**Chapter 5**

# A parallel dose-escalation study of weekly and twice-weekly bortezomib in combination with gemcitabine and cisplatin in the first-line treatment of patients with advanced solid tumors

Jens Voortman, Egbert F Smit, Richard Honeywell, Bart C Kuenen, Godefridus J Peters, Helgi van de Velde and Giuseppe Giaccone

*Clinical Cancer Research 2007, 13:3642-51*

## Abstract

**Purpose:** To establish maximum tolerated dose (MTD) and tolerability of two schedules of bortezomib in combination with cisplatin and gemcitabine as first-line treatment of patients with advanced solid tumors.

**Experimental Design:** Patients were assigned to increasing doses of bortezomib days 1 and 8 (weekly schedule), or days 1, 4, 8, and 11 (twice-weekly schedule), in addition to gemcitabine 1,000 mg/m$^2$ days 1 and 8 and cisplatin 70 mg/m$^2$ day 1, every 21 days. Maximum of 6 cycles. Plasma pharmacokinetics of cisplatin and gemcitabine were determined at MTD.

**Results:** Thirty-four patients were enrolled of whom 27 had non–small cell lung cancer (NSCLC). Diarrhea, neutropenia and thrombocytopenia were dose-limiting toxicities (DLTs) leading to an MTD of bortezomib 1.0 mg/m$^2$ in the weekly schedule. Febrile neutropenia and thrombocytopenia with bleeding were DLTs in the twice-weekly schedule, leading to an MTD of bortezomib 1.0 mg/m$^2$ as well. Most common ≥ Grade 3 treatment-related toxicities were thrombocytopenia and neutropenia. No Grade ≥ 3 treatment-related sensory neuropathy was reported. Of 34 evaluable patients 13 achieved partial responses, 17 stable disease, and 4 progressive disease. Response and survival of NSCLC patients treated with twice weekly or weekly bortezomib were similar. However, increased dose-intensity of bortezomib led to increased gastro-intestinal toxicity as well as myelosuppression. Pharmacokinetic profiles of cisplatin and gemcitabine were not significantly different in patients receiving either schedule.

**Conclusions:** Weekly bortezomib 1.0 mg/m$^2$ plus gemcitabine 1,000 mg/m$^2$ and cisplatin 70 mg/m$^2$ is the recommended phase 2 schedule, constituting a safe combination, with activity in NSCLC.

## Introduction

The ubiquitin-proteasome pathway plays a pivotal role in the degradation of most intracellular proteins in eukaryotic cells, including those regulating apoptosis, cell cycle progression, transcription factor activation and angiogenesis [Palombella et al., 1994; Read et al., 1995; King et al., 1996]. Bortezomib (VELCADE; Millennium Pharmaceuticals Inc, Cambridge, MA; Johnson & Johnson Pharmaceutical Research and Development, LLC, Raritan, NJ), a dipeptide proteasome inhibitor, is a novel anti-neoplastic agent presently approved for the treatment of patients with relapsed and refractory multiple myeloma and relapsed mantle-cell lymphoma [Twombly, 2003]. Inhibition of the chymotryptic-like proteolytic activity of the proteasome by bortezomib suppresses tumor survival through multiple mechanisms, including induction of $G_2$-M phase cell cycle arrest, cleavage of bcl-2, upregulation and/or accumulation of BH3-only proteins, activation of caspases, and inhibition of nuclear factor-κB (NF-κB) activation [Beg and Baltimore, 1996; Ling et al., 2002; Adams, 2004; Fernandez et al., 2005; Perez-Galan et al., 2006]. In preclinical and clinical studies, it has shown a unique and promising cytotoxicity profile in a variety of solid tumors as well [Adams et al., 1999; Ludwig et al., 2005].

When the recommended schedule for multiple myeloma patients, twice-weekly administration of bortezomib 1.3 mg/m$^2$, was administered as second-line treatment to non-small cell lung cancer (NSCLC) patients, 8% achieved a partial response [Richardson et al, 2005; Fanucchi et al, 2006].

Gemcitabine in combination with cisplatin is a widely used chemotherapeutic regimen for the treatment of advanced non–small cell lung cancer (NSCLC), urothelial cell cancer and other solid tumors [Sandler et al., 2000; Lehmann et al., 2003]. Preclinical and clinical studies indicate synergistic or additive activity when bortezomib is combined with gemcitabine and/or platinum agents [Teicher et al., 1999; Mortenson et al., 2004; Kamat et al., 2004; Aghajanian et al., 2005; Davies et al., 2006]. Inhibition of NF-κB activation, a factor thought to play a role in resistance to chemotherapy, and accumulation of proteins, misfolded or damaged by the effects of chemotherapy, might play important contributory roles [Imai et al., 2003; Fahy et al., 2005]. A sequence-specific interaction of bortezomib and several chemotherapeutics has been demonstrated in some preclinical studies, suggesting administration

of chemotherapy prior to bortezomib increases apoptosis induction in cell lines, as compared to administrating chemotherapy after bortezomib administration [Mack et al., 2003; Mortenson et al., 2004].

The present phase 1B study was designed to establish safety and maximum tolerated dose (MTD) of bortezomib in combination with cisplatin and gemcitabine in patients with advanced solid tumors, especially NSCLC, in order to determine a recommended phase 2 dose. Considering potentially overlapping toxicities between bortezomib and chemotherapy, and as an attempt to develop a more patient-friendly schedule, a weekly schedule of bortezomib was evaluated next to a twice-weekly schedule.

## Patients and methods

***Patients.*** Chemonaive adult patients with inoperable, locally advanced or metastatic cancer, for whom gemcitabine and cisplatin therapy was an acceptable therapeutic option, were eligible for this study. Tumors were cytologically or histologically confirmed. Other eligibility criteria included: Karnofsky performance status (KPS) ≥ 70; life expectancy > 3 months; measurable or evaluable disease. An adequate method of birth control had to be used, and women of childbearing potential had to have a negative urine pregnancy test. Patients were excluded if they had received prior treatment with chemotherapy or bortezomib; had received treatment with monoclonal antibodies, other biologic therapies or investigational agents ≤ 4 weeks before enrollment; underwent major surgery ≤ 4 weeks before enrollment; underwent prior extensive radiation therapy (>25% of bone marrow reserve); underwent radiation therapy within 4 weeks prior to enrollment (except for limited radiation of bone metastases with 1-2 fractions); had inadequate bone marrow and/or organ function, defined as creatinine clearance <60mL/minute (calculated according to Cockcroft-Gault formula), total bilirubin ≥2 times the upper limits of normal, aspartate transaminase ≥3 times the upper limits of normal, alanine transaminase ≥3 times the upper limits of normal, hemoglobin ≤9.0 g/dL, platelet count $<100\times 10^9$/L, absolute neutrophils count $<1.5\times 10^9$/L; had grade ≥ 2 peripheral neuropathy or grade ≥ 3 hearing loss as defined by NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0; had symptomatic central nervous system (CNS) metastases (corticosteroid use was allowed to suppress symptoms); were hypersensitive for boron or mannitol; had serious uncontrolled medical disease, active infection, significant cardiovascular disorder, or any psychiatric illness or other disorders that could potentially impair compliance. This study was reviewed and approved by the institutional review board of the study center. Informed consent was obtained from all patients before undergoing any study-related procedures.

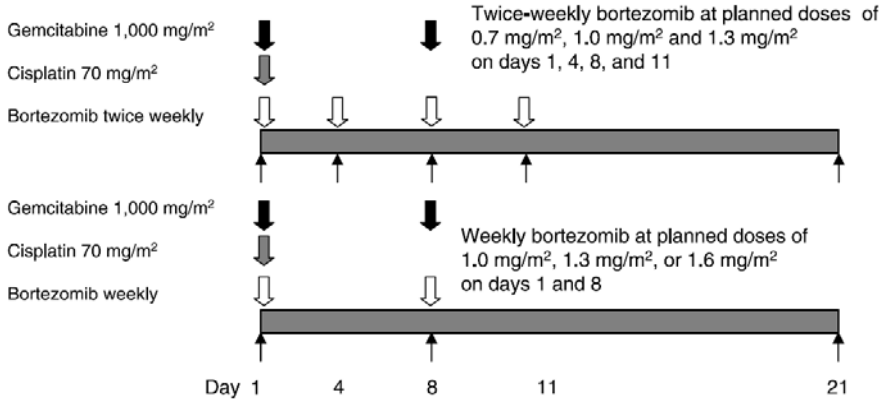


**Figure 1.** Treatment schemas.

***Study design.*** This was a phase 1B, open-label, dose-escalation study conducted in one study center. Two different schedules of bortezomib were evaluated in combination with gemcitabine and cisplatin. Patients were alternately assigned to either schedule. In the twice-weekly schedule bortezomib was administered on days 1, 4, 8, and 11, followed by gemcitabine on days 1 and 8, and cisplatin on day 1. In the weekly schedule bortezomib was administered on days 1 and 8, followed by gemcitabine on days 1 and 8 and cisplatin on day 1 (see Figure 1). Planned bortezomib dose levels were 1.0, 1.3 and 1.6 mg/m$^2$ for the weekly schedule and 0.7, 1.0 and 1.3 mg/m$^2$ for the twice-weekly schedule. Doses of cisplatin and gemcitabine were chosen at 70 and 1,000 mg/m$^2$, respectively. If well tolerated at maximum planned bortezomib dose, cisplatin dose was to be increased to 80 and 100 mg/m$^2$.

At least three patients were enrolled per dose level. Maximum tolerated dose (MTD) determination was based on occurrence of dose limiting toxicity (DLT) in cycle 1. DLT was defined as: any drug-related non-hematologic toxicity grade 3 or 4 (excluding nausea and vomiting or diarrhea responding to symptomatic management); grade 4 neutropenia lasting more than 5 days; grade 3 or 4 febrile neutropenia, grade 4 thrombocytopenia of any duration; and toxicity causing a delay in the start of the next cycle of more than 2 weeks. When a DLT was encountered, the cohort was expanded to six patients. Dose escalation was continued until a DLT was observed in two out of two to six patients.

Initially, doses of gemcitabine and cisplatin were reduced by 25% in case of grade 4 neutropenia and/or grade 3 thrombocytopenia or occurrence of any non-hematologic DLT in the previous cycle. In a protocol amendment the threshold for thrombocytopenia was lowered to grade 4. In case of a DLT in the previous cycle, bortezomib dose was reduced to the next lower dose level. If the patient was receiving 0.7 mg/m$^2$, bortezomib was discontinued. Day 8 gemcitabine was reduced by 50% in case of neutrophils of 0.75-1.5x $10^9$/L and/ or platelets of 50-100x $10^9$/L. Drug-specific dose-modifications were made in

case of neuropathy (cisplatin, bortezomib), nephrotoxicity (cisplatin) or ototoxicity (cisplatin).

Patients could receive a maximum of 6 cycles until disease progression, occurrence of an unacceptable adverse event (AE), death, or meeting of any criterion for withdrawal from treatment. When deemed beneficiary, patients were allowed to continue with bortezomib monotherapy for a maximum of one year.

***Drug administration.*** Bortezomib was provided as a sterile lyophilized powder for reconstitution in vials containing 3.5 mg bortezomib and 35 mg mannitol. Cisplatin and gemcitabine were provided using available commercial supplies. Bortezomib was administered as an intravenous (i.v.) 3-5 sec bolus injection, gemcitabine as an i.v. infusion over 30 minutes, cisplatin as an i.v. infusion over 3 hours. Cisplatin pre- and posthydration consisted of a 1 L and 4 L 0.9% NaCl infusion over 2 and 21 hours, respectively, with 2 g $MgSO_4$ and 20 mmol KCl added per liter. The anti-emetic regimen consisted of dexamethasone 8 mg twice-daily day 1, and ondansetron 8 mg twice-daily days 1 through 4, 8, and, in the twice-weekly schedule, day 11 as well. When deemed beneficiary, aprepitant was added days 1 (125 mg) and days 2 and 3 (80 mg). For the remainder of the cycle metoclopramide was provided on an as needed basis.

***Patient evaluation.*** Patients were evaluated at scheduled visits during screening, treatment and follow-up. At screening, a complete medical history, including KPS, Functional Assessment of Cancer Therapy/ Gynecologic Oncology Group (FACT-GOG) neurotoxicity questionnaire version 4.0, chest x-ray, audiometry, ECG, and laboratory samples for hematology, coagulation tests, clinical chemistry, serum tumor markers (when applicable), and urinalysis (including pregnancy test) were obtained. A physical examination was performed. Target and non-target lesions were identified and measured by spiral-computed tomography scan and/or magnetic resonance imaging. Follow-up assessments were conducted weekly (days 1, 8 and 15) or twice-weekly (days 1, 4, 8, 11 and 15), depending on the treatment schedule, until the end of treatment. One additional visit was planned 6 weeks after ending treatment ("6-week follow-up"). Safety evaluations included symptom-directed physical examination, KPS, laboratory analyses, FACT-GOG neurotoxicity questionnaire (at screening, start of every cycle, end of treatment and at 6-week follow-up) and audiometry (at screening and at least every 3 cycles). Upon occurrence of severe left ventricular dysfunction in one patient, left ventricular ejection fraction (LVEF) measurement by multiple-gated acquisition (MUGA) scan was conducted in subsequently enrolled patients at screening and end of treatment. All adverse events were documented. Toxicity was assessed using the NCI-CTCAE, version 3.0. Patients were evaluated for response using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines at screening, every two cycles, at end of treatment and at 6-weeks follow-up [Therasse et al, 2000]. An objective response was to be confirmed after at least 6 weeks. Patients were observed for disease and survival assessment until death.

***Blood sampling and pharmacokinetic analysis.*** At the MTD, blood plasma samples were drawn from 12 patients (6 patients in each group) for determination of total platinum, gemcitabine, dFdC (2',2'-difluoro-2'-deoxycytidine, gemcitabine), its metabolite dFdU (2',2'-difluoro-2'-deoxyuridine), and endogenous deoxycytidine (CdR) at the following time points: cycle 1, day 1, before infusion of gemcitabine, at the end of gemcitabine infusion, just before starting the cisplatin infusion, and at 30, 60, 120, and 180 minutes after cisplatin infusion and 24 hours after gemcitabine infusion; day 8, before infusion of gemcitabine, at the end of gemcitabine infusion, and at 30 minutes after gemcitabine infusion. Processing of samples and determination of compounds were as described previously [van Moorsel et al., 1999; Honeywell et al., 2006; Honeywell et al., 2007]. Briefly, 150 µL of plasma was extracted and stored at −20°C until analysis. Separation and quantification of gemcitabine and dFdU from the plasma was achieved with an isocratic reversed-phase high-performance liquid chromatography (HPLC) system using a µBondapak C18 column. Peak areas were quantified using the data acquisition program Chromeleon (version 3.02; Chromeleon Chromatography Data Systems, Gynkotek HPLC, Germering, Germany). For CdR measurement, plasma extracts were prepared by protein precipitation and an ethyl acetate/water back extraction. Quantitation was performed by multi-reaction monitoring (MRM) tandem mass spectrometry, using $^{15}N_3$ CdR as an isotopic internal standard. For total plasma platinum (Pt) samples were diluted ten times with 0.38 M NaCl/0.5 M HCL and 0.2% triton + 0.2% antifoam prior to measurement by flameless atomic absorption spectrophotometry (spectra AA−300 Zeeman AAS Varian, Houten, The Netherlands).

***Statistical analysis.*** Descriptive statistics were used for baseline characteristics, safety assessment, and pharmacokinetic data. The response rate was calculated for all response-evaluable patients along with the 95% confidence interval (CI). Median duration of response, stable disease, progression free survival and overall survival was calculated using the Kaplan-Meier method, along with their 95% CI.

## Results

Patients and treatment. A total of 34 chemonaive patients were enrolled between August 2004 and June 2005. All patients were included in the safety and efficacy analyses and received at least one dose of study drug. Table 1 shows patient baseline characteristics. Most patients (n = 27) had NSCLC. Twenty-six out of 27 NSCLC patients (96%) were current or former smokers and 4 patients (15%) had received prior treatment with erlotinib.

Twenty-seven patients (79%) had metastatic disease, 10 of whom with involvement of the CNS (37%). At study entry, one patient was diagnosed with adenocarcinoma of unknown primary, which was later diagnosed as breast carcinoma. Thirty-four patients received a total of 142 cycles. In total

15 patients received twice-weekly bortezomib in combination with chemotherapy, at a dose of 0.7 mg/m$^2$ (3 patients) and 1.0 mg/m$^2$ (12 patients). Nineteen patients received weekly bortezomib in combination with chemotherapy, at a dose of 1.0 mg/m$^2$ (12 patients) and 1.3 mg/m$^2$ (7 patients). Five patients experienced drug-related dose limiting toxicity (DLT) during cycle 1 of treatment. Two of those five patients were treated with twice-weekly bortezomib 1.0 mg/m$^2$. The first patient had NSCLC, KPS 70% on study entry, with liver, bone and CNS metastases, who experienced grade 4 neutropenic sepsis and grade 4 symptomatic thrombocytopenia (gastro-intestinal bleeding). The second patient had grade 4 asymptomatic thrombocytopenia. Therefore the MTD of twice-weekly bortezomib in combination with gemcitabine and cisplatin was established at 1.0 mg/m$^2$. Three patients treated with weekly bortezomib 1.3 mg/m$^2$ developed grade 4 asymptomatic thrombocytopenia during cycle 1, which coincided with grade 4 neutropenia in two patients and grade 3 diarrhea in one patient. This led to an MTD of 1.0 mg/m$^2$ bortezomib in the weekly schedule as well. Exposure to treatment is listed in Table 2. Median number of cycles was four in all dose groups. Median cumulative bortezomib dose was highest in patients treated with twice-weekly bortezomib 1.0 mg/m$^2$ and lowest in patients treated with weekly bortezomib 1.0 mg/m$^2$: 13.7 and 7.2 mg/m$^2$ respectively. Seventy-nine percent of patients had study drugs (cisplatin, gemcitabine and/ or bortezomib) reduced during treatment. Reasons for dose reduction were thrombocytopenia (38%), neutropenia

**Table 1.** Patient characteristics

| | All patients | NSCLC patients |
|---|---|---|
| No. patients | 34 | 27 |
| Median age, y (range) | 55 (35-71) | 53 (35-67) |
| Gender, male/female | 21/13 | 15/12 |
| Karnofsky performance status 70%/≥80% | 6/28 | 4/23 |
| Locally advanced | 7 | 5 |
| Metastatic | 27 | 22 |
| Central nervous system | 10 | 10 |
| Bone | 12 | 10 |
| Liver | 9 | 7 |
| Lung | 15 | 13 |
| Primary tumor | | |
| Lung (NSCLC) | 27 | |
| Urothelium | 4 | |
| Breast* | 1 | |
| Pancreas | 1 | |
| Liver | 1 | |
| Histology (NSCLC patients) | | |
| Squamous cell carcinoma | | 6 |
| Adenocarcinoma | | 12 |
| Mixed adenosquamous cell carcinoma | | 2 |
| Undifferentiated non-small cell carcinoma | | 7 |

*Later diagnosis, at study entry, diagnosed as adenocarcinoma of unknown primary.

(37%), neutropenia combined with thrombocytopenia (10%), asthenia (8%), ototoxicity (3%) and other (5%). Comparing patients receiving weekly and twice-weekly bortezomib 1.0 mg/m$^2$ reasons for dose reduction were: neutropenia, 60% vs. 53%; thrombocytopenia 6% vs. 37%; combined neutropenia and thrombocytopenia, 0% vs. 5%. In the weekly schedule of bortezomib 1.3 mg/m$^2$ reasons for dose reduction were thrombocytopenia 29%, combined neutropenia and thrombocytopenia 29%, neutropenia 18%, and asthenia 24%. In 41% of patients, start of one or more treatment cycles was delayed due to toxicity. Delays were caused by neutropenia (75%) or non-hematologic toxicity (25%).

**Table 2.** Dose intensity per bortezomib dose group

| Dose level | Median number of cycles (range) | Median time to treatment failure, d (range) | Cisplatin projected/ actual dose, mg/m$^2$* | Gemcitabine projected/actual dose, mg/m$^2$/wk* | Bortezomib projected/actual dose, mg/m$^2$/wk* |
|---|---|---|---|---|---|
| Twice-weekly bortezomib 0.7 mg/m$^2$ | 4 (4-6) | 126 (91-133) | 23.3/17.3 (74%) | 667/470 (70%) | 0.93/0.88 (95%) |
| Twice-weekly bortezomib 1.0 mg/m$^2$ | 4 (1-6) | 87.5 (28-136) | 23.3/21.5 (92%) | 667/547.2 (82%) | 1.33/1.14 (86%) |
| Weekly bortezomib 1.0 mg/m$^2$ | 4 (2-6) | 84 (36-126) | 23.3/21.0 (90%) | 667/570.9 (86%) | 0.67/0.60 (90%) |
| Weekly bortezomib 1.3 mg/m$^2$ | 4 (2-6) | 89.5 (55-148) | 23.3/17.7 (76%) | 667/531.5 (80%) | 0.87/0.74 (85%) |

*Actual administered doses per square meter of study drugs were divided by the actual number of weeks between first dose of study drugs and day 21 of the final cycle. Projected doses were calculated as the projected dose per cycle per square meter divided by 3 (weeks).

Common treatment-related AEs occurring throughout the trial are shown in Table 3 and included asthenia (100%), nausea (91%), taste alteration (82%), anorexia (79%), constipation (79%), tinnitus (71%) and sensory neuropathy (62%). Weekly bortezomib 1.0 mg/m$^2$ was generally better tolerated than twice-weekly 1.0 mg/m$^2$ treatment. We observed a trend towards higher incidence of (severe) myelotoxicity as well as gastro-intestinal toxicity and asthenia with increased dose-intensity of bortezomib. Due to grade 3 gastro-intestinal toxicity, 1 (8%) and 3 (25%) patients treated with bortezomib 1.0 mg/m$^2$ weekly or twice-weekly, respectively, were treated with aprepitant. For anemia treatment or prophylaxis, 17 (50%) of patients received (darb)epoetin. Three patients (9%) repeatedly experiencing neutropenia received pegfilgrastim injections. Sixteen (47%) of patients developed a low-grade rash, which was typically acneiform. Fifteen (44%) patients experienced facial/ peri-orbital edema. One patient, who was being treated with gemcitabine and bortezomib only, after discontinuation of cisplatin due to asthenia and gastro-intestinal toxicity, experienced severe, grade 3 peri-

orbital edema which recurred to a lesser degree on later treatment with gemcitabine only.

**Table 3.** Adverse events attributed to cisplatin, gemcitabine, and/or bortezomib (any cycle)

| Schema | Twice-weekly bortezomib 0.7 mg/m² (*n* = 3) | | | Twice-weekly bortezomib 1.0 mg/m² (*n* = 12) | | | Weekly bortezomib 1.0 mg/m² (*n* = 12) | | | Weekly bortezomib 1.3 mg/m² (*n* = 7) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Toxicity grade | 1-2 | 3 | 4 | 1-2 | 3 | 4 | 1-2 | 3 | 4 | 1-2 | 3 | 4 |
| Constitutional | | | | | | | | | | | | |
| Asthenia | 67% | 33% | | 33% | 67% | | 67% | 33% | | 71% | 29% | |
| Flu-like syndrome | 67% | | | 50% | | | 50% | | | 29% | | |
| Hot flush | 33% | | | 42% | | | 8% | | | 43% | | |
| Hematologic | | | | | | | | | | | | |
| Anemia | 100% | | | 92% | 8% | | 92% | 8% | | 72% | 14% | 14% |
| Neutropenia | 33% | 67% | | 8% | 33% | 25% | 8% | 50% | 8% | | 43% | 57% |
| Trombocytopenia | | 100% | | 42% | 33% | 25% | 25% | 50% | 17% | 29% | 14% | 57% |
| Digestive | | | | | | | | | | | | |
| Nausea | 100% | | | 92% | 8% | | 75% | | | 100% | | |
| Taste alteration | 100% | | | 83% | | | 83% | | | 71% | | |
| Anorexia | 100% | | | 83% | | | 75% | | | 57% | 14% | |
| Vomiting | 100% | | | 42% | 33% | | 50% | 8% | | 86% | | |
| Constipation | 67% | | | 92% | | | 83% | | | 57% | | |
| Diarrhea | | 33% | | 67% | | | 42% | | | 57% | 14% | |
| Stomatitis | 33% | | | 25% | | | 25% | | | | 14% | |
| Heart burn | 33% | | | 33% | | | 25% | | | 14% | | |
| Dry mouth | | | | 25% | | | | | | 43% | | |
| Nervous system | | | | | | | | | | | | |
| Sensory neuropathy | 67% | | | 75% | | | 50% | | | 57% | | |
| Tinnitus | 67% | | | 67% | | | 67% | | | 86% | | |
| Headache | 33% | | | 33% | 8% | | 33% | | | 43% | | |
| Orthostasis/dizziness | 33% | | | 50% | | | 17% | | | 29% | | |
| Skin and appendages | | | | | | | | | | | | |
| Alopecia | 33% | | | 50% | | | 67% | | | 57% | | |
| Rash | 33% | | | 50% | | | 58% | | | 29% | | |
| Injection site reaction | | | | 58% | | | 25% | | | 29% | | |
| Dry skin | 33% | | | 25% | | | 8% | | | 14% | | |
| Respiratory system | | | | | | | | | | | | |
| Cough | 33% | | | 33% | | | 67% | | | 43% | | |
| Dyspnea | 33% | | | 17% | | | 33% | 25% | | | | |
| Infection | 67% | | | 33% | | | 25% | 8% | | 29% | | |
| Epistaxis | | | | 33% | | | 8% | | | 43% | | |
| Hiccoughs | | | | 8% | | | 25% | | | 14% | | |
| Ocular/visual | | | | | | | | | | | | |
| Conjunctivitis | 67% | | | 42% | | | 17% | | | 14% | | |
| Blurred vision | 33% | | | 42% | | | 8% | | | 14% | | |
| Fluid homeostasis | | | | | | | | | | | | |
| Edema (except facial) | 67% | | | 42% | | | 67% | | | 57% | | |
| Periorbital edema | 33% | | | 58% | | | 25% | | | 43% | 14% | |
| Pain | | | | | | | | | | | | |
| Pain abdomen | 33% | | | 33% | | | 33% | | | 29% | | |
| Myalgia | | | | 33% | | | 8% | | | 29% | | |
| Skeletal pain | | | | | | | 25% | | | 29% | | |
| Nutritional/metabolic | | | | | | | | | | | | |
| Weight loss | 67% | | | 58% | | | 67% | | | 57% | | |
| Elevated ALT/AST | 67% | | | 58% | | | 42% | | | 29% | 14% | |

NOTE: Events reported by 15% or more of patients, considered possibly, probably, or certainly related to cisplatin, gemcitabine, and/or bortezomib by the treating physician and the investigator are presented. Several episodes in the same patient are counted as one adverse event and only the worst grade is mentioned.
Abbreviations: ALT, alaninine aminotransferase; AST, aspartate aminotransferase.

Results of additional assesments of neuro-, oto-, nephro-, and cardiotoxicity are listed in Table 4. Of 91% of patients, baseline and end-of-treatment neurotoxicity questionnaires were available for assessment. Surprisingly, we did not observe a significant difference of baseline vs. end of treatment scores combining bortezomib and cisplatin-based chemotherapy. Nevertheless, mild, low-grade neuropathy, typically characterized by paresthesias in fingers and toes, was present in 62% of patients. Sensory neuropathy, as well as orthostasis/ dizziness, which might represent autonomic dysfunction, was

slightly more frequent in patients treated with twice-weekly bortezomib 1.0 mg/m$^2$ than in other treatment cohorts. No neuropathic pain was reported.

Six (18%) patients experienced subjective hearing loss, 24 (71%) experienced tinnitus during treatment and in 2 patients (6%) cisplatin was discontinued due to progressive ototoxicity. Renal function, as calculated by the Cockcroft-Gault formula, was significantly reduced at end of treatment in all cohorts. In one patient, the cisplatin dose was reduced due to nephrotoxicity. One patient developed severe left ventricular dysfunction, which restored gradually after discontinuation of treatment. This deterioration of LVEF upon treatment was documented by NT-proBNP measurements in archived serum samples as well as by LVEF assessment by echocardiography and MUGA-scan [Voortman and Giaccone, 2006]. Because bortezomib might have been the cause for the observed cardiac effect in this patient, subsequently enrolled patients underwent LVEF measurement by MUGA-scan at baseline and end of treatment. In total 18 patients were evaluated at screening. Three patients, without signs of cardiac failure, were not evaluated after completing treatment due to rapid disease progression (n=2) and death (n=1). No significant decline in LVEF in patients receiving weekly or twice-weekly bortezomib 1.0 mg/m$^2$ was observed.

**Table 4.** Baseline and end of treatment assessment of neurotoxicity, ototoxicity, nephrotoxicity, and cardiotoxicity

| Assessment | All cohorts | Weekly bortezomib 1.0 mg/m$^2$ (MTD) | Twice-weekly bortezomib 1.0 mg/m$^2$ (MTD) |
|---|---|---|---|
| Neurotoxicity questionnaire (score) | | | |
| Baseline | 3.0 ($n$ = 31) | 2.2 ($n$ = 11) | 3.1 ($n$ = 10) |
| EOT | 3.5 ($n$ = 31) | 2.6 ($n$ = 11) | 3.9 ($n$ = 10) |
| ΔEOT | +0.5 ($P$ = 0.62) | +0.4 ($P$ = 0.73) | +0.8 ($P$ = 0.70) |
| Hearing loss (dB) | | | |
| Baseline | 38.6 ($n$ = 31) | 47.1 ($n$ = 10) | 31.3 ($n$ = 11) |
| EOT | 46.0 ($n$ = 31) | 58.3 ($n$ = 10) | 35.7 ($n$ = 11) |
| ΔEOT | +7.4 ($P$ = 0.24) | +10.8 ($P$ = 0.34) | +4.4 ($P$ = 0.66) |
| Renal function (GFR, mL/min) | | | |
| Baseline | 93.9 ($n$ = 34) | 91.9 ($n$ = 12) | 91.1 ($n$ = 12) |
| EOT | 83.9 ($n$ = 34) | 85.0 ($n$ = 12) | 80.8 ($n$ = 12) |
| ΔEOT | -10.0 ($P$ < 0.05) | -7.0 ($P$ < 0.05) | -10.3 ($P$ < 0.05) |
| Left ventricular function (%) | | | |
| Baseline | 61.2 ($n$ = 15) | 60.9 ($n$ = 7) | 61.6 ($n$ = 8) |
| EOT | 58.7 ($n$ = 15) | 60.0 ($n$ = 7) | 57.6 ($n$ = 8) |
| ΔEOT | -2.5 ($P$ = 0.20) | -0.9 ($P$ = 0.80) | -4.0 ($P$ = 0.096) |

NOTE: Data are Functional Assessment of Cancer Therapy/Gynecologic Oncology Group neurotoxicity questionnaire, version 4.0, scores; average hearing loss at 4 and 8 kHz by audiometric measurement; glomerular filtration rate as calculated by the Cockcroft-Gault formula; and LVEF by multiple-gated acquisition scan. Only patients from whom baseline and end of treatment assessments were available were included. Reported values are averages. Three patients had no follow-up audiometric measurements due to disease progression. LVEF was routinely measured in all patients after incidence of a possible treatment-related grade 4 left ventricular dysfunction in a patient. $P$ value expresses significance.

Abbreviations: EOT, end of treatment; ΔEOT, difference between baseline value and end of treatment value; GFR, glomerular filtration rate.

Two patients experienced grade 4 non-hematologic toxicity. One NSCLC patient, who was being treated with corticosteroids because of CNS metastases, had a gastric perforation from which she recovered without surgical intervention. Another patient, who had a partial response, presented

with acute abdominal pain. Explorative laparotomy showed a large intra-abdominal collection of pus without signs of gastro-intestinal perforation at that time.

One NSCLC patient died during treatment. A bronchial stent placed before start of chemotherapy had migrated, most probably due to tumor shrinkage. During an endoscopic replacement procedure an acute fatal pulmonary hemorrhage occurred.

Tumor response. All enrolled patients were evaluable for response. As shown in Table 5, overall response rate was 38% (95% CI, 21-55%). Response rate in NSCLC patients combining all dose groups and both treatment schedules was 33% (95% CI, 15-51%). In NSCLC patients, twice-weekly administration of bortezomib resulted in a response rate of 25% (95% CI, 0-50 %) compared to 40% (95% CI, 15-65 %) in patients who received weekly administration of bortezomib. Disease control rate (responses plus stable disease) in NSCLC patients was 89% with a median stable disease duration of 3.0 months (95% CI, 2.0-4.0 months) and median response duration of 5.6 months (95% CI, 4.1-7.1 months). Of 4 patients with urothelial cell cancer, three had a partial response. Only one patient, with pancreatic cancer, who experienced stable disease after 6 cycles of therapy, opted to continue on monotherapy bortezomib, which was discontinued after two cycles due to progressive disease. No other responding patients or patients with stable disease opted to continue with bortezomib monotherapy. In patients with progressive disease we observed an average weight loss of 6% at end of treatment compared to 2% in patients with stable disease and a weight gain of 2% in patients responding to treatment.

**Table 5.** Treatment efficacy in all patients and in NSCLC patients

| Investigator-assessed response | All patients (*N* = 34) | NSCLC all (*N* = 27) | NSCLC weekly bortezomib (*N* = 15)* | NSCLC twice-weekly bortezomib (*N* = 12)† |
|---|---|---|---|---|
| PR | NSCLC (9)<br>Urothelial cell cancer (3)<br>ACUP‡ (1) | 9 (33%) | 6 (40%) | 3 (25%) |
| SD§ | NSCLC (15)<br>Urothelial cell cancer (1)<br>Pancreatic cancer (1) | 15 (56%) | 8* (53%) | 7 (58%) |
| PD | NSCLC (3)<br>Hepatocellular cancer (1) | 3 (11%) | 1 (7%) | 2 (17%) |

Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease.
* Ten patients treated at 1.0 mg/m$^2$; five patients treated at 1.3 mg/m$^2$ bortezomib.
† One patient treated at 0.7 mg/m$^2$; 11 patients treated at 1.0 mg/m$^2$ bortezomib.
‡ Identified as breast carcinoma at a later stage.
§ Including a NSCLC with a partial response that could not be confirmed because of death due to fatal bleeding during broncoscopic procedure.

As the study population constituted a heterogenous group of patients with various solid tumor types, we performed a subgroup analysis for time to progression and survival in NSCLC patients (n =27) only. In an intention to treat analysis, median follow up was 19 months. One NSCLC patient was lost to follow-up at 7 months due to emigration. As shown in Figure 2, median overall survival (OS) and time to progression (TTP) were 315 days (95% CI 206-424 days; 6 patients censored) (10.4 months) and 152 days (95% CI 101-203 days; 1 patient censored) (5.0 months), respectively. The 1-year survival probability for NSCLC patients was 41%. In patients treated with bortezomib twice-weekly (n = 12) median survival and TTP were 299 days (95% CI 90-508 days; 4 patients censored) (9.8 months) and 120 days (95% CI 110-130 days; 1 patient censored) (3.9 months), respectively. In patients treated weekly with bortezomib (n = 15) median survival and TTP were 315 days (95% CI 168-462 days; 2 patients censored) (10.4 months) and 162 days (95% CI 129-194 days) (5.4 months), respectively.

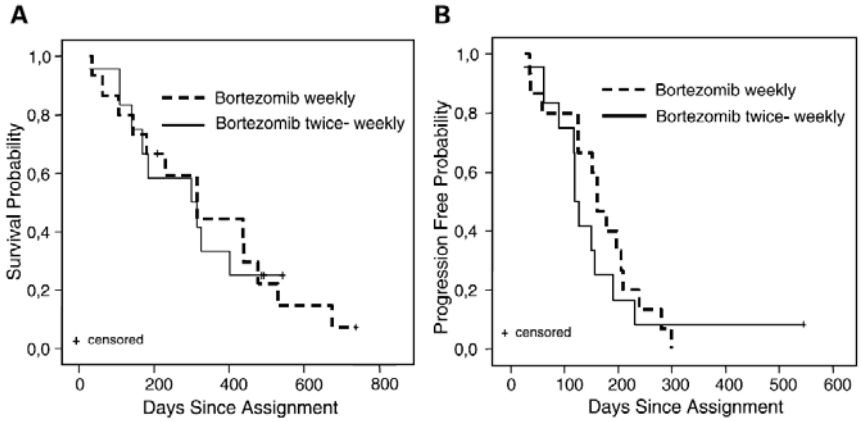


**Figure 2.** Kaplan-Meier (A) survival and (B) time to progression curves in NSCLC patients, by treatment schedule (intent-to-treat population, n = 27).

Pharmacokinetic analysis. Table 6 and Figure 3 show the plasma pharmacokinetic parameters for cisplatin (platinum) and gemcitabine (dFdC and gemcitabine's inactive metabolite dFdU). Addition of bortezomib did not seem to alter these parameters compared to historical controls generated in our laboratory (van Moorsel et al, 1999; Kroep et al, 2006].

**Table 6.** Pharmacokinetic variables for cisplatin (platinum) and gemcitabine (dFdC and dFdU)

| Compound | All patients mean ± SD ($n$ = 12) | Twice-weekly bortezomib 1.0 mg/m², mean ± SD ($n$ = 6) | Weekly bortezomib 1.0 mg/m², mean ± SD ($n$ = 6) | $P$* |
|---|---|---|---|---|
| Platinum (plasma) | | | | |
| $C_{max}$, μmol/L | 11.0 ± 2.2 | 10.7 ± 1.4 | 11.3 ± 3.0 | 0.63 |
| AUC, min mmol/L | 50.0 ± 12.8 | 47.0 ± 8.8 | 52.9 ± 16.3 | 0.45 |
| dFdC (plasma) | | | | |
| $C_{max}$ day 1, μmol/L | 48.7 ± 14.9 † | 47.6 ± 15.1 | 50.0 ± 16.4 ‡ | 0.81 |
| $C_{max}$ day 8, μmol/L | 50.5 ± 24.7 | 44.6 ± 17.9 | 56.3 ± 30.6 | 0.44 |
| AUC day 1 (0-60 min) min•μmol/L§ | 1,441 ± 376 † | 1,398 ± 357 | 1,491 ± 434 ‡ | 0.71 |
| AUC day 8 (0-60 min) min•μmol/L§ | 1,516 ± 666 † | 1,369 ± 515 | 1,662 ± 812‖ | 0.45 |
| dFdU (plasma) | | | | |
| $C_{max}$ day 1, μmol/L | 98.6 ± 13.8 | 100.1 ± 11.1 | 97.2 ± 17.1 | 0.74 |
| $C_{max}$ day 8, μmol/L | 102.1 ± 23.2 | 106.6 ± 23.2 | 97.7 ± 24.5 | 0.53 |
| AUC day 1 (0-60 min) min•μmol/L¶ | 3,850 ± 641 | 3,938 ± 564 | 3,763 ± 754 | 0.66 |
| AUC day 8 (0-60 min) min•μmol/L¶ | 4,163 ± 1,109 | 4,189 ± 1,205 | 4,137 ± 1,120 | 0.94 |

Abbreviation: AUC, area under the concentration curve.
*Twice-weekly compared with weekly bortezomib.
† $n$ = 11.
‡ $n$ = 5.
§Partial area under the concentration curve (0-60 min) was used to compare the pharmacokinetic profiles of dFdC and dFdU on days 1 and 8.
‖ $n$ = 6.
¶Partial area under the concentration curve (0-60 min) was used to compare the pharmacokinetic profiles of dFdC and dFdU on days 1 and 8.

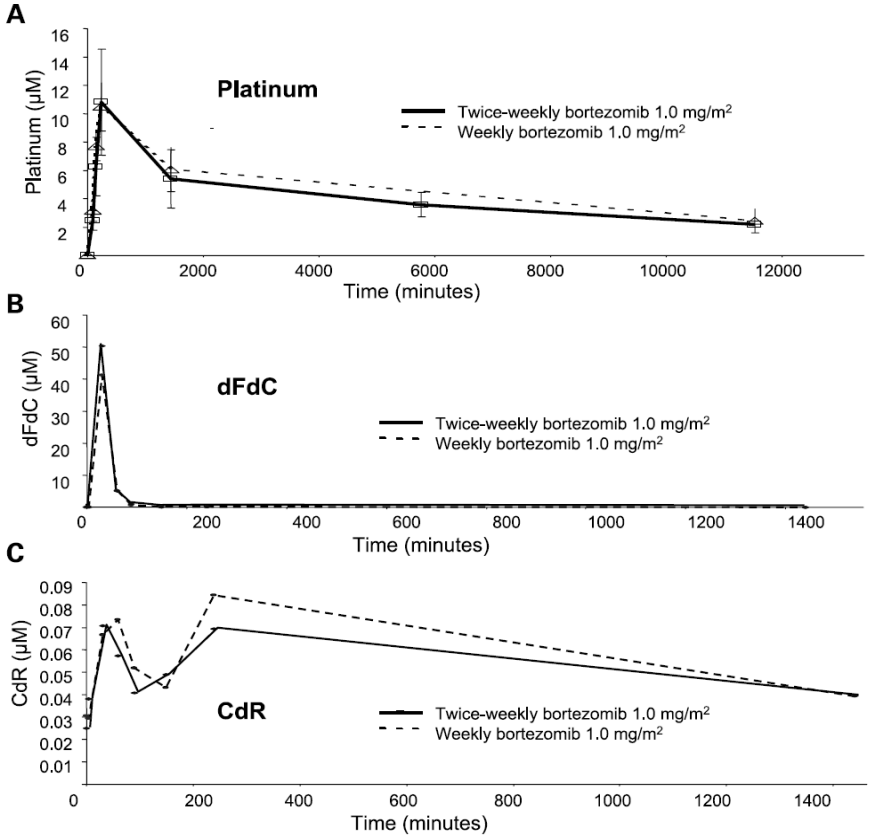


**Figure 3.** Mean plasma concentration time curves for (**A**) cisplatin (platinum), (**B**) gemcitabine (dFdC) and (**C**) endogenous deoxycytidine (CdR), by treatment schedule

No significant difference was found in parameters comparing patients treated with weekly or twice-weekly bortezomib.

In addition to measurements of the deoxycytidine-analog gemcitabine, the plasma level of endogenous deoxycytidine (CdR) was also determined. We observed an unexpected, transient drop in the level of plasma CdR in the current combination of bortezomib, gemcitabine and cisplatin.

## Discussion

Bortezomib is a novel and promising anti-neoplastic agent, presently approved for the treatment of second-line multiple myeloma and mantle-cell lymphoma patients. Many studies are being conducted in solid tumor patients and as a single agent bortezomib has shown modest activity in NSCLC patients [Fanucchi et al., 2006]. As inhibition of proteasome activity might sensitize for chemotherapy-induced cytoxicity, combinations of bortezomib and chemotherapy are being investigated [Scagliotti et al., 2006).

In this study we combined bortezomib and cisplatin-gemcitabine chemotherapy as a first-line treatment for patients with advanced solid tumors, preferentially including NSCLC patients. We evaluated the tolerability of two schedules of bortezomib, a standard twice-weekly schedule and an alternative weekly schedule. Overall, treatment was well tolerated in both schedules with an equal MTD of bortezomib of 1.0 mg/m$^2$.

The toxicity profile of gemcitabine and cisplatin combined with bortezomib appears comparable to gemcitabine and cisplatin chemotherapy without bortezomib. Hematologic toxicity was prominent and non-hematologic toxicity relatively mild. However, increased dose-intensity of bortezomib led to higher grade hematologic as well as non-hematologic toxicity, notably gastro-intestinal toxicity and asthenia. In general, incidences of neutropenia and especially thrombocytopenia, in patients treated with cisplatin, gemcitabine and bortezomib appear to be higher compared to reported incidences in larger groups of patients treated with a three-week regimen of gemcitabine (1200-1250 mg/m$^2$) and cisplatin (75-80 mg/m$^2$) alone [Scagliotti et al., 2002; Zatloukal et al., 2003]. However, the cause and kinetics of bortezomib-induced thrombocytopenia differ from conventional chemotherapy-induced thrombocytopenia. Bortezomib-induced thrombocytopenia is due to a reversible effect on megakaryocytic function rather than a direct cytotoxic effect on megakaryocytes. Consequently, bortezomib-induced

thrombocytopenia is characterized by rapid recovery during the wash-out period and is associated with a low incidence of bleeding, which was also our experience in the combination with cisplatin and gemcitabine [Lonial et al., 2005].

Surprisingly, combining cisplatin and bortezomib, we did not observe treatment-emergent neuropathy, though the majority of patients experienced low-grade neuropathy. Refractory multiple myeloma patients treated with twice-weekly bortezomib 1.0 mg/m$^2$ were reported to have a 21% incidence of treatment-emergent neuropathy [Richardson et al., 2006]. This might be due to the fact that patients in our study were not pretreated with neurotoxic drugs, patients with ≥ grade 2 neuropathy were excluded and, in multiple myeloma patients, paraproteinemic associated neuropathy might contribute to the relatively high incidence of observed neuropathy [Ropper et al., 1998].

Neutropenia was the primary cause of treatment delay. Three patients (9%) experienced repeatedly prolonged neutropenia, causing unacceptable treatment delay. Growth factor support with G-CSF injections was effective in preventing persisting neutropenia in these patients and could therefore be considered in patients experiencing prolonged neutropenia following the first treatment cycle. We do not recommend standard use of G-CSF injections with this combination treatment as these patients formed a small subgroup of the total study population.

Plasma pharmacokinetic parameters of cisplatin and gemcitabine were not affected by the addition of bortezomib. As the effectiveness of deoxycytidine (CdR) analogs, such as gemcitabine, can be linked to the direct competition with active forms of endogenous CdR, we also determined the plasma level of endogenous deoxycytidine in patient samples [Honeywell et al., 2006]. Endogenous deoxycytidine plasma levels showed an unexpected, transient drop. This was not observed in other patients treated with cisplatin-gemcitabine in our hospital (data not shown). The significance of this finding, notably if there might be an effect of bortezomib co-administration on intracellular gemcitabine metabolism, is unclear and is currently being investigated.

A recently published phase 1 study reported that the inhibition of 20S proteasome activity in PBMCs by bortezomib was unaffected by gemcitabine coadministration [Ryan et al., 2006]. As for pharmacodynamic activity of bortezomib in combination with gemcitabine and cisplatin, a pilot experiment,

measuring proteasome activity in a few remaining PBMC samples, showed a decrease in proteasome activity upon treatment [Lightcap et al., 2000]. Furthermore we observed throughout the study population a typical bortezomib-associated cyclical thrombocytopenia pattern whilst on treatment.

The achieved overall response rate in NSCLC patients in our study is 33%. Notably, as much as 10 (37%) of our 27 NSCLC patients presented with brain metastases, 4 patients (15%) using corticosteroids at study entry and 2 additional patients (7%), early progressive on treatment, started shortly after study entry with corticosteroids. The response rate appears to be similar to those observed in advanced NSCLC patients treated with only cisplatin and gemcitabine, generally at higher doses of up to 80 mg/m$^2$ and up to 1,250 mg/m$^2$, respectively [Cardenal et al., 1999; Sandler et al., 2000; Scagliotti et al., 2002; Zatloukal et al., 2003; Mazzanti et al., 2003; Gebbia et al., 2003]. Although this study was not powered to show a difference between the two schedules, response rates were similar in the weekly regimen compared to the twice-weekly regimen and overall survival as well as progression free survival curves seemingly superimposable.

Recently, final results were presented from a phase 2 study in which efficacy of bortezomib twice-weekly 1.0 mg/m$^2$, carboplatin AUC 5 and gemcitabine 1,000 mg/m$^2$, was assessed in 114 chemonaive stage advanced NSCLC patients [Davies et al., 2006]. At a median follow-up of 13 months PFS and OS were 5 and 11 months, respectively. The 11-month median survival achieved was regarded as unprecedented by the authors. In that trial chemotherapy was administered prior to bortezomib, based on preclinical results indicating this sequence might favor efficacy [Mack et al., 2003; Mortenson et al., 2004]. In our trial we administered chemotherapy after bortezomib, achieving comparable PFS and median OS duration in advanced NSCLC patients. Furthermore, a weekly schedule of bortezomib in combination with carboplatin and gemcitabine was not investigated by Davies et al. [Davies et al, 2006], nor were pharmacokinetic variables for gemcitabine or carboplatin determined. Interestingly, a weekly administration of bortezomib is currently being studied as a more convenient alternative in multiple myeloma and non-Hodgkin lymphoma as well [Greco et al., 2006; Suvannasankha et al., 2006].

In conclusion, bortezomib can be safely combined with cisplatin-gemcitabine chemotherapy and constitutes an active regimen in advanced stage NSCLC

patients. Though this is a non-randomized, phase 1 study does not allow comparison between the two schedules, the weekly schedule of bortezomib appears to be favorable over a twice-weekly schedule, based on lower toxicity and no indication of inferior activity compared to the twice-weekly schedule.

## Acknowledgments

We thank Adrie C. Laan for his valuable contribution to the pharmacokinetic studies, Chantal Dinkgreve for assisting in data management, and Atie van Wijk for her help at the Department of Pulmonology, all at VU University Medical Center, Amsterdam, the Netherlands; and Deborah Brouwer at Janssen-Cilag,Tilburg, the Netherlands, for her contribution to this study.

## References

Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD, Maas J, Pien CS, Prakash S, Elliott PJ (1999) Proteasome inhibitors: a novel class of potent and effective antitumor agents. *Cancer Res* 59: 2615-22

Adams J. The proteasome: a suitable antineoplastic target (2004) *Nat Rev Cancer* 4: 349-60

Aghajanian C, Dizon DS, Sabbatini P, Raizer JJ, Dupont J, Spriggs DR (2005) Phase I trial of bortezomib and carboplatin in recurrent ovarian or primary peritoneal cancer. *J Clin Oncol* 23: 5943-9

Beg AA, Baltimore D (1996) An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* 274: 782-4

Cardenal F, Lopez-Cabrerizo MP, Anton A, Alberola V, Massuti B, Carrato A, Barneto I, Lomas M, García M, Lianes P, Montalar J, Vadell C, González-Larriba JL, Nguyen B, Artal A, Rosell R (1999) Randomized phase III study of gemcitabine-cisplatin versus etoposide-cisplatin in the treatment of locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 17: 12-8

Davies AM, McCoy J, Lara PN, Gumerlock PH, Crowley J, Grandara GR (2006) Bortezomib + gemcitabine (Gem)/carboplatin (Carbo) results in encouraging survival in advanced non-small cell lung cancer (NSCLC): Results of a phase II Southwest Oncology Group (SWOG) trial. *Int J Clin Oncol* 24: 18S, (suppl;abstr 7017)

Fahy BN, Schlieman MG, Mortenson MM, Virudachalam S, Bold RJ (2005) Targeting BCL-2 overexpression in various human malignancies through NF-kappaB inhibition by the proteasome inhibitor bortezomib. *Cancer Chemother Pharmacol* 56: 46-54

Fanucchi MP, Fossella FV, Belt R, Natale R, Fidias P, Carbone DP, Govindan R, Raez LE, Robert F, Ribeiro M, Akerley W, Kelly K, Limentani SA, Crawford J, Reimers HJ, Axelrod R, Kashala O, Sheng S, Schiller JH (2006) Randomized phase II study of bortezomib alone and bortezomib in combination with docetaxel in previously treated advanced non-small-cell lung cancer. *J Clin Oncol* 24: 5025-33

Fernandez Y, Verhaegen M, Miller TP, Rush JL, Steiner P, Opipari AW, Lowe SW, Soengas MS (2005) Differential regulation of noxa in normal melanocytes and melanoma cells by proteasome inhibition: therapeutic implications. *Cancer Res* 65: 6294-304

Gebbia V, Galetta D, Caruso M, Verderame F, Pezzella G, Valdesi M, Borsellino N, Pandolfo G, Durini E, Rinaldi M, Loizzi M, Gebbia N, Valenza R, Tirrito ML, Varvara F, Colucci G; Gruppo Ocologico Italia Meridionale (2003) Gemcitabine and cisplatin versus vinorelbine and cisplatin versus ifosfamide+gemcitabine followed by vinorelbine and cisplatin versus vinorelbine and cisplatin followed by ifosfamide and gemcitabine in stage IIIB-IV non small cell lung carcinoma: a prospective randomized phase III trial of the Gruppo Oncologico Italia Meridionale. *Lung Cancer* 39:179-89

Greco FA, Spigel DR, Barton JH, Farley C, Schreeder MT, Hermann RC, Hainsworth JD (2006) Weekly bortezomib in the treatment of patients (pts) with previously treated multiple myeloma: a phase II trial of the Minnie Pearl Cancer Research Network. *J Clin Oncol* 24: 18S (suppl;abstr 7547)

Honeywell R, van Groeningen CJ, Laan AC, Strocchi E, Ruiter R, Giaccone G, Peters GJ (2006) Analysis of deoxycytidine accumulation in gemcitabine treated patients. *Nucleosides Nucleotides Nucleic Acids* 25:1225-32

Honeywell R, Laan AC, van Groeningen CJ, Strocchi E, Ruiter R, Giaccone G, Peters GJ (2007) The determination of gemcitabine and 2'-deoxycytidine in human plasma and tissue by APCI tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 847:142-52

Imai J, Yashiroda H, Maruya M, Yahara I, Tanaka K (2003) Proteasomes and molecular chaperones: cellular machinery responsible for folding and destruction of unfolded proteins. *Cell Cycle* 2: 585-90

Kamat AM, Karashima T, Davis DW, Lashinger L, Bar-Eli M, Millikan R, Shen Y, Dinney CP, McConkey DJ (2004) The proteasome inhibitor bortezomib synergizes with gemcitabine to block the growth of human 253JB-V bladder tumors in vivo. *Mol Cancer Ther* 3:279-90

King RW, Deshaies RJ, Peters JM, Kirschner MW (1996) How proteolysis drives the cell cycle. *Science* 274:1652-9

Kroep JR, Smit EF, Giaccone G Van der Born K, Beijnen JH, Van Groeningen CJ, Van der Vijgh WJ, Postmus PE, Pinedo HM, Peters GJ (2006) Pharmacology of the paclitaxel-cisplatin, gemcitabine-cisplatin, and paclitaxel-gemcitabine combinations in patients with advanced non-small cell lung cancer *Cancer Chemother Pharmacol* 58:509-16

Lehmann J, Retz M, Steiner G, Albers P, Jaeger E, Knuth A, Lippert C, Koser M, Stockamp K, Otto C, Melchior H, Fassmann C, Potratz C, Loch T, Derigs HG, Becker T, Kälble T, Piechota HJ, Hertle L, Weinknecht S, Weissbach L, Al-Mwalad M, Hamza A, Henss H, Brkovic D, Pomer S, Roloff J, Walz P, Muschter R, Tunn U, Winter E, Bub P, Kaldenbach U, Roth S, Brauers A, Jakse G, Richter AE, Wirth M, Hartlapp J, Van Ahlen H, Stöckle M. (2003) [Gemcitabine/cisplatin vs. MVAC. 5 year survival outcome of the phase III study of chemotherapy of advanced urothelial carcinoma in Germany]. *Urologe* A 42:1074-86

Lightcap ES, McCormack TA, Pien CS, Chau V, Adams J, Elliott PJ (2000) Proteasome Inhibition Measurements: Clinical Application. *Clin Chem* 46: 673-83

Ling YH, Liebes L, Ng B, Buckley M, Elliott PJ, Adams J, Jiang JD, Muggia FM, Perez-Soler R (2002) PS-341, a Novel Proteasome Inhibitor, Induces Bcl-2 Phosphorylation and Cleavage in Association with $G_2$-M Phase Arrest and Apoptosis. *Mol Cancer Ther* 1: 841-9

Lonial S, Waller EK, Richardson PG, Jagannath S, Orlowski RZ, Giver CR, Jaye DL, Francis D, Giusti S, Torre C, Barlogie B, Berenson JR, Singhal S, Schenkein DP, Esseltine DL, Anderson J, Xiao H, Heffner LT, Anderson KC; SUMMIT/CREST Investigators (2005) Risk factors and kinetics of thrombocytopenia associated with bortezomib for relapsed, refractory multiple myeloma. *Blood* 106: 3777-84

Ludwig H, Khayat D, Giaccone G, Facon T (2005) Proteasome inhibition and its clinical prospects in the treatment of hematologic and solid malignancies. *Cancer* 104: 1794-807

Mack PC, Davies AM, Lara PN, Gumerlock PH, Gandara DR (2003) Integration of the proteasome inhibitor PS-341 (Velcade) into the therapeutic approach to lung cancer. *Lung Cancer* 41 Suppl 1:S89-96

Mazzanti P, Massacesi C, Rocchi MB, Mattioli R, Lippe P, Trivisonne R, Buzzi F, De Signoribus G, Tuveri G, Rossi G, Di Lullo L, Sturba F, Morale D, Catanzani S, Pilone A, Bonsignori M, Battelli T (2003) Randomized, multicenter, phase II study of gemcitabine plus cisplatin versus gemcitabine plus carboplatin in patients with advanced non-small cell lung cancer. *Lung Cancer* 41:81-9

Mortenson MM, Schlieman MG, Virudachalam S, Bold RJ (2004) Effects of the proteasome inhibitor bortezomib alone and in combination with chemotherapy in the A549 non-small-cell lung cancer cell line. *Cancer Chemotherapy and Pharmacology* 54:343-53

Palombella VJ, Rando OJ, Goldberg AL, Maniatis T (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 78:773-85

Perez-Galan P, Roue G, Villamor N, Montserrat E, Campo E, Colomer D (2006) The proteasome inhibitor bortezomib induces apoptosis in mantle-cell lymphoma through generation of ROS and Noxa activation independent of p53 status. *Blood* 107:257-64

Read MA, Neish AS, Luscinskas FW, Palombella VJ, Maniatis T, Collins T (1995) The proteasome pathway is required for cytokine-induced endothelial-leukocyte adhesion molecule expression. *Immunity* 2:493-506
Richardson PG, Briemberg H, Jagannath S, Wen PY, Barlogie B, Berenson J, Singhal S, Siegel DS, Irwin D, Schuster M, Srkalovic G, Alexanian R, Rajkumar SV, Limentani S, Alsina M, Orlowski RZ, Najarian K, Esseltine D, Anderson KC, Amato AA (2006) Frequency, characteristics, and reversibility of peripheral neuropathy during treatment of advanced multiple myeloma with bortezomib. *J Clin Oncol* 24:3113-20

Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, Harousseau JL, Ben-Yehuda D, Lonial S, Goldschmidt H, Reece D, San-Miguel JF, Bladé J, Boccadoro M, Cavenagh J, Dalton WS, Boral AL, Esseltine DL, Porter JB, Schenkein D, Anderson KC; Assessment of Proteasome Inhibition for Extending Remissions (APEX) Investigators (2005) Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med* 352:2487-98

Ropper AH, Gorson KC(1998) Neuropathies associated with paraproteinemia. *N Engl J Med* 338:1601-7

Ryan DP, Appleman LJ, Lynch T, Supko JG, Fidias P, Clark JW, Fishman M, Zhu AX, Enzinger PC, Kashala O, Cusack J Jr, Eder JP (2006) Phase I clinical trial of bortezomib in combination with gemcitabine in patients with advanced solid tumors. *Cancer* 107:2482-9

Sandler AB, Nemunaitis J, Denham C, von Pawel J, Cormier Y, Gatzemeier U, Mattson K, Manegold C, Palmer MC, Gregor A, Nguyen B, Niyikiza C, Einhorn LH (2000) Phase III trial of gemcitabine plus cisplatin versus

cisplatin alone in patients with locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 18:122-30

Scagliotti G (2006) Proteasome inhibitors in lung cancer. *Crit Rev Oncol Hematol* 58:177-89

Scagliotti GV, De Marinis F, Rinaldi M, Crinò L, Gridelli C, Ricci S, Matano E, Boni C, Marangolo M, Failla G, Altavilla G, Adamo V, Ceribelli A, Clerici M, Di Costanzo F, Frontini L, Tonato M; Italian Lung Cancer Project (2002) Phase III randomized trial comparing three platinum-based doublets in advanced non-small-cell lung cancer. *J Clin Oncol* 20: 4285-91

Suvannasankha A, Smith GG, Juliar BE, Abonour R (2006) Weekly bortezomib/methylprednisolone is effective and well tolerated in relapsed multiple myeloma. *Clin Lymphoma Myeloma* 7:131-4

Teicher BA, Ara G, Herbst R, Palombella VJ, Adams J (1999) The Proteasome Inhibitor PS-341 in Cancer Therapy. *Clin Cancer Res* 5:2638-45

Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New Guidelines to Evaluate the Response to Treatment in Solid Tumors. J *Natl Cancer Inst* 92:205-16

Twombly R (2003) First proteasome inhibitor approved for multiple myeloma. *J Natl Cancer Inst* 95:845

van Moorsel CJ, Kroep JR, Pinedo HM, Veerman G, Voorn DA, Postmus PE, Vermorken JB, van Groeningen CJ, van der Vijgh WJ, Peters GJ (1999) Pharmacokinetic schedule finding study of the combination of gemcitabine and cisplatin in patients with solid tumors. *Ann Oncol* 10:441-8

Voortman J, Giaccone G (2006) Severe reversible cardiac failure after bortezomib treatment combined with chemotherapy in a non-small cell lung cancer patient: a case report. *BMC Cancer* 6:129

Zatloukal P, Petruzelka L, Zemanová M, Kolek V, Skricková J, Pesek M, Fojtů H, Grygárková I, Sixtová D, Roubec J, Horenková E, Havel L, Průsa P, Nováková L, Skácel T, Kůta M (2003) Gemcitabine plus cisplatin vs. gemcitabine plus carboplatin in stage IIIb and IV non-small cell lung cancer: a phase III randomized trial. Lung Cancer 41:321-31