SPANDIDOS PUBLICATIONS

A phase II trial with gemcitabine and paclitaxel for the treatment of refractory and relapsed multiple myeloma patients

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Abstract. Multiple myeloma (MM) is an incurable disease with a 10-year survival of <20%. We have previously shown that the combination of gemcitabine and paclitaxel acts synergistically to induce apoptosis of myeloma cells *in vitro*. Based on these preclinical studies and phase I-II clinical trials in patients with solid tumors, we initiated a phase II clinical trial of paclitaxel 150 mg/m^2 IV over 3 h followed by gemcitabine 3000 mg/m^2 IV over 30-60 min in patients with relapsed or refractory MM. This regimen was administered every two weeks for a total of six cycles. Twelve patients enrolled, 3 discontinued treatment after 1 or 2 cycles because of severe neutropenia. As a result the protocol was modified to reduce the starting dose of gemcitabine to 2,000 mg/m^2. This resulted in tolerable hematological and mild non-hematological toxicities in the rest of the patients. One patient died before the onset of treatment. Of the 8 remaining patients treated with a reduced dose of gemcitabine, 1 achieved a durable CR, 3 had PR, 1 had minor response (MR), 1 had stable disease and 2 had progressive disease. The CR patient had a 98% reduction in the M-protein, ß2-microglobulin and plasma cells. His CR continued for more than 6 months. The 3 PR patients had a >50% reduction in the M-protein and >40% reduction in ß2-microglobulin. Bone marrow plasma cells were reduced by >50% in these patients. Treatment with the combination of paclitaxel and gemcitabine is an active and well-tolerated regimen in patients with relapsed or refractory multiple myeloma.

Introduction

Multiple myeloma is an incurable, clonal, B-cell malignancy (1-3). Little progress has been made during the last 25 years in the overall survival of patients with MM using standard chemotherapy (4). High dose chemotherapy with autologous peripheral blood stem cell (PBSC) rescue has resulted in an initial CR rate of >50% but 10-year survival remains <20% (5-9). Relapse of MM patients following transplantation is attributed to residual myeloma cells in the host following the myeloablative treatment, and/or to the emergence of a resistant clone of myeloma cells present in the host as was shown by us and by others (10,11).

Newer therapies have been developed in the past 5 years to treat relapse and refractory MM patients with some promising results. These therapies include thalidomide (12-14), lenalidomide (CC-5013) (15) and bortezomib (16).

Gemcitabine (2',2'-difluorodeoxycytidine; dFdC) is a pyrimidine analog that possesses a broad range of antitumor activity against solid tumors and leukemias *in vitro* and *in vivo* (17,18). The triphosphate form of the drug competes with dCTP for incorporation into DNA resulting in DNA chain termination (19). Gemcitabine cytotoxicity is proportional to the intracellular concentration of dFdCTP and its incorporation into DNA. The diphosphate of gemcitabine exerts a time and concentration dependent inhibition of ribonucleotide reductase, thereby reducing intracellular dCTP and enhancing the incorporation of dFdCTP into DNA (20,21).

In phase I clinical trials, gemcitabine was evaluated in a variety of schedules: daily x5 every 21 days (22), twice weekly x3 every 28 days (as a 30 min infusion and as a 5 min bolus) (23), once weekly x3 every 28 days (24), once every 2 weeks (25), and prolonged intravenous infusion weekly x3 every 4 weeks (26). Objective responses were observed in patients with various solid tumors in the phase I trials. Subsequent evaluation of gemcitabine in phase II and III clinical trials revealed significant clinical activity in a variety of tumors, including pancreatic, bladder, breast, ovarian, and non-small cell lung cancer.

Paclitaxel is an antimitotic drug that promotes microtubular aggregation and affects cellular functions such as cell transport and motility with little effect on DNA, RNA, or protein synthesis (27,28). Paclitaxel has demonstrated a broad range of activity against a number of solid tumors and is widely used in the treatment of non-small cell lung, epithelial ovarian, urothelial transitional cell, and head and neck cancers (29-33). There has been little clinical evaluation of paclitaxel in multiple myeloma. One study utilizing relatively low doses of paclitaxel (125 mg/m^2 over 24 h or 135 mg/m^2 over 3 h) reported responses in 5/33 patients with

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newly diagnosed multiple myeloma (response rate=15%; 95% CI= 5-32%) (33).

A sound theoretical rationale exists for evaluating the combination of gemcitabine and paclitaxel. i) Gemcitabine and paclitaxel have different cellular targets (DNA synthesis and microtubules, respectively) and act at different phases of the cell cycle (S phase for gemcitabine and mitosis for paclitaxel). ii) Their different mechanisms of action are likely to induce distinct, non-overlapping patterns of resistance. A challenging aspect to the development of a gemcitabine/paclitaxel combination is the differing drug administration schedules used for each of these agents: A 4-week treatment cycle for gemcitabine and a 3-week treatment cycle for paclitaxel. Adoption of one schedule would require a compromise of the other. A 4-week treatment cycle would preserve the gemcitabine treatment schedule, but would reduce the paclitaxel dosing frequency by 33% (from q 3 weeks to q 4 weeks). This schedule could also lead to difficulties in the administration of the Day 15 gemcitabine due to the timing of the paclitaxel-induced granulocyte nadir. A 3-week treatment schedule would preserve the paclitaxel dosing interval but would require omission of the Day 15 gemcitabine dose in exchange for a week of rest. This would reduce the number of gemcitabine treatments from 3 per cycle to 2 per cycle and could potentially reduce the effectiveness of this component of therapy. Fortunately, both gemcitabine and paclitaxel have been tested using a biweekly schedule. In a phase I study conducted in Italy, the MTD of gemcitabine was 4,560 mg/m^2 (25). This represents the highest MTDs for gemcitabine achieved in phase I using any schedule. Paclitaxel, infused over 3 h, has been administered on a q 2-week schedule in doses up to 90 to 100 mg/m^2 (with cisplatin) (35). This was achieved without the routine use of hematopoietic growth factors. We performed a phase I trial of the combination of gemcitabine and paclitaxel in 42 patients with refractory solid tumors (36). Paclitaxel doses of 75-175 mg/m^2 IV over 3 h were followed by gemcitabine 1,500-3,500 mg/m^2 IV over 30 min administered once every two weeks. Dose-limiting toxicities included grade 3 transaminase elevation and grade 4 neutropenia lasting for more than 5 days. The recommended phase II dose was paclitaxel 150 mg/m^2 and gemcitabine 3,000 mg/m^2 administered once every two weeks. None of the 6 patients treated at this level experienced dose-limiting toxicity.

We have previously shown that gemcitabine and paclitaxel induce effective cytotoxicity, *in vitro* against various myeloma cell lines as single agents, and act synergistically at very low doses (34). The time and dose titration of gemcitabine and paclitaxel revealed an IC50 of 5 μM for gemcitabine in myeloma cell lines expressing either high, or low levels of bcl-2. Paclitaxel, on the other hand was found more effective in cells with low levels of bcl-2 compared to the same cells expressing high levels of bcl-2, with IC50 of <0.1 μM and >1 μM, respectively. The combination of the two drugs, at 5-10-fold lower doses, was effective in killing >95% of myeloma cells following 24 h of treatment, regardless of the level of expression of bcl-2 (34). Pharmacokinetic studies of paclitaxel administered to cancer patients at 135 mg/m^2 (3 h infusion), revealed a peak plasma concentration of 2.5 μM (29). Pharmacokinetic studies of gemcitabine, at 3,000 mg/m^2 (1.5 h infusion) revealed a peak plasma concentration of 100 μM (37). These concentrations are >50-fold higher than the IC50 we observed *in vitro* for the combination gemcitabine and paclitaxel in myeloma cell lines (34). Based on this broad spectrum of clinical activity and the encouraging activity of gemcitabine against resistant myeloma cells *in vitro*, we felt that further evaluation of this drug in patients with relapsed and refractory myeloma is warranted. Hence, we designed a phase II trial of gemcitabine and paclitaxel in patients with relapsed and refractory multiple myeloma. The goals of this study were: To determine the objective response-rate to the combination of gemcitabine and paclitaxel for patients with MM who were resistant to or had relapsed after conventional therapy, and to assess the toxicity and tolerability of the regimen in this group of patients.

Patients and methods

Patients were enrolled from Audie L. Murphy Veterans Administration Medical Center, University Hospital and Wilford Hall Medical Center at San Antonio, TX; Northwestern University Medical Center, Chicago, IL and Vanderbilt Medical Center, Nashville, TN. Informed consent was obtained by the participating investigators in the different institutions. The protocol was approved by the local IRB of all the institutions involved in this study. Informed consent was obtained from all patients before entry into the study. Patients could have received prior high dose chemotherapy and stem cell transplantation and relapsed after multiple lines of chemotherapy. Other inclusion criteria were Zubrod Performance Score of 0-2, serum creatinine of <2.5 mg/dl; bilirubin ≤2.5 mg/dl; transaminases ≤2x institutional upper limit of normal levels with no evidence of active bacterial infection; ANC ≥1500/μl and platelets ≥l00,000/μl. While on trial, patients were allowed to receive concurrent bisphosphonate treatment for bone lesions and hematopoietic growth factor (G-CSF) support according to ASCO guidelines. No prophylactic growth factor support was allowed.

Prior to the initiation of therapy, patients underwent baseline evaluation which included CBC, serum chemistries, SPEP, quantitative immunoglobulin, ß2-microglobulin and M-protein. Bone marrow aspirates were collected for routine histopathology. Patients underwent a bone survey, and 24 h urine collection for assessment of Bence-Jones proteinuria. Any lytic lesions not previously treated within 3 months of entering the study by radiotherapy were noted as index lesions, but were not used to assess response. Patients also had a coagulation profile (PTT/PT), chest X-ray, EKG, routine urine analysis and a pregnancy test performed, if applicable. In order to prevent severe hypersensitivity reactions, all patients were premedicated prior to receiving paclitaxel with dexamethasone 20 mg po 12 and 6 h prior to paclitaxel, diphenhydramine 50 mg IV and cimetidine 300 mg po 30 to 60 min prior to paclitaxel.

All patients were placed on a Dynamap monitor during paclitaxel infusion and their blood pressure and pulse rate were checked every 15 min. All patients began treatment by receiving an infusion of 150 mg/m^2 of paclitaxel (Mead-Johnson, Princeton, NJ) through a peripheral IV access over a period of 3 h. This was followed by an IV infusion of

ose modification due to hematological toxicities.

ANC (x10^9/l)	Platelets (x10^9/l)	Paclitaxel	Gemcitabine
≥1.5	and ≥75	Full dose	Full dose[a]
1.0-1.49	50-74.9	-1 dose	Full dose
0-0.99	0-49.9	-1 dose	-1 dose

[a]A dose level reduction was 500 mg/m^2 for gemcitabine and 50 mg/m^2 for paclitaxel.

gemcitabine (Gemzar; LY 188011, 2',2'-difluorodeoxycytidine, Eli Lilly), 3000 mg/m^2 over 30-60 min. This treatment was repeated every two weeks. The planned course of therapy was 6 cycles. Actual body weight was used to determine BSA throughout this study. Due to the hematological toxicities associated with the high dose of gemcitabine used in the first 4 patients enrolled, the dose of gemcitabine was subsequently reduced to 2000 mg/m^2 (see below).

Dose adjustment was based on the complete blood count and differential blood count obtained in preparation for the day of scheduled therapy. A new cycle of treatment was begun only when granulocytes count was >1.5x10^9/l and platelets >75x10^9/l. Treatment was delayed for up to 2 weeks to allow sufficient time for recovery. Upon recovery, the patient was retreated using the guidelines outlined in Tables I and II. In cases where the cut-off for granulocytes or platelets was not met after a two-week delay, the patient was removed from the study and considered to have DLT. G-CSF at 5 μg/kg was administered when neutrophils were <0.5x10^9/l for at least 5 days or in cases of neutropenic fever. Blood and platelet support was given as needed. Packed RBCs were given as needed to keep Hgb >8g/dl. For the platelets, either single or multiple apheresis collections were given to maintain a platelet count of >10,000 if patient was clinically bleeding.

At baseline and after each cycle, blood samples were tested for CBC and differential count; Serum Protein Electrophoresis (SPEP, Albumin, Alpha-1 Fraction, Alpha-2 Fraction, Beta Fraction, Gamma Fraction); and serum chemistries. The M-protein was quantitated by electrophoresis and immunofixation methods in urine and serum and ß2-microglobulin was quantitated in the serum. The plasma cell content in bone marrow was also determined every other cycle, when possible. In addition, EKG; chest X-ray and bone survey were performed at baseline and after completion of the study (3 months).

This phase II study was designed to determine the efficacy of gemcitabine and paclitaxel in patients with relapsed or refractory MM. The study design was based on the Fleming two-stage design (38). In stage I, 15 patients are treated. If no response is seen in the first 15 patients, then one can rule out a ≥20% response-rate and stop the trial. If 3 or more responses are obtained in the first group of 15 patients, then the trial is stopped and the drug is accepted. However, if 1-2 responses are obtained in the first group of 15 patients, an additional 20 patients are entered. If 1-3 responses are obtained among the 35 patients, then the drug is accepted if the response rate is ≥11.4% (4/35 patients), and the drug is rejected if the response-rate is ≤8.6% among the 35 patients (3/35 patients). Of the intended first phase of 15 patients only 12 patients were enrolled in this study because of slow accrual due to competitive new chemotherapy regimes for refractory MM patients, such as thalidomide and bortezomib.

Objective response rate was determined according to Southwest Oncology Group criteria: Sustained decrease in

Table II. Dose modification due to non-hematological toxicities.

Toxicity	Toxicity level	Paclitaxel	Gemcitabine
Bilirubin	0-2	[a]Full dose	[a]Full dose
	3	-2 dose levels	-1 dose level
	4	-2 dose levels	-2 dose levels
Transaminases	0-1	Full dose	Full dose
	2	Full dose	-1 dose level
	3-4	Full dose	-2 dose levels
Myalgias or peripheral neuropathy	0-2	Full dose	Full dose
	3	-1 dose level	Full dose
	4	-2 dose levels	Full dose
Skin rash	0-2	Full dose	Full dose
	3	Full dose	-1 dose level
	4	-1 dose level	-2 dose levels
Other toxicities except nausea/vomiting	0-3	Full dose	Full dose
	4	-1 dose level	-1 dose level

[a]A dose reduction for gemcitabine was 500 mg/m^2 and 50 mg/m^2 for paclitaxel.

Table III. Patient characteristics.

Patient no. and Ig	Age/ Sex	Disease duration and status	Prior therapy	Cycles of treatment	[a]Response and outcome	Comments
1/kLC	76/F	5y/PD	Mel+P (x2); INF; VAD	2	Pan cytopenia; off protocol	3 g/m^2 GEM
2/IgG/l	76/M	5y/progressive	Relapse post ASCT (TBI+Mel)	0	Died before treatment of liver disease	No treatment
3/IgG/k	54/M	3y/Relapsed post Tx	VAD (x3); ASCT (TBI+Mel)	1	Pan cytopenia; off protocol	3 g/m^2 GEM
4/kLC	66/F	4y/ Relapse post Tx	VAD (x2); Mel; ASCT (Mel)	1	Pan cytopenia; refused to continue treatment	3 g/m^2 GEM
5/IgG/k	53/M	2y/refractory	Multiple cycles of VAD; ASCT (Mel+TBI); Thal	4	Progressive disease; off protocol	2 g/m^2 GEM
6/IgG/k	63/M	8y/Relapse post Tx	VAD; INF; Mitoxantrone; ASCT (TBI+Mel)	6	MR, continued on thalidomide	2 g/m^2 GEM
7/IgG/k	61/M	hRefractory	Multiple cycles of VAD; Mel+P; INF; Mel	6	Achieved PR, relapsed	2 g/m^2 GEM
8/IgG/k	59/M	3y/Relapse post Tx	Multiple Chemotherapy; XRT; ASCT (TBI+Mel)	6	Achieved PR; went on thalidomide	2 g/m^2 GEM
9/IgG/k	74M	4y/Smouldering MM	Multiple XRT; Mel+P; INF	6	Achieved CR	2 g/m^2 GEM
10/IgG/k	56/M	3y/Relapse post Tx	Multiple XRT; VAD; ASCT (TBI+Mel)	6	Achieved PR	2 g/m^2 GEM
11/IgG/k	66/F	5y/Relapse post Tx	Multiple XRT; High DEX	6	Remained in SD	2 g/m^2 GEM
12/l/LC	58/M	1y/refractory	Mel+P; high DEX; XRT	6	Died of PD	2 g/m^2 GEM

[a]Response by SWOG Criteria; Mel, melphalan; XRT, radiation; Thal, thalidomide; INF, interferon; P, prednisone; DEX, dexamethasone; VAD, vincristine/adriamycin/dexamethasone; ASCT, autologous stem cell transplantation; MR, minimal response; PR, partial response; CR, complete clinical response; SD, stable disease; GEM, gemcitabine; Tx, transplant; PD, progressive disease.

serum M-protein to ≤25% of the pretreatment value and a sustained decrease in the 24-h urine M-protein to ≤10% of pretreatment value with a ≥50% decrease in bone marrow plasma cells.

Results

Twelve relapsed or refractory MM patients were enrolled into the study. The median age was 61 (53-76) years and 6 patients had relapsed after autologous stem cell transplantation. Others were refractory MM patients following multiple regimens of conventional chemotherapy with or without radiotherapy (Table III).

Response to treatment. Of the 12 patients enrolled in this trial, 1 patient died before the onset of treatment and 3 were taken off protocol because of sustained grade 4 neutropenia following the first or second cycle of treatment. The protocol was amended and the dose of gemcitabine was reduced from 3,000 mg/m^2 to 2,000 mg/m^2 in the subsequent 8 patients. This resulted in much more tolerable myelosuppression. Of the 8 remaining patients, 1 patient received only 4 cycles of treatment and was discontinued because of disease progression and grade 4 neutropenia, despite filgrastim support and dose reduction of gemcitabine and paclitaxel, according to the dose reduction schema (Table I). All the remaining 7 patients completed 6 cycles of treatment with 1 patient achieving a durable CR, 3 achieving PR, 1 achieving MR, 1 with SD and 1 with PD who experienced grade 4 neutropenia and died of progressive disease shortly after completing the treatment. The results are summarized in Table IV. The CR patient (no. 9) had a steady decrease in the serum M-protein during the course of treatment reaching 2% of the initial level of 1,140 mg/dl after the 6th cycle of therapy. His ß2-microglobulin levels decreased during the course of treatment from 2.5 mg/l to 0.1 mg/l after the 6th cycle of treatment. Percent bone marrow plasma cells (PC) declined from 39% to 1% (Table IV). This patient continued treatment off protocol, at his request, for another 6 cycles and remained in CR for the duration of treatment with all 3 myeloma indicators approaching undetectable values (Fig. 1). The levels of Ca^{++} remained practically the same for the duration of the treatment starting at 9.1 mg/dl and ending at 8.7 mg/dl (Fig. 1). His WBC fluctuated between 5,500 and 3,000 cells/μl with nadirs of 1,000 and 1,500 cells/μl after the 1st and 6th cycles on protocol and with sustained WBC of 1,900 to 4,800 cells/μl in the next 6 cycles off protocol (Fig. 2). The patient's hemoglobin ranged between 12.7 g/dl to 11.6 g/dl at completion of protocol with only 1 nadir of 7.8 g/dl where the patient received RBCs support. The patient completed

Follow-up of Gem-Tax protocol patients for myeloma markers.

[a]Pt	ß2-microglobulin/cycle of treatment				M-protein/cycle of treatment				% Plasma cells/cycle of treatment			
	0	II	IV	VI	0	II	IV	VI	0	II	IV	VI
6	3.2	ND	2.4 (75)	2.2 (69)	5,170	ND	3,906 (75)	3,674 (71)	49	ND	39 (79)	36 (73)
7	2.7	0.5 (81)	1.4 (51)	1.7(63)	760	170 (22)	150 (20)	120 (16)	35	15 (43)	8 (23)	12 (34)
8	3.3	1.1 (33)	1.9 (57)	1.6 (48)	500	ND	162 (32)	125 (25)	23	ND	12 (52)	9 (39)
9	2.5	2 (80)	1.8 (84)	0.1 (4)	1,140	450 (39)	290 (25)	26 (2)	40	19 (47)	11 (27)	1 (2)
10	3.5	3.6 (103)	3.5 (97)	2.4 (68)	1,420	1,010 (71)	830 (58)	690 (48)	42	40 (95)	36 (85)	20 (49)
11	4.6	3.5 (76)	3.0 (65)	3.7 (80)	7,100	6,400 (90)	6,100 (89)	5,700 (80)	70	61 (87)	30 (83)	37 (52)
12	6.6	11.1 (167)	6.4 (97)	ND	4,130	4,073 (98)	4,560 (110)	5,990 (145)	54	46 (85)	ND	50 (92)

[a]Patients who completed 6 cycles of therapy are depicted; the numbers in parentheses represent the percent of the baseline values; ND, note done.

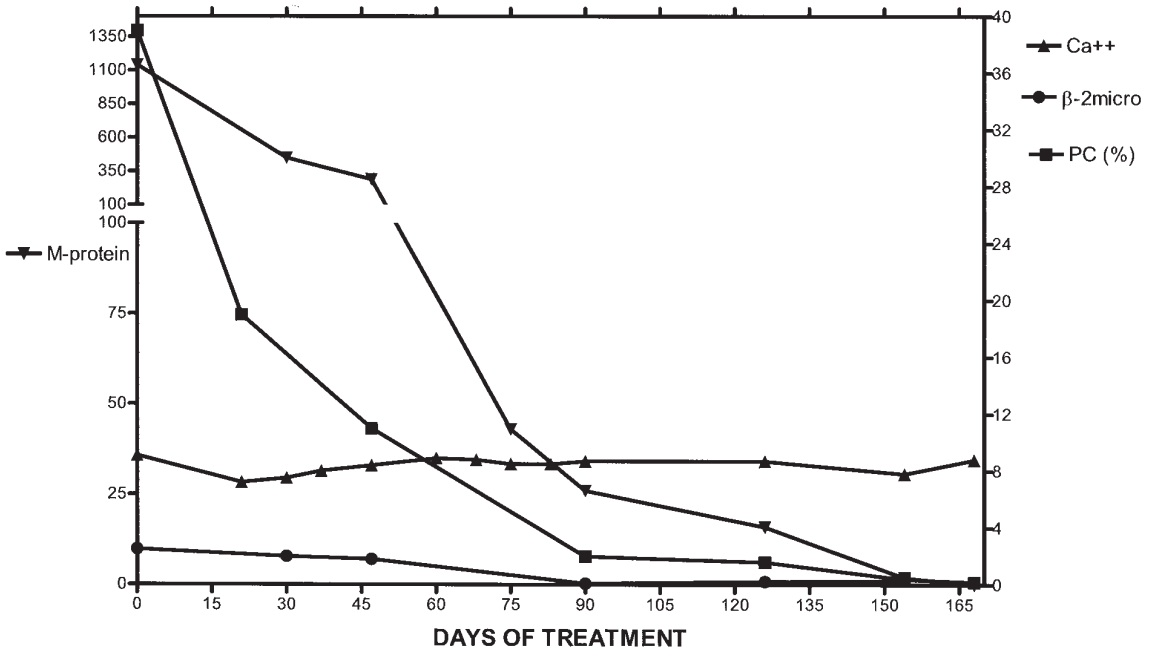

Figure 1. Decrease in the M-protein, ß2-microglobulin and bone marrow plasma cells following Gem-Tax treatment of patient no. 9. Patient no. 9 was treated for 6 cycles on protocol and additional 6 cycles off protocol. PC, plasma cells (% of total nucleated cells in bone marrow smears). The M-protein was measured in plasma by immunofixation. For experimental details see materials and methods.

additional 6 cycles off protocol with Hgb of 11.7 g/dl (Fig. 2). Similarly, his platelet (plt) count was around 200,000 plts/μl with only 1 nadir of 98,000 plts/μl. AST values were within the normal range, between 25 to 22 IU/l for 5 cycles of therapy with only a single spike to 40 IU/l after the first cycle, after which his AST stabilized around 20-25 IU/l for the next 6 cycles off protocol. ALT values followed similar pattern described for AST (Fig. 2). The patient's baseline Ca^{++} level was 9.1 mg/dl, decreased to 7.2 during treatment and increased back to 8.7 mg/dl at the end of the first 6 cycles. The patient remained at the same Ca^{++} levels for the duration of the next 6 cycles off protocol (Fig. 1). He initially had a regression of his skull plasmacytoma for the duration of the treatment. Upon stopping the treatment the patient had a recurrence of his skull lesions and progression of his myeloma disease. He was then treated with radiotherapy to the skull for pain relief and with thalidomide for his myeloma disease. The patient did not tolerate thalidomide and the treatment was stopped and he died 1 year later from slow progression of his disease.

The 3 PR patients (nos. 7, 8 and 10) had a 45% to 84% reduction in M-spike with 2 patients achieving >75% reduction in the M-spike with consistent decrease in ß2-microglobulin of 32% to 52% of baseline values and a 63% to 51% reduction in the percent of bone marrow plasma cells (PC) compared to baseline values (Table IV). In 1 PR patient (no. 7), the baseline serum Ca^{++} level was 9.7, dropped to 8.2 and increased back to 9.8 mg/dl at the end of treatment. In another PR patient (no. 8) baseline Ca^{++} level was 9.7, dropped to 8.6 and increased to 8.9 at the end of treatment.

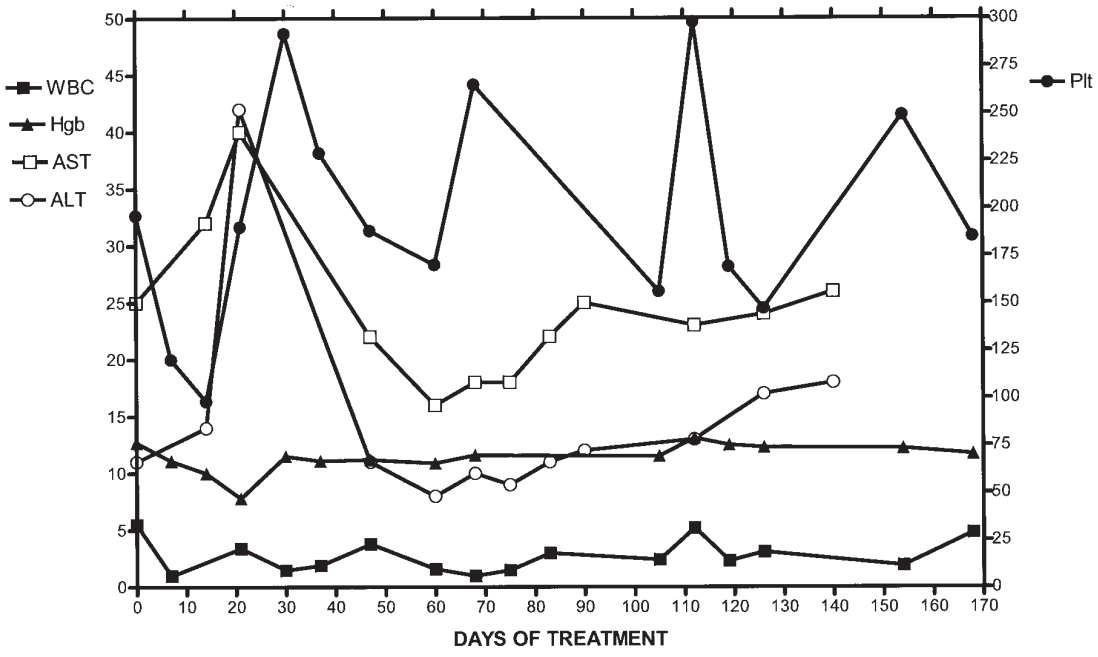

Figure 2. Fluctuations in blood counts, hemoglobin and liver enzymes following the Gem-Tax treatment of patient no. 9. He was treated for 6 cycles on protocol and additional 6 cycles off protocol. AST and ALT are liver enzymes. For experimental details see Materials and methods.

Table V. Follow-up of Gem-Tax protocol patients for non-hematological toxicities.

[a]Pt/	Alopecia		Pain			Weakness			Mucositis			Skin rash			Fever				Neuropathy		
grade	I	II	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	IV	I	II	III
6	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	6	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0
8	0	2	5	1	0	4	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
10	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
12	0	2	0	2		0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0

[a]Pt, patients. Only patients who completed 6 cycles of therapy were included. Toxicity was graded by SWOG criteria. The numbers represent cycles of observed toxicities. No grade IV toxicities were observed for pain, weakness, mucositis, skin rash and neuropathy.

The 3rd PR patient (no. 10) had Ca^{++} baseline of 12.1, which dropped to 9.8 and bounced back to 10.8 mg/dl. The patient with MR (no. 6) had >25% consistent reduction in the 3 myeloma indicators including a reduction in skull plasmacytoma. His baseline Ca^{++} was 11.6, then dropped to 9.1 and increased to 10.7 at the end of treatment. All patients eventually progressed and died 1-2 years after treatment on this protocol.

Hematological toxicities. Following the dose reduction of gemcitabine, we generally observed transient and manageable hematological toxicities. Of the 8 patients treated with 2 g/m^2 of gemcitabine, 2 had grade 4 neutropenia and 1 had grade 3 neutropenia, all of which were resolved by conventional RBCs, platelet and G-CSF support. All but 1 patient with grade 4 neutropenia failed hematological support (patient no. 5).

Non-hematological toxicities. Generally, non-hematological toxicities were minor and transient. Of the 7 patients who completed all 6 cycles of therapy, grade II alopecia was observed in 3/7 patients during 1-2 cycles. Level I pain was observed in 3 patients during 1-6 cycles of therapy. Grade I weakness was observed in 2 patients for 2-4 cycles and grade III weakness was observed in 1 patient for 1 cycle. Grade I mucositis was observed in 3 patients, for 1 cycle. Grade II fever was also rare with 2 patients experiencing grade II for 1 cycle. Grade I neuropathy was observed in 4 patients for 1-2 cycles and grade II neuropathy was observed in 1 patient for

treatment. No skin rush was observed in any of the who completed 6 cycles of therapy. No grade IV toxicities were observed for alopecia, pain, weakness, mucositis, skin rash and neuropathy (Table V).

Discussion

Extensive preclinical studies of a possible *in vitro* synergy between paclitaxel and gemcitabine suggested a highly significant synergy between gemcitabine and paclitaxel and a variety of myeloma cell lines (34). Based on these *in vitro* results and the efficacy of this combination in a great variety of solid tumors, we launched a phase II clinical trial in refractory multiple myeloma patients. Of the 12 patients enrolled in this study, 4 heavily-pretreated multiple myeloma patients experienced objective responses, including one patient who achieved a durable CR. All objective responses were observed in the cohort of patients treated with a reduced starting dose of gemcitabine. This dose-reduction to 2 g/m^2 of gemcitabine resulted in tolerable and manageable hematological toxicities in the rest of the patients. Only one of the 8 patients treated at this dose could not receive the full 6 planned cycles of treatment, and this was due to disease progression, not drug toxicity. Of the intended first phase of 15 patients only 12 patients were enrolled in this study because of slow accrual due to competitive new chemotherapy regimes for refractory MM patients, such as thalidomide and bortezomib.

In recent years new treatment modalities have been developed to treat MM including thalidomide (19) and bortezomib (Velcade) (16). The first thalidomide study of 169 refractory MM patients resulted with ~30 responses and 1 CR. However, peripheral neuropathy was a major treatment-limiting toxicity affecting 50% to 80% of the patients, the severity and reversibility of which were related to both dose and duration of drug administration of thalidomide. In other studies, thalidomide doses of >400 mg resulted in grade 3 neurotoxicity in approximately one third of patients (12-14). In another ongoing randomized study of 550 patients receiving Total Therapy with or without thalidomide, the 4-year estimate of grade ≥3 peripheral neuropathy has been about 16% in the thalidomide arm vs only 5% in the Total Therapy arm with no thalidomide treatment (9). In other studies, the combination of thalidomide and doxorubicin was tested resulting in manageable treatment related thrombo-embolic complications (42). Early trials of thalidomide and dexamethasone are ongoing (43).

CC-5013 (Revimid) is a thalidomide-related, newly discovered drug with lower neurotoxic side effects. Responses have been reported in one third of patients with advanced and refractory myeloma (44), however, unlike thalidomide, CC-5013 causes myelosuppression especially when combined with other drugs sharing a similar toxicity profile (9).

Another newly developed drug for the treatment of MM is bortezomib (Velcade) which has been approved for the treatment of MM (16,39). This drug is a proteosome inhibitor (40) which induced 35% overall response in 202 relapsed and refractory MM patients (SUMMIT; CREST studies). The most common side effects of bortezomib (25-55%) include nausea, diarrhea, fatigue, thrombocytopenia, neutropenia, peripheral neuropathy, vomiting and anorexia. The dose limiting toxicities (DLTs) include grade 3 hypo-natremia, fatigue, thrombocytopenia and neutropenia (19,39). The overall response with this drug has been about 35% including 4% CR and 6% near CR according to the criteria proposed by Blade *et al* (40-41). A combination of bortezomib and dexamethasone in patients who did not respond to bortezomib resulted in an additional response in 13/74 patients (39). In a recent study (APEX), Richardson *et al* (45) reported the results from randomized study of 669 patients with relapsed myeloma receiving either bortezomib followed by dexamethasone, or with dexamethasone alone. In a follow-up of 1 year, patients treated with bortezomib and dexamethasone had higher response rates, a longer time to progression and a longer survival than patients treated with dexamethasone (CR+PR= 38% vs 18%, respectively) and 6% of patients receiving bortezomib plus dexamethasone achieved CR compared to 1% in the dexamethasone arm. Toxicities remained a problem with grade 3 or 4 adverse events observed in 75% of patients treated with bortezomib plus dexamethasone and in 60% of patients receiving dexamethasone alone (45).

In other studies, bortezomib synergizes with gemcitabine *in vivo* in a xenograft model of human bladder tumor (46). This recent observation and our results merit a phase I clinical trial of the combination of bortezomib and gemcitabine in refractory myeloma patients.

In summary, in this phase II trial of the combination of paclitaxel and gemcitabine for refractory and relapsed MM patients we observed objective responses in 30% (4/12) of patients (3 PR and 1 durable CR by SWOG criteria). Hence, the responses in this small study are similar to the responses observed for bortezomib and thalidomide with fewer and manageable toxicities described above for thalidomide, CC-5013, bortezomib or combinations of these drugs with other drugs. Our results justify additional investigation of this drug combination in a larger number of refractory MM patients.

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