Phase I Trial of Fixed-Dose Rate Gemcitabine in Combination with Bortezomib in Advanced Solid Tumors*

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Abstract. *Background: Bortezomib demonstrates synergism with gemcitabine via a fixed-dose rate (FDR). The aim of this phase I trial in solid tumors was to establish the maximum tolerated dose (MTD) and safety data for this combination. Patients and Methods: Twenty-nine patients with a median age of 63 (range 36-84) years and median Karnofsky Performance Status of 90 (range 60-100) were enrolled and treated with bortezomib (1.0 or 1.3 mg/m²) on days 1, 4, 8 and 11 and FDR gemcitabine (750, 1,000, or 1,250 mg/m²) on days 1 and 8 of each 21-day cycle. Response was evaluated every two cycles. Results: Dose-limiting toxicities were grade 4 thrombocytopenia and neutropenia and grade 3 liver function test abnormalities. The MTD was bortezomib 1 mg/m2 and FDR gemcitabine 1,250 mg/m². The median number of cycles delivered was 3 (range 1-28). There was one partial response and six cases of stable disease. The median duration of response was 8.5 (range 3-20) months. Conclusion: FDR gemcitabine and bortezomib combination can be delivered effectively with acceptable toxicity.*

Bortezomib (Velcade®; PS-341, LDP 341, MLN341) is a small molecule proteasome inhibitor approved by the United States Food and Drug Administration for the treatment of refractory multiple myeloma (1). By inhibiting a single molecular target, the proteasome, bortezomib affects multiple signaling pathways that involve several distinct mechanisms, *i.e.* inhibition of cell growth and survival, induction of apoptosis genes, and suppression of cellular adhesion, migration and angiogenesis.

Gemcitabine (Gemzar; 2',2'-difluorodeoxycytidine, dFdC) is a nucleoside analog that has therapeutic activity against lung, breast, pancreatic, bladder, ovarian and hematological malignancies. Gemcitabine diphosphate blocks DNA synthesis by inhibiting ribonucleotide reductase, the enzyme responsible for production of deoxynucleotides required for DNA replication and repair. This inhibition results in lower levels of deoxycytidine triphosphate (dCTP) and high levels of gemcitabine triphosphate (dFdCTP) accumulation. Gemcitabine triphosphate is then incorporated into newly synthesized DNA, thereby causing masked-chain termination that interferes with excision repair of the gemcitabine monophosphate from the DNA. Gemcitabine must be metabolized to the active triphosphate to cause cell death by impairing DNA synthesis (2-4). It requires intracellular uptake and phosphorylation by deoxycytidine kinase, which acts as a rate-limiting step. Increasing triphosphate improves antitumor activity because more tumor cells are recruited into S-phase during this prolonged half-life.

In the clinic, the routine standard administration of gemcitabine is *via* a short 30-min infusion not fixed rate that allows plasma levels peak within 1 hour and subsequently decline. In contrast, administration of gemcitabine at a fixed dose rate (FDR) of 10 mg/m²/min achieves steady-state plasma levels of 15 μM-20 μM, giving maximal rates of dFdCTP accumulation (5). This was further confirmed in a phase II trial comparing activity of gemcitabine given as a 30-min bolus *versus* FDR of 10 mg/m²/min that resulted in a 1.4-fold increase in the maximal intracellular dFdCTP concentration for the latter method (6, 7). The maximum tolerated dose (MTD) for single-agent FDR gemcitabine was established in 27 solid tumors at 1,500 mg/m² with myelosuppression as a dose-limiting toxicity (DLT) (8). The clinical benefit of FDR over bolus gemcitabine was seen in a

phase II trial in metastatic pancreatic carcinoma (92 patients) which resulted in a better median survival (7.2 months *versus* 4.9 months), and 1-year (28.8% *versus* 9%) and 2-year survival(18.3% *versus* 2.2%). The MTD for combination of bortezomib and bolus gemcitabine was established at 1 mg/m^2 for bortezomib and 1,000 mg/m^2 for gemcitabine on days 1 and 8 of every 21 days (9-11). Based on our preliminary laboratory data, we hypothesized that the addition of bortezomib would potentiate the cytotoxic affect of the FDR gemcitabine. Consequently, our phase I trial was designed to determine the safety, toxicity, and MTD of FDR gemcitabine at 10 $mg/m^2/min$ with bortezomib given 4 hours after gemcitabine on days 1 and 8 of a 21-day cycle.

Materials and Methods

Preliminary in vitro study.

Cell lines and culture conditions. Human breast cancer cell lines (ZR-75 and MCF-7 from American Type Culture Collection, Rockville, MD, USA) were maintained in culture (RPMI-1640 or DMEM high glucose medium supplemented with 10% heat-inactivated fetal calf serum [FCS], respectively) as adherent cells in a humidified atmosphere at 37°C and 5% CO_2.

Growth inhibitory effect of single and combination therapy with bortezomib and gemcitabine. ZR-75 and MCF-7 cells (~5,000 cells/well) were plated in a 96-well plate in their respective culture media. After 24 hours, increasing concentrations of bortezomib (0.01-1.0 µM) and/or gemcitabine (0.1-1.0 µM) were added simultaneously. The cytotoxicity of the drugs was measured using Digital Imaging Microscope SCAN (DIMSCAN) (12) or the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium (inner salt; MTS) and the electron coupling reagent, phenazine methosulfate (PMS) assay. MTS assay (13) was performed 72 hours after the addition of the drugs: MTS reagent (20 µl) was added to each well and the color intensity was read at 490 nm after two hours incubation at 37oC. For DIMSCAN, 40 µg/ml fluorescein diacetate in 0.5% Eosin Y solution was added at 72 hours after incubation, and plates were scanned by a digital imaging microscope after 20 minutes. 50% Inhibitory concentration (IC_{50}) values were calculated from dose-response curves and combination indices (CI) were derived using Calcusyn software (Biosoft, UK).

Phase I study of combination therapy of gemcitabine and bortezomib.

Patient eligibility criteria. Patients were eligible if they had histologically or cytologically confirmed metastatic solid tumors for which standard curative measures were no longer effective, age ≥18 years, Karnofsky performance status ≥60, organ and marrow functions within 14 days of receiving the first study drug dose must have been adequately defined as: absolute neutrophil count (ANC) ≥1,500/mm^3, platelet count ≥100,000/mm^3, hemoglobin ≥8 g/dl, serum creatinine ≤1.5 units, total bilirubin ≤2 mg/dl, aspartate transaminase (AST) ≤3× upper limit of normal values. Patients must not have received a previous chemotherapy, immunotherapy, or radiotherapy within 4 weeks of enrolling. All enrolled patients signed

a written informed consent. Because of the unknown risks to a developing fetus, patients were required to use an acceptable method of birth control if they were of child-bearing age. Prior treatment with gemcitabine was allowed. Patients were excluded if they had ≥grade 2 peripheral neuropathy within 14 days before enrollment; uncontrolled intercurrent illness; uncontrolled brain metastases; were HIV-positive; or were hypersensitive to bortezomib, boron, mannitol, or gemcitabine. This trial was reviewed and approved by the Scientific Review and Institutional Review Board prior patient enrollment. The protocol design and conduct followed all applicable regulations, guidance, and local policies.

Study design and drug administration. This was an open-labeled, single-arm dose-escalation phase I study of bortezomib with FDR gemcitabine. Bortezomib was supplied by Millennium, Inc. Gemcitabine was commercially available.

The starting dose of bortezomib was 1.0 mg/m^2 on days 1, 4, 8, and 11 and FDR gemcitabine was 750 mg/m^2 on days 1 and 8 of every 21 day cycle as illustrated in the schema (Table I). Bortezomib was administered 4 hours after gemcitabine in accordance with the findings from the preliminary *in vitro* study (cf Results). Dose escalation was performed using a modified Fibonacci dose escalation scheme, with three to six patients entered per dose level (additional patients were accrued to dose levels 3 and 4). Concurrent accrual was allowed within the same dose level. Dose escalation did not occur until all three patients in a cohort were observed for at least 4 weeks following initiation of the first cycle of therapy.

Toxicity was graded according to the NCI common toxicity criteria (CTCAE) version 3.0 (14). DLTs in a given patient were defined as any treatment-related grade 3 non-hematological of toxicity not reversible to grade 2 or less within 96 hour, or any grade 4 non-hematological toxicity, except inadequately treated nausea, vomiting or diarrhea. Hematological DLT was defined as grade 4 thrombocytopenia (platelet count ≤10,000/mm^3), or grade 3 thrombocytopenia (platelet count ≤50,000/mm^3) associated with any bleeding, and/or a requirement for transfusion and/or lasting greater than 7 days; febrile neutropenia (per NCI CTCAE: ANC <1.0×10^9/l and fever ≥38.5°C; by definition a ≥grade 3 toxicity); and/or grade 4 neutropenia (ANC <0.5×10^9/l) without fever of ≥7 days duration. DLT was based on the first course of treatment. All patients who were not evaluable for toxicity were replaced.

Rules for dose escalation. Three patients were treated at each dose level, and if no DLT occurred, the dosage was escalated. If DLT was experienced in 1/3 patients, three additional patients (for a total of
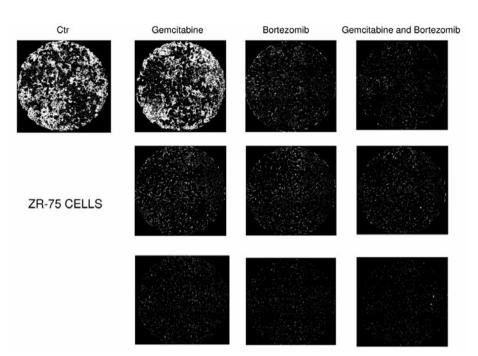
Table I. *Dose escalation schema.*

Schema

Dose group	Bortezomib (mg/m^2)	Gemcitabine (mg/m^2)
1	1.0	750
2	1.0	1000
3	1.0	1250
4	1.3	1000
5	1.3	1250
6	1.3	1500

Figure 1. *DIMSCAN images of ZR-75 cells. ZR-75 cells were treated with the indicated concentrations of gemcitabine and/or bortezomib for 72 h and then stained with fluorescein diacetate. The combination of the two drugs was more cytotoxic as compared to either of the drugs alone.*

six) were accrued at that dose level. If no additional DLT was observed in the expanded dose level group (*i.e.*, no more than 1/6 patients with DLT), the dose then was escalated. Escalation was terminated when two patients experienced any DLT attributable to the study drug(s) and then was de-escalated until a total of six patients were treated, with a DLT observed in at most 1/6 evaluable patients. MTD was defined at the dose at which ≤1/6 patients experienced a first-course DLT. Treatment was continued in an individual patient at the same dose level if no DLT was observed and if benefit was observed; patients discontinued protocol therapy if excessive toxicity was experienced. No intrapatient dose escalation was permitted.

Safety and efficacy evaluations. Patients were seen and examined at the beginning of each cycle with a complete blood count and differential, serum chemistry weekly on days 7 and 14 during the first cycle and for each cycle thereafter. Radiographic assessments were carried out every two cycles to assess tumor response by the Response Evaluation Criteria in Solid Tumor (RECIST) (15).

Treatment modification.
Bortezomib. Hematological toxicity: On day 1, administration of both drugs (gemcitabine and bortezomib) required an ANC of ≥1,500/mm^3 and platelet count of ≥100,000/mm^3. Bortezomib was given on day 4 as planned. On days 8 and 11, if the ANC was ≥500/mm^3, bortezomib was given at full dose, whether or not the parameters of the trial permitted dosing of gemcitabine on day 8. If the ANC was <500/mm^3, the treating physicians could opt to abort the dose of bortezomib on days 8 and 11. If unacceptable hematological toxicity persisted following a gemcitabine dose reduction, the bortezomib dose was reduced to the next lower dose level. If the day 1 treatment was held >7 days due to hematological

toxicity, the gemcitabine dose was reduced first then that of bortezomib if toxicity persisted.

Non-hematological toxicity: Any intolerable grade 2 or ≥grade 3 non-hematological toxicity attributed to bortezomib had to be resolved before a new cycle of treatment was started. Subsequent doses of bortezomib were permanently reduced to the next lower dose level. If the toxicity recurred at the lower dose or treatment delay was ≥4 weeks because of toxicity due to bortezomib, the drug was discontinued and the patient was removed from the study. Any grade 2 neurological toxicity resulted in bortezomib being reduced to the next lower dose level since bortezomib can cause irreversible painful neuropathy. All dose reductions were permanent.

Gemcitabine. Hematological toxicity: Day 8 gemcitabine dose adjustments consisted of a 25% dose reduction for an ANC of 1,000-1,499/mm^3 or platelet count of 75,000-99,999/mm^3 and held if ANC <1000/mm^3 or platelets <75,000/mm^3. There were no make-up doses, and if day 8 was held, then gemcitabine was reduced by 25% at the next cycle. If gemcitabine was held for >7 days, its dose was reduced by 25% at the next cycle; however, bortezomib remained at the same dose level. If there were conflicting dose attenuations due to hematological and non-hematological toxicities, the greater dose reduction was applied.

Non-hematological toxicity: Gemcitabine was held until recovery with a 25% dose reduction for grade 3 and 50% for grade 4 toxicity. If treatment was delayed ≥4 weeks because of toxicity, the patient was removed from the study. If there were conflicting dose attenuations due to hematological and non-hematological toxicities, the greater dose reduction was applied. All dose modifications were permanent. The patient was removed from the study for disease progression, toxicity or withdrawal.
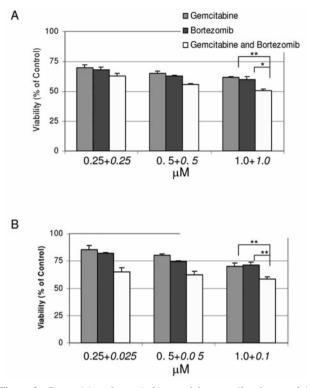
Figure 2. *Cytotoxicity of gemcitabine and bortezomib, alone and in combination using DIMSCAN. Each bar represents the mean±standard deviation of 3 independent experiments for (A) MCF-7 and (B) ZR-75 cells (*p<0.05 and **p<0.001). Concentrations written under each bar in plain text are those for gemcitabine and those in italics are for bortezomib.*

Table II. *Patient characteristics.*

Total number of patients	29
Number of evaluable patients	25
Median age (range), years	63 (36-84)
Gender	
Male	16
Female	13
Ethnicity	
Caucasian	19
Asian	6
Hispanic	4
Median performance status (KPS)	90 (60-100)
Tumor types	
Breast	4
Lung	11
Prostate/urinary	2
Unknown primary	2
Skin	2
Salivary gland	2
Colon	1
Liver	1
Gastrointestinal	2
Nasopharynx/Trachea	2
Prior chemotherapy regimens	
0	1
1	3
2	10
3	5
4	8
5	1
6	1

Results

Pre-clinical growth inhibition of bortezomib and FDR gemcitabine. ZR-75 and MCF cells were incubated in the presence of gemcitabine and/or bortezomib for 72 hours. DIMSCAN analysis and MTS assay were used to determine the cytotoxicity. Both gemcitabine and bortezomib inhibited cell growth in a dose dependent manner; however, the effect was more pronounced for the combination (Figures 1 and 2). ZR-75 cells were more sensitive to bortezomib. The DIMSCAN results were confirmed with an MTS assay (Figure 3). Isobolograms for the combination showed that bortezomib and gemcitabine have combination indices of 0.63 for ZR-75 and 0.55 for MCF-7 cells, indicating synergistic effect (Figure 3C and D). Furthermore, this synergistic effect was seen as early as and more pronounced when the cells were treated with gemcitabine 4 hours prior to the addition of bortezomib and stayed the same at 8 and 12 hours. This effect was not significantly different in MCF-7 cells (Figure 4A) but was strongly indicated in ZR-75 cells (Figure 4B). Thus we designed this phase I trial with the administration of bortezomib 4 hours after FDR gemcitabine.

Safety of FDR gemcitabine and bortezomib. A total of 29 patients were enrolled from July 2005 to May 2007. Patient characteristics are summarized in Table II. There were a variety of solid tumors, with the largest group being those for lung cancer patients. Twenty-four of the patients were evaluable for toxicity (having completed cycle 1 per protocol). The five patients not evaluable for toxicity either had evidence of clinical progression, refused to continue treatment, or had toxicity unrelated to the studied drugs prior to completing cycle 1. All patients but one had already received prior chemotherapy, and ten patients had received more than three prior regimens.

There was one grade 3 DLT with elevated liver function tests (LFT) at dose level 3; however, in the expanded cohort, no further DLTs were observed (Table II). The trial was then escalated. At dose level 5, two patients developed grade IV hematological toxicities of thrombocytopenia and febrile neutropenia. It should be noted that these two patients belonged to the most heavily pretreated patients. The first one had metastatic disease from an unknown primary that was previously treated with cisplatin, etoposide, irinotecan and paclitaxel, and this patient developed grade 4
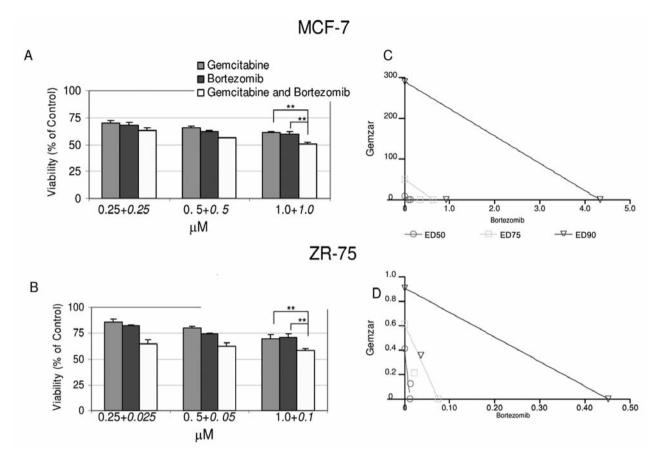
Figure 3. *Cytotoxicity of gemcitabine and bortezomib, alone and in combination, using MTS assay. Each bar represents the mean±standard deviation of 3 independent experiments for (A) MCF-7, (B) ZR-75 cells. C and D represent the isobolograms for MCF-7 and ZR-75 respectively, generated by Calcysyn software. The combination of the two drugs showed a strong synergy in both cell lines (**p<0.001). Concentrations written under each bar in plain text are for gemcitabine and in italics are for bortezomib.*

thrombocytopenia on day 15 requiring platelet transfusion. The second patient, with metastatic nasopharyngeal carcinoma, was treated with carboplatin/paclitaxel, then concurrent cisplatin/5-fluorouracil/radiation, and developed febrile grade 4 neutropenia of 0.2×10^9/l, requiring granulocyte colony-stimulating factor support (baseline WBC was 2.9×10^9/l with an ANC of 1.9×10^9/l. Dose escalation was halted and level 4 was expanded.

In the expanded cohort of dose level 4, one neurotoxicity DLT and excessive dose modifications due to course 1 toxicities (6/8 evaluable patients) were observed. In particular, one unexpected autonomic neuropathy DLT deemed related to bortezomib (prior to cycle 2) occurred in one patient and another developed grade 3 thrombocytopenia (patient had had >9 prior chemotherapy regimens for breast cancer) that required platelet support (DLT). Both level 3 and 4 doses satisfied the condition for MTD, with one formal DLT in each expanded cohort, although all patients that experienced a DLT were able to receive multiple cycles of therapy thereafter. The combination of dose modifications

and the ability to deliver multiple cycles resulted in our choice for the recommended phase II dose as dose level 3, *i.e.* with bortezomib at1.0 mg/m^2 and FDR gemcitabine at 1,250 mg/m^2.

Grade 3 toxicity for all evaluable patients for all couses included fatigue (7/24), elevated transaminases (7/24), neuropathy (3/24), anemia (4/24), neutropenia (9/24) and thrombocytopenia (9/24).

Tumor response. Twenty-five out of the 29 patients were evaluable for response. A clinical benefit rate, defined as the number of patients with objective disease response (1 patient) plus patients having stable disease for at least 4 months (6 patients) was observed as 7/25 patients. The partial response (PR) was in a patient with metastatic breast cancer who received a total of 16 cycles of treatment. Six patients with stable disease (SD) received a median of 11.5 cycles (range 4-28). A median of 2 cycles (range 1-28) were administered to the entire cohort. None of the patients that had a clinical benefit had received prior gemcitabine. Grade
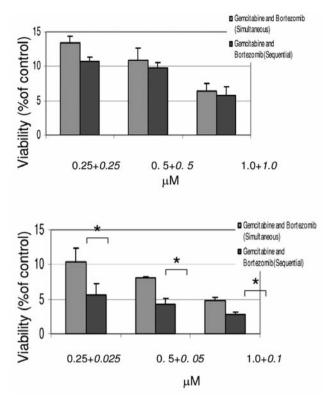
Figure 4. *Comparison of simultaneous and sequential addition of gemcitabine and bortezomib. For sequential conditions, gemcitabine was added 4 h prior to the addition of bortezomib in (A) MCF-7 and (B) ZR-75 cells. Each bar represents the mean±SD of 3 independent experiments. *p<0.05 vs. simultaneous addition. Concentrations written under each bar in plain text are for gemcitabine and those in italics are for bortezomib.*

3/4 toxicities of the 7 evaluable patients treated at the recommended phase II dose (dose level 3) over the course of therapy included anemia (1patient/0patient), neutropenia (3/0), thrombocytopenia (4/1), fatigue (1/0), elevated transaminases (2/0), sensory neuropathy (1/0) and thrombosis (0/1).

We selectively analyzed the four patients with breast cancer in this trial because of a longer duration of response. In addition to the patient with a partial response for a total of 16 cycles, two had SD for 10 and 14 cycles after six and three prior regimens for metastasis respectively. The fourth patient, who was previously treated with gemcitabine, progressed after 2 cycles.

Discussion

Bortezomib exhibits a novel pattern of cytotoxicity in both *in vitro* and *in vivo* assays and in xenograft tumor models, both as a single agent and in combination with chemotherapy and radiation (16-19). Notably, it induces apoptosis in cells that overexpress bcl-2, a genetic trait that confers unregulated growth and resistance to conventional chemotherapeutics (20). In multiple myeloma, the efficacy of bortezomib was attributed to its inhibition of nuclear factor κB activation, its attenuation of interleukin-6 (IL-6)-mediated cell growth, a direct apoptotic effect, and anti-angiogenesis. In animal models, proteasome inhibition in peripheral blood had a half-life of less than 24 hours and intermittent but high inhibition (>70%) of proteasome activity was better tolerated than sustained inhibition (21,

Table III. *Treatment-related toxicities at each dose level.*

Bortezomib/ Gemcitabine (mg/m²)	No. of patients	Patients evaluable for toxicity	No. Median cycles	No. of DLTs /No. DM[d]	DLT description	Best responses
Level 1 (1.0/7500)	3	3	2 (1-16)	0/2	None	1 PR
						2 PD
Level 2 (1.0/1000)	3	3	8 (2-13)	0/1	None	2 SD
						1 PD
Level 3 (1.0/1250)	8	7[a]	3 (1-28)	1/4		3 SD
					Gr 3 LFT	5 PD
Level 4 (1.3/1000)	10	8[b]	2 (1-4)	1/6	Gr 3 PLT	1 SD
						7 PD
						2 INEVAL
Level 5 (1.3/1250)	5	3[c]	2 (1-4)	2/3	Gr 4 PLT	3 PD
					Gr 4 ANC	2 INEVAL

DLT, dose limiting toxicitiy; DM, dose modification; PR, partial response; PD, progression of disease; SD, stable disease; LFT, liver function test; PLT, platelets; INEVAL, inevaluable; ANC, absolute neutrophil count. [a]Course one treatment was not completed due to disease-related chest pain; [b]one patient did not complete day 8 of treatment due to disease-related complications. One patient did not complete course one treatment due to unrelated hyperglycemia; [c]one patient died due to progressive disease, another refused further treatment and did not complete course one. [d]Number of patients who required dose modifications (DM) due to cycle 1 treatment-related toxicities. Patients excluded from toxicity evaluation are not counted.

Table IV. *Major treatment related toxicities in all courses*.*

Toxicity	Grade			
	1	2	3	4
Non-hematological				
Fatigue	6	12	7	0
Diarrhea	9	2	0	0
Nausea/Vomiting	14	3	0	0
Elevated LFTs	14	5	7	0
Neuropathy:Autonomic	0	0	1	0
Neuro: Sensory	5	0	2	0
Arrhythmia	6	1	0	0
Thrombosis	0	0	0	1
Hypotension	0	1	1	0
Constipation	7	3	0	0
Diarrhea	9	2	0	0
Cough	6	2	0	0
Hyperglycemia	4	7	2	0
Hypoglycemia	2	1	0	0
Hyponatremia	8	0	3	1
Hypernatremia	1	0	1	0
Hypophosphatemia	4	4	1	0
Hypokalemia	6	1	0	0
Hyperkalemia	2	1	0	0
Hypomagnesemia	8	1	0	0
Hypermagnesemia	1	0	0	0
Hypocalcemia	10	1	0	0
Hypercalcemia	7	0	0	0
Insomnia	6	0	0	0
Edema	5	3	0	0
Pain	5	7	3	0
Dyspnea	8	1	1	0
Syncope	0	0	2	0
Mucositis/stomatitis esophagitis	4	1	0	0
Myelodysplasia	0	1	0	0
Hematological				
Anemia	8	14	4	1
Thrombocytopenia	3	3	9	9
Neutropenia	5	5	9	1
Lymphopenia	4	3	7	1
Low WBC	2	16	4	1

*Other observed toxicities not listed included: dizziness, weight loss, proteinuria, dysphagia, and visual changes. LFTs: Liver function tests; WBC: white blood cell count.

22). In patients with advanced malignancies, the maximum pharmacodynamic effect (inhibition of 20S activity) occurred within the first hour post-dose, with a mean proteasome inhibition of approximately 61% (1.3 mg/m^2) (23). When bortezomib is given on a day 1, 4, 8, and 11 schedule, variable (10%-30%) levels of proteasome inhibition have been observed at the next scheduled dosing.

The recommended phase II dose for bortezomib was established here at 1.0 mg/m^2 and FDR gemcitabine at 1,250 mg/m^2 at dose level 3. This dose combination was selected over dose level 4 with bortezomib of 1.3 mg/m^2 and FDR gemcitabine of 1,000 mg/m^2. This selection was based on the number of dose modifications required (4/7 on dose 3 *versus* 6/8 on dose 4), the increased number of cycles delivered (a higher median and a much higher maximum number of cycles) and the near DLT toxicities experienced at dose level 4.

By using FDR gemcitabine administration, we delivered a higher dose of gemcitabine (1,250 mg/m^2) *versus* the established standard 30-min bolus of 1,000 mg/m^2 (11, 24). Hematological toxicities were dose-limiting for this trial. Although, the logistics of administering bortezomib on days 1, 4, 8 and 11 and 4 hours after gemcitabine on days 1 and 8, created inconvenience for patients being in the clinic twice weekly, only two patients refused further treatment. The total hematological toxicity with 17% anemia, 37.5% neutropenia, and 37.5% thrombocytopenia were considered acceptable in this heavily pre-treated group of patients. For non-hematological toxicity, it is notable for neuropathy, fatigue and elevated liver function tests. We reported a clinical benefit (PR and SD) in 7/25 patients with a median of 11.5 cycles of chemotherapy delivered prior to progression. This is a very favorable result for a phase I trial patients and we incidentally observed an excellent response in patients with metastatic breast cancer (10-16 cycles in three patients without prior gemcitabine and 2 cycles in a patient with prior gemcitabine). The median number of cycles in these four patients was 10.5, with a clinical benefit (PR+SD) of 3/4. In this heavily pre-treated group of breast cancer patients (median number of prior chemotherapy regimens of 4), this result is intriguing and needs to be evaluated further. Of note, single-agent gemcitabine at 1200 mg/m^2 in metastatic breast cancer in multiple phase II trials has a reported response rate from 14% to 37% for first-line, and 12% to 30% as second-line therapy after prior taxane or anthracycline (25).

In conclusion, in this phase I trial, we established the recommended phase II dose for the combination of FDR gemcitabine 1,250 mg/m^2 followed 4 hours later with bortezomib at 1.0 mg/m^2. Given the preclinical and this phase I clinical data, the benefit rate seen in this highly heterogeneous and heavily pre-treated patient population encourages further phase II trials to further evaluate the preclinical hypothesis that the addition of bortezomib will block further cell survival responses.

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