatment schedules with either paclitaxel and carboplatin (IV d1) followed by bortezomib (IV d2, d5, d8) [schedule A] or bortezomib (IV d1, d4, d8) followed by paclitaxel and carboplatin (IV d2) [schedule B], were evaluated.

Patients and methods

Patient selection

Patients with histologic proof of metastatic or locally advanced solid tumors were eligible for this study. Eligibility criteria included age ≥ 18 years; Eastern Cooperative Oncology Group performance status ≤ 2; adequate bone marrow (platelets $\geq 100 \times 10^9$ cells/l, absolute neutrophil count [ANC] $\geq 1.5 \times 10^9$ cells/l), hepatic (total bilirubin $\leq 1.5 \times$ upper limit of normal and AST $\leq 1.5 \times$ upper limit of normal) and renal (serum creatinine ≤ 1.5 times the upper limit of normal) functions; life expectancy ≥ 12 weeks; no chemotherapy, radiotherapy, immunotherapy, biologic, or investigational drug therapy within 4 weeks prior to study entry; no nitrosourea or mitomycin C chemotherapy within 6 weeks prior to study entry and no prior bone marrow transplant. Excluded from this study were patients with radiation therapy to $> 30\%$ of the bone marrow; grade 2 or more peripheral neuropathy; uncontrolled infection; known HIV-positivity; brain metastasis, unless disease had been resected by surgery or radiosurgery and patient had been stable for at least 8 weeks. Written informed consent was obtained according to federal and institutional guidelines.

Experimental treatment

This study consisted of two groups (schedules A and B). Patients were alternately assigned to either schedule A to receive escalating doses of paclitaxel and carboplatin on day 1 followed by bortezomib on days 2, 5, and 8 or schedule B to receive bortezomib on days 1, 4 and 8 with paclitaxel and carboplatin given on day 2. Cycle length was 21 days. Table 2 shows the dose escalation

scheme. At the time of scheduled re-treatment of each cycle, bortezomib, carboplatin and paclitaxel were to be held if ANC < 1,500, PLT < 100k, or grade 3/4 non-hematologic toxicities occur until ANC > 1,500, PLT > 100k and non-hematologic toxicities return to base line or normal limits. Within the treatment cycle (days 5 and 8 every cycle of schedule A and days 4 and 8 every cycle of schedule B), bortezomib was to be omitted if ANC < 1,000, PLT < 75k, grade 2 or higher vomiting or diarrhea despite maximal supportive care, grade 2 peripheral neuropathy or grade 3 or higher of all other non-hematologic toxicities were experienced and restarted at lower dose levels with carboplatin and paclitaxel to be reduced by 20% at scheduled retreatment for the next cycle. If grade 4 non-hematologic toxicities were experienced, the dose of carboplatin and paclitaxel was to be reduced by 40%. Protocol treatment was to be discontinued if the patient develops grade 3/4 peripheral neuropathy.

Bortezomib was supplied by the Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI) as a 3.5 mg vial for injection. The drug was given without further dilution as an intravenous bolus (over 3–5 s). Paclitaxel was given as an IV infusion over about 3 h, followed by carboplatin IV over 30 min. Standard premedications with dexamethasone, diphenhydramine, and ranitidine or cimetidine were given prior to paclitaxel infusion.

The cohorts-of-three phase I design was used for dose escalation to assess the MTD of each schedule for this regimen. For each cohort, three patients were treated initially at a dose level and assessed for dose limiting toxicity (DLT) at the end of cycle 1 (at 3 weeks). The next three patients are placed on either: (1) one dose level higher; (2) the same dose level; or (3) one level lower depending on the number of DLTs observed.

Once the MTD was determined for a given schedule, up to 10 additional patients were accrued in each schedule for performance of ancillary and pharmacodynamic studies.

Dose limiting toxicities

All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). The maximum tolerated dose (MTD) was defined as one dose level below the dose that induced dose-limiting toxicities in one-third or more of patients (at least two of a maximum of six patients). MTD was defined based on toxicities documented in the first cycle of treatment only. Severe or life-threatening non-hematologic toxicity (grade 3 or 4), with the exception of nausea, vomiting or diarrhea, was considered dose-limiting. Grade 3 or 4 nausea and vomiting with maximal anti-emetic treatment and grade 3 or 4 diarrhea in spite of maximal antidiarrheal therapy were considered dose-limiting. Grade 4 neutropenia associated with fever or lasting for 5 days or more, grade 4 anemia or platelet counts $\leq 25 \times 10^9$/l of any duration were likewise deemed dose-limiting. In addition, a treatment delay of a week or more with bortezomib during treatment weeks 1–2 because of toxicity was also considered a DLT.

Pretreatment and follow-up studies

Complete patient histories, physical examination, complete blood counts, serum electrolytes, and chemistries were obtained at baseline and prior to each course of treatment. Laboratory studies were performed weekly. Serum pregnancy test was performed for women of childbearing potential. Chest X-ray was performed at base line. Evaluation of indicator lesion (computed axial tomographic scans, or magnetic resonance imaging, etc.) for patients with measurable or evaluable disease was performed at baseline and at the end of every other cycle to assess tumor response. Confirmatory scans were obtained 6 weeks following initial documentation of objective response. Modified Response Evaluation Criteria in Solid Tumors (RECIST) was used to document treatment response.

Full supportive care, such as anti-emetics, anti-diarrhea, blood products, use of recombinant erythropoietin to maintain adequate hemoglobin and avoid blood transfusions, etc. were rendered as necessary. Routine or prophylactic use of colony-stimulating factors G-CSF or GM-CSF was not allowed. Therapeutic use of G-CSF or GM-CSF in patients with serious neutropenic complications were allowed at the physician's discretion.

20S proteasome inhibition studies

Preclinical studies in animal models demonstrated that bortezomib was rapidly removed from the circulation and distributed widely to non-vascular tissues, making detection in serum difficult. Thus, a pharmacodynamic assay measuring the amount of inhibition of the 20S proteasome in whole blood, developed by Millennium Pharmaceuticals, Inc. [22], was employed. For patients enrolled at MTD, two 5-ml venous blood samples were obtained prior to the study treatment to establish a baseline, one sample each on the day prior to bortezomib administration and then at 1, 6, and 24 h following completion of the bortezomib administration on days 2 and 8 (Schedule A) or days 1 and 4 (Schedule B)

during the first cycle. Blood samples were collected in sodium heparin-containing tubes and inverted several times before freezing at −80°C. Samples were sent frozen to Millennium Pharmaceuticals, Inc. for 20S proteasome inhibition determination. The blood cells were lysed with 5 mM EDTA (pH 8.0) for 1 h and then centrifuged at 6,600×*g* for 10 min at 4°C. The resultant whole blood lysate samples were then used in the 20S proteasome assay as described previously [22]. Briefly, samples (10 ml) were added to 2 ml of buffered substrate (20 mM HEPES, 0.5 mM EDTA, 0.05% SDS, and 60 mM Ys substrate-Suc-Leu-Leu-Val-Tyr-AMC; Bachem, King of Prussia, PA, USA). The reaction was carried out at 37°C for 5 min; and the rate of substrate cleavage by the 20S proteasome was determined. The protein content of the samples was estimated using a Coomassie protein assay (Pierce Corp., Rockford, IL, USA).

Results

Patient demographics

A total of 53 patients (A 25 and B 28) were treated between Dec 5, 2001 and July 13, 2004 at Mayo Clinic, Rochester, MN. The overall median age was 54 (range 23–76) years. Forty-seven percent of patients were females. Majority of patients had ECOG performance status 0 or 1. The most common tumor types were melanoma, lung, sarcoma, and gastro-intestinal malignancies. All patients were off active treatment as of December 16, 2004. Table 1 lists the patient characteristics overall and by schedule.

Table 1 Patient characteristics

Characteristic, *N* (%)	Schedule A (*N* = 25)	Schedule B (*N* = 28)	Overall (*N* = 53)
Age (yr), median (range)	52 (23,76)	56.5 (24,70)	54 (23,76)
Gender			
Female	13 (52%)	12 (42.9%)	25 (47.2%)
Male	12 (48%)	16 (57.1%)	28 (52.8%)
Race			
White	24 (96%)	26 (92.9%)	50 (94.3%)
Unclassified	1 (4%)	2 (7.1%)	3 (5.7%)
Performance score			
0	12 (48%)	13 (46.4%)	25 (47.2%)
1	11 (44%)	14 (50%)	25 (47.2%)
2	2 (8%)	1 (3.6%)	3 (5.6%)
Tumor type			
Melanoma	7 (28.%)	7 (25%)	14 (26.4%)
Lung	4 (16%)	9 (32.1%)	13 (24.5%)
Breast	2 (8%)	0 (0%)	2 (3.8%)
Sarcoma	4 (16%)	3 (10.7%)	7 (13.2%)
Vagina	0 (0%)	1 (3.6%)	1 (1.9%)
Colon	2 (8%)	0 (0%)	2 (3.8%)
Ovary	1 (4%)	1 (3.6%)	2 (3.8%)
Uterine	1 (4%)	0 (0%)	1 (1.9%)
Thyroid	1 (4%)	0 (0%)	1 (1.9%)
Adrenal	1 (4%)	1 (3.6%)	2 (3.8%)
Stomach	1 (4%)	0 (0%)	1 (1.9%)
Esophageal	0 (0%)	3 (10.7%)	3 (5.7%)
Prostate	0 (0%)	1 (3.6%)	1 (1.9%)
Gall bladder	0 (0%)	1 (3.6%)	1 (1.9%)
Head and neck	1 (4%)	1 (3.6%)	2 (3.8%)
Prior treatments			
Chemotherapy	20 (80%)	18 (64.3%)	38 (71.7%)
Radiation therapy	10 (40%)	10 (35.7%)	20 (27.7%)
Surgery	25 (100%)	28 (100%)	53 (100%)

Toxicities

Thirty-three patients (A 15, B 18) were enrolled into the dose escalation portions and 20 patients (A 10, B 10) were enrolled at the MTD (Table 2). The median number of cycles administered in schedules A and B were 3 (range 1–8) and 3.5 (range 1–10), respectively. All 33 patients enrolled for the dose escalation portion were evaluable for DLT. In schedule A, 2 out of the 6 patients experienced DLT at dose level 4 (one patient had platelet counts of less than 25k and another patient missed a treatment during cycle 1 due to low platelet counts). In schedule B, 2 out of the 3 patients experienced DLT at dose level 6 (one patient missed day 8 of cycle 1 treatment due to platelet counts of less than 25k and a grade 4 neutropenia, the other patient had a grade 4 thrombocytopenia with a platelet count of 24k). As a result, dose level 3 (bortezomib 1.2 mg/m^2, paclitaxel 135 mg/m^2 and carboplatin AUC = 6) was declared the MTD for schedule A and dose level 5 (bortezomib 1.2 mg/m^2, paclitaxel 175 mg/m^2 and carboplatin AUC = 6) was declared as MTD for schedule B. Among the ten additional patients treated at the MTD of each schedule, one patient in schedule A had a grade 4 neutropenia that resulted in a dose reduction during cycle 1 and one patient in schedule B also had a grade 4 neutropenia during cycle 1. No grade 5 toxicities were seen in this study. Also, there were no grade 4 non-hematologic toxicities during cycle 1 in both groups.

Hematologic toxicity

Grade 3 or higher hematologic toxicities during cycle 1 included neutropenia (A 24%, B 28.6%), thrombocytopenia (A 4%, B 3.6%) and anemia (A 0%, B 3.6%), and during all cycles of treatment included neutropenia

Table 2 Dose escalation scheme and treatment data for schedules A and B

Dose level	Bortezomib (mg/m^2)	Paclitaxel (mg/m^2)	Carboplatin (AUC)	Schedule A		Schedule B	
				No. Patients	No. DLTs	No. Patients	No. DLTs
1	0.7	135	6	3	0	3	0
2	0.9	135	6	3	0	3	0
3	1.2	135	6	3 (10[a])	0 (1[a])	3	0
4	1.2	150	6	6	2	3	0
5	1.2	175	6	0	0	3 (10[a])	0 (1[a])
6	1.5	175	6	0	0	3	2

[a] Additional patients treated at the MTD

(A 52%, B 50%), anemia (A 12%, B 7.1%) and thrombocytopenia (A 16%, B 17.9%).

Non-hematologic toxicity

The non-hematologic side effects of bortezomib in combination with carboplatin and paclitaxel were mostly mild to moderate, with majority being grade 1 and 2 (Figs. 1, 2). Alopecia, fatigue, anorexia, nausea, vomiting, diarrhea and peripheral neuropathy are among the most common toxicities experienced for both schedules as shown in Figs. 1, 2.

Comparison of toxicity profiles

Overall, a higher percentage of patients on schedule B experienced severe (grade 3 or higher) toxicities both in cycle 1 and over all treatment cycles, although none was statistically significantly different between the two schedules (Table 3). It is important to note, however, that 19 (68%) of the 28 patients on schedule B were treated at dose levels higher than the MTD of schedule A. A further breakdown of the severe toxicities listed in Table 3 by dose levels indicated that majority of the toxicities within each schedule occurred at their respective MTD or higher dose levels. Specifically, 4 of 6 patients (cycle 1) and 15 out of 19 patients (all cycles) who experienced grade 3 or higher toxicities were treated at dose levels 3 (MTD) and 4 in schedule A; 6 out of 10 patients (cycle 1) and 11 out of 21 patients (all cycles) who experienced grade 3 or higher toxicities were treated at dose levels 5 (MTD) and 6. A similar pattern was observed when breakdown for any grade 4, hematologic and non-hematologic toxicities.

Antitumor activity

All 53 patients (A 25 and B 28) were evaluable for efficacy. No CR was obtained. Six (21.4%) PRs were seen in schedule B (2 in previously treated malignant melanoma, 3 in NSCLC and 1 in a patient with gynecologic cancer). Five of these patients maintained PR for 5 cycles. In contrast, only 1 (4%) PR was achieved in schedule A in a patient with previously treated melanoma, which lasted for 6 cycles. SD was observed in 14 (56%) and 13 (46.4%) of patients in schedules A and B, respectively. Five patients on schedule A and 2 patients on schedule B had SD for 5 cycles. None of the patients with PR had previous exposure to paclitaxel. Of the 34 patients (A 15, B 19) who went off study due to disease progression only 4 (A 3, B 1) had been treated with paclitaxel before study entry.

20S proteasome inhibition

The 20S proteasome inhibition study was performed at various time points following administration of bortezomib during the first cycle of treatment for the 20 patients (Schedule A 10, Schedule B 10) enrolled at MTD (1.2 mg/m^2 of bortezomib). As shown in Table 4, regardless of the treatment schedule, proteasome inhibition was achieved for all patients and was comparable to inhibition seen in other studies in the first hour (ranging from 64.8 to 73.7%) [17]. Recovery of the proteasome activity was approximately 80% in 24 h following the administration of bortezomib.

Discussion

The Ubiquitin–proteasome pathway plays an important role in neoplastic cell growth and metastasis. Bortezomib, a potent proteasome inhibitor, has shown antitumor activity in a variety of tumor types [2]. Recent studies also indicate that bortezomib potentiates the sensitivity of tumor cells to chemotherapeutic agents, likely through regulating the intracellular levels of molecules important in mediating chemotherapy resistance [2]. Interestingly, the sequence of administration for the combination of bortezomib and different chemotherapeutic agents appears to be important for the synergistic anti-tumor effect, although the underlying

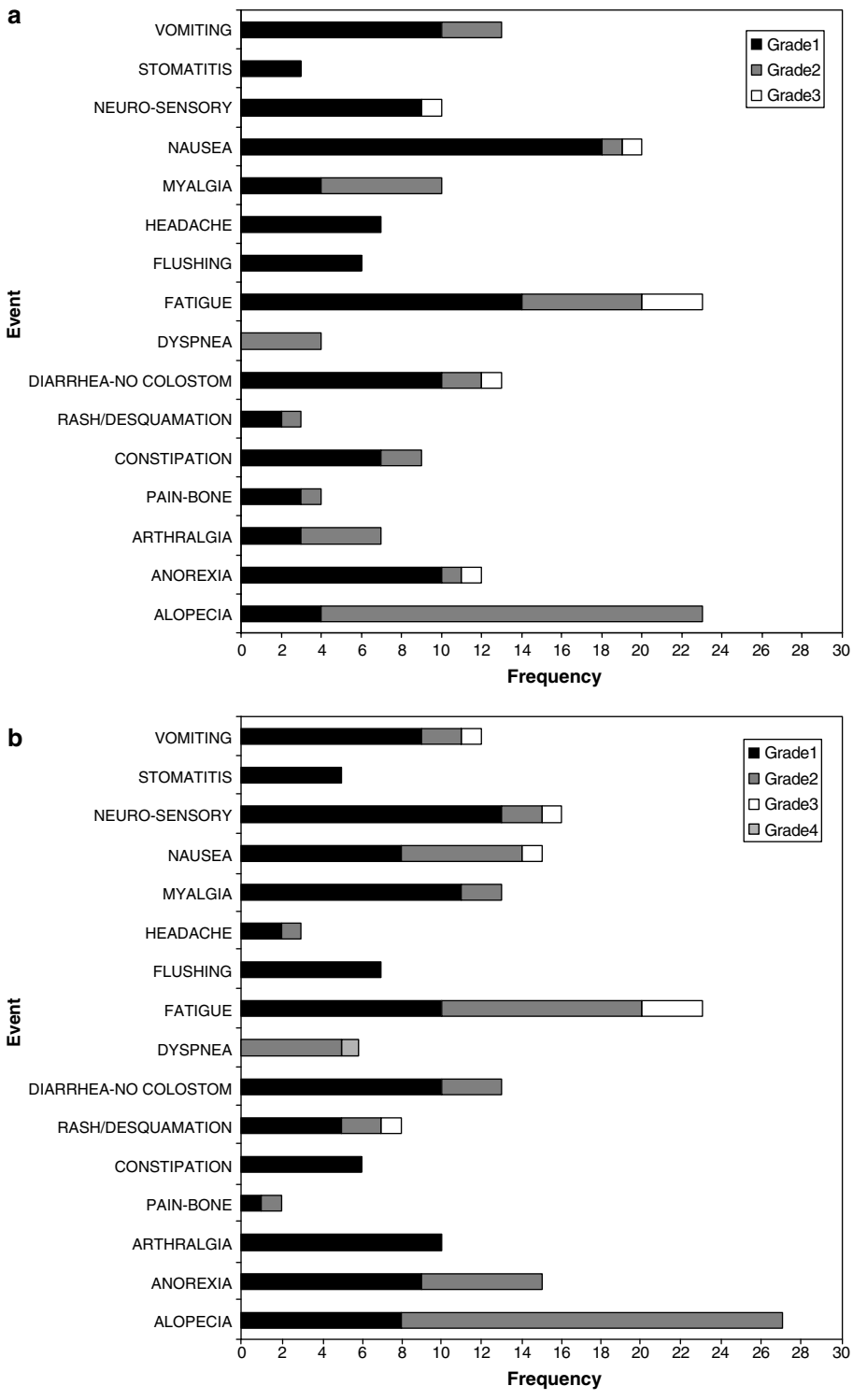

Fig. 1 All cycles non-hematologic treatment-related toxicities occurring in at least 10% of patients in schedule A (**a**) and schedule B (**b**). "*Event*" refers to treatment-related toxicity, with the greatest grade shown for each patient. "*Frequency*" refers to the number of patients who experienced the particular "event"

mechanism is unclear. The present study evaluated two different treatment schedules (A and B) of bortezomib in combination with carboplatin and paclitaxel to explore the importance of the sequence of administration on the MTD and bortezomib pharmacodynamic parameters. Patients were alternately assigned to either schedule A to receive escalating doses of paclitaxel and carboplatin on day 1 followed by bortezomib on days 2, 5, and 8 or schedule B to receive bortezomib on days 1, 4 and 8 with paclitaxel and carboplatin given on day 2 on a 21-day cycle.

In this combination study of bortezomib and carboplatin/paclitaxel with two different treatment schedules, schedule B appeared to be better tolerated with

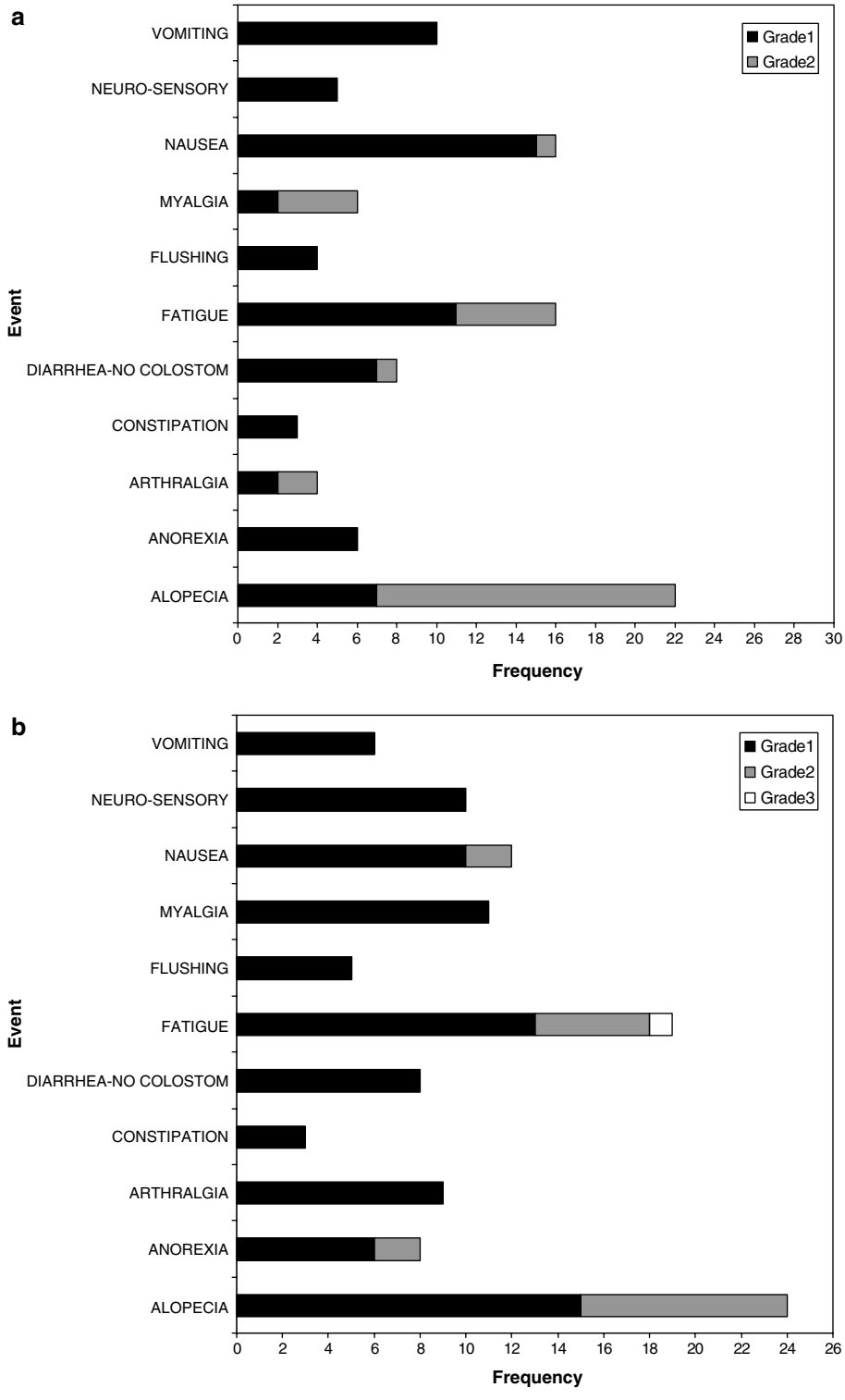

Fig. 2 Cycle 1 non-hematologic treatment-related toxicities occurring in at least 10% of patients in schedule A (**a**) and schedule B (**b**). "*Event*" refers to treatment-related toxicity, with the greatest grade shown for each patient. "*Frequency*" refers to the number of patients who experienced the particular "event"

an apparently higher response rate. The MTD for schedule B was defined at dose level 5 (bortezomib 1.2 mg/m^2, paclitaxel 175 mg/m^2 and carboplatin AUC = 6) compared to the MTD obtained at dose level 3 (bortezomib 1.2 mg/m^2, paclitaxel 135 mg/m^2 and carboplatin AUC = 6) for schedule A. For both treatment schedules, hematologic toxicities including neutropenia and leukopenia were the most common grade 3 or higher treatment related adverse events. Thrombocytopenia and treatment delays due to thrombocytopenia were the observe DLTs, for both treatment schedules. Non-hematologic toxicities were fairly

Table 3 Comparison of toxicities by schedule

	Schedule A (25[a]) *N* (%)	Schedule B (28[a]) *N* (%)	*P* values[b]
Cycle 1 toxicity			
Any grade 3 or higher	6 (24%)	10 (35.7%)	0.35
Any grade 4 or higher	1 (4%)	4 (14.3%)	0.20
Grade 3 or higher hematologic	6 (24%)	9 (32.1%)	0.51
Grade 4 or higher hematologic	1 (4%)	4 (14.3%)	0.20
Grade 3 or higher non-hematologic	0 (0%)	1 (3.6%)	0.34
All cycles of toxicity			
Any grade 3 or higher	19 (76%)	21 (75%)	0.93
Any grade 4 or higher	4 (16%)	9 (32.1%)	0.17
Grade 3 or higher hematologic	16 (64%)	19 (67.9%)	0.77
Grade 4 or higher hematologic	4 (16%)	8 (28.6%)	0.28
Grade 3 or higher non-hematologic	8 (32%)	8 (28.6%)	0.79

[a] Includes 10 additional patients treated at MTD

[b] Fisher's exact/chi-square test

Table 4 Inhibition of 20S proteasome in peripheral whole blood

	% Inhibition (mean ± SE)		
	1 h	6 h	24 h
Schedule A			
Day 2	69.7 ± 4.1	51.8 ± 6.3	22.2 ± 2.3
Day 8	68.0 ± 5.0	47.6 ± 2.4	23.6 ± 3.7
Schedule B			
Day 1	64.8 ± 4.1	45.2 ± 3.4	20.6 ± 2.8
Day 4	73.7 ± 3.4	46.6 ± 2.6	28.8 ± 7.4

mild. Interestingly, significantly higher response rate (PR) was observed in patients treated with schedule B (21.4%) than schedule A (4%).

Although schedule B appeared to have shown superior tolerability and efficacy when compared to schedule A, future randomized studies are needed to evaluate this further. Nonetheless, the results from this phase I study are valuable in establishing the MTD and providing initial evidence on potential differences in the two administration schedules for the combination of bortezomib, carboplatin and paclitaxel.

The 20S proteasome activity of peripheral whole blood was assayed at MTD for both schedules to evaluate any potential differences in the pharmacodynamic properties of bortezomib. Because bortezomib is rapidly cleared from the vascular compartment and distribute widely, measurement of its plasma concentration for pharmacokinetics studies was not feasible. The assay of the 20S proteasome inhibition of peripheral whole blood has been shown to be an accurate and reproducible pharmacodynamic indicator of its target activity. The levels of proteasome inhibition in this study with the combination of bortezomib and carboplatin/paclitaxel are similar to what was obtained with single agent bortezomib [23]. In addition, similar levels of proteasome inhibition were achieved at the same time point on different days of treatment for both treatment schedules. This indicates that the pharmacodynamics of bortezomib was probably not affected by the administration of carboplatin and paclitaxel in our study and would not explain the clinical differences observed for the two treatment schedules.

Consistent with our result, carboplatin was found to have no effect on bortezomib pharmacodynamics as measured by percent inhibition of the 20S proteasome in a recently reported phase I trial of bortezomib (days 1, 4, 8, and 11) and carboplatin (day 1) in recurrent ovarian or primary peritoneal cancer [24]. In the same study, NF-kB induced by carboplatin was decreased by the use of bortezomib [24]. Similarly, bortezomib has shown to sensitize cells to Taxol induced apoptosis through the regulation of NF-kB in preclinical studies [25]. It is reasonable to speculate that the sequence of administration might have resulted in differences in the intracellular kinetics of molecules such as NF-kB that are important in mediating chemotherapy resistance for carboplatin and paclitaxel. One other possibility which was not addressed by the current study is the potential impact of administration schedules on the pharmacokinetic parameters of paclitaxel and/or carboplatin, which could affect the tolerability of one schedule over the other.

Further studies to address these possibilities and to elucidate the mechanisms of the sequence dependent synergism of bortezomib with chemotherapeutic agents are needed.

In summary, the toxicity and efficacy of bortezomib in combination with carboplatin and paclitaxel appeared to be schedule dependent. The maximum tolerated dose and the recommended doses for future phase II trials are bortezomib 1.2 mg/m^2, paclitaxel 135 mg/m^2 and carboplatin AUC = 6 for schedule A and bortezomib 1.2 mg/m^2, paclitaxel 175 mg/m^2 and carboplatin AUC = 6 for schedule B.

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