A Randomized Phase II Trial of the Matrix Metalloproteinase Inhibitor BMS-275291 in Hormone-Refractory Prostate Cancer Patients with Bone Metastases

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Abstract **Background:** BMS-275291 is a selective matrix metalloproteinase inhibitor (MMPI) that does not inhibit sheddases implicated in the dose-limiting arthritis of older MMPIs. We conducted a randomized phase II trial of two doses of BMS-275291 (1,200 versus 2,400 mg) in hormone-refractory prostate cancer (HRPC) patients with bone metastases to probe for a dose-response relationship and to assess differential toxicities. Serial serum and urine specimens were collected to assess for markers of bone metabolism.

Methods: The primary end point was 4-month progression-free survival (PFS). Eligibility criteria included documentation of androgen-independent disease (including anti-androgen withdrawal), skeletal metastasis, adequate end-organ function and performance status, and no more than one prior chemotherapy regimen. Patients were randomized to 1,200 mg orally once daily (arm A) or 1,200 mg orally twice daily (arm B). Response was assessed every 56 days.

Results: Eighty patients were enrolled: 39 in arm A and 41 in arm B. There were no responders by prostate-specific antigen or measurable disease to treatment. Stable disease was noted at 8 weeks in 39% of patients in arm A and in 17% of patients in arm B. Progression of disease at 8 weeks was seen in 61% of patients in arm A versus 83% of patients in arm B. Median survival time was 21.6 months (95% confidence interval, 17.5; not reached), whereas median PFS time was 1.8 months (95% confidence interval 1.74; 2) for all patients. Patients in arm A had a median survival time that was not reached, whereas patients on arm B has a median survival time of 21 months ($P = 0.2$). PFS at 4 months favored arm A: 22% versus 10% (log-rank, $P = 0.008$). Grade 3 toxicities occurred in 5 (13%) patients in arm A and in 9 (22%) patients in arm B. Grade 4 toxicities were uncommon (only 4% of patients): one each of thrombosis, fatigue, and motor neuropathy was seen in the arm B. Bone marker studies showed that baseline serum levels of *N*-telopeptide, osteocalcin, procollagen I NH_2-terminal propeptide, and PICP had prognostic significance for PFS and/or overall survival.

Conclusions: Regardless of dose schedule, BMS-275291 was well tolerated in HRPC patients and had no dose-limiting arthritis. Toxicities differed modestly according to the dose schedule employed. As overall survival and PFS favored the once daily schedule, this dose schedule is recommended for future studies. Baseline markers of bone metabolism may have prognostic value in HRPC patients with bone metastases.

Prostate cancer is a leading cause of cancer-related deaths in men, second only to lung cancer (1). Metastatic prostate cancer accounts for the vast majority of deaths. It is a virulent disorder for which no curative therapy exists. Presently, treatment consists of androgen ablation, which is associated with significant toxicity, such as vasomotor instability, deterioration of muscular strength, and progressive fatigue. The relative ineffectiveness of this therapy is evidenced by the fact that

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following hormonal intervention, the 2-year survival for patients with prostate cancer is only 50%. Disease progression occurs despite total androgen blockade because a clone of cells that is unresponsive to androgen ablation obtains a growth advantage, or a clone of cells with initial partial responsiveness adapts to the new milieu. This so-called "androgen-independent" prostate cancer has been shown to persistently express the human androgen receptor despite maximal androgen blockade and to be unresponsive to conventional cytotoxic chemotherapy (2). Despite recent advances with taxane-based therapy, results remain suboptimal (3, 4). New approaches to therapy are therefore necessary to improve outcomes in this disease.

BMS-275291 is a novel, non-hydroxamate matrix metalloproteinase inhibitor (MMPI), prospectively designed to inhibit a broad spectrum of MMPs (including MMP-2 and MMP-9). In contrast to hydroxamate-based MMPIs, BMS-275291 does not inhibit sheddases, which are felt to play a role in the dose-limiting arthritis seen with older compounds. Newer MMPIs that lack antisheddase activity are expected to be less toxic. Preclinical studies have shown that BMS-275291 exhibits dose-dependent inhibition of angiogenesis in *in vivo* Matrigel mouse models and dose-dependent inhibition of metastasis and tumor progression in murine tumor models. In a phase I trial, BMS-275291 was found to be well tolerated, and a maximum tolerated dose was not determined despite the administration of doses as high as 2,400 mg/d in patients with advanced or refractory solid tumors (5). Nevertheless, the investigators recommended a dose of 1,200 mg once a day for further study. Although no objective tumor responses were observed, 12 of 44 patients received treatment for ≥4 months, 6 for ≥8 months, and 3 for over a year.

Bone metabolism is distinguished by two seemingly opposing activities: the formation of new bone by osteoblasts and the resorption of old bone by osteoclasts. These processes are tightly coupled in space and time. Ultimately, bone mass is dependent upon the balance between formation and resorption. In metastatic prostate cancer, both osteoblastic and osteoclastic activities are activated, but the homeostatic balance is tipped, such that osteoblastic activity predominates, resulting in sclerotic bone metastases. Prostate cancer cells interact directly with bone cells to activate specific MMPs that allow malignant cells to invade the skeletal environment (6, 7).

Bone metastasis is a very common event in patients with prostate cancer and is a frequent source of morbidity, including bone pain or fracture. Assessment of skeletal bone metastases in these patients has traditionally been with imaging modalities, such as nucleotide bone scintigraphy scans. However, bone scintigraphy is not highly specific and fails to detect areas of bone degradation, a feature common to bony metastases (8, 9). In addition, many patients with a clinical response to therapy characterized by a declining prostate-specific antigen (PSA) and improved well-being may not have an immediate corresponding improvement in the bone scintigraphy scan.

Thus, biochemical markers of bone metabolism in blood have been explored as indicators of bone turnover for their potential as prognostic and/or predictive variables (10, 11). In this study, we sought to prospectively evaluate the prognostic and predictive significance of markers of bone metabolism in the context of a randomized clinical trial in hormone-refractory prostate cancer (HRPC).

Patients and Methods

Eligibility criteria

To be eligible for the phase II trial, all patients must have had a histologic diagnosis of adenocarcinoma of the prostate (stage D_2) that was unresponsive or refractory to hormone therapy (despite androgen deprivation and anti-androgen withdrawal when applicable) as defined by at least one of the following criteria: (*a*) progression of unidimensionally measurable disease assessed within 28 days before initial administration of drug; (*b*) progression of evaluable but not measurable disease assessed within 28 days before initial administration of drug for PSA evaluation and within 42 days for imaging studies; and (*c*) rising PSA, defined as at least two consecutive increases in PSA to be documented over a reference value (measure 1). The first increasing PSA (measure 2) should be taken at least 7 days after the reference value. A third confirmatory PSA measure (2nd beyond the reference level) should be greater than the second measure, and it must be obtained at least 7 days after the 2nd measure. If this is not the case, a fourth PSA is required to be taken and be greater than the second measure. A minimum PSA was not required. All patients must have bone metastases as documented by X-ray, bone scan, magnetic resonance imaging, or biopsy. Androgen ablation was maintained throughout the trial. Patients were required to have a Karnofsky performance status of 60% to 100% and have adequate hematologic, hepatic, and renal function. No other chemotherapeutic agents, biological response modifiers, herbal therapies, radiation therapy, corticosteroid, or hormonal concomitant therapy (other than continuing luteinizing hormone–releasing hormone treatment) were allowed during protocol treatment. Patients were not allowed to begin bisphosphonate therapy during trial participation, but those already receiving bisphosphonates at the time of enrollment were allowed to continue as long as progressive disease was documented before entry. No more than one prior chemotherapy regimen was allowed. At least 21 days must have elapsed because the completion of chemotherapy, and the patient must have recovered from the side effects of such therapy. All patients were informed of the investigational nature of the study and provided written informed consent in accordance with institutional and federal guidelines. The protocol was approved by the respective institutional review boards of all participating sites.

Treatment plan

Patients in the trial were randomized to receive BMS-275291 either 1,200 mg orally once daily (arm A) or 1,200 mg orally twice daily (arm B). Cycle length was 4 weeks. Toxicities were assessed using the National Cancer Institute's Common Toxicity Criteria (version 2). In the absence of unacceptable toxicity or clear clinical progression, the patient received a minimum of two cycles of treatment. If a patient developed progressive disease after two cycles of therapy, that patient was removed from the protocol. In the absence of progression, the patient continued on protocol until unacceptable toxicity or other reason for discontinuation occurred. Criteria for removal from protocol therapy included progression of disease or after a minimum of two cycles of treatment if progression is by PSA, unacceptable toxicity, withdrawal of patient consent, and delay of treatment >4 weeks from planned date of therapy due to toxicity.

Response assessment

Response was assessed using Response Evaluation Criteria in Solid Tumors, which was modified to incorporate recent consensus recommendations on assessing response by PSA levels (12). All disease must have been assessed using the same technique as baseline. Complete response was defined as "complete disappearance" of all measurable and nonmeasurable disease with no new lesions, no disease-related symptoms, normalization of markers and other abnormal lab values, and PSA ≤ 0.2 ng/mL. Partial response was defined by either of the two sets of criteria: (*a*) ≥30% decrease

under baseline of the sum of longest diameters of all target measurable lesions, with no new lesions and no unequivocal progression of nonmeasurable disease; or (*b*) a decline in PSA by at least 50%, confirmed by a second PSA value ≥4 weeks later with the reference PSA for these declines being a PSA measured within 3 weeks before starting therapy and patients not showing clinical or radiographic evidence of progression of measurable or nonmeasurable disease during this time period. Stable disease was defined as not qualifying for complete response, partial response, progression, or symptomatic deterioration. Progressive disease was noted when one or more of the following occurred: (*a*) 20% increase in the sum of longest diameters of target measurable lesions over smallest sum observed (over baseline if no decrease during therapy) using the same techniques as baseline; (*b*) increase in PSA by at least 25% (by at least 5 ng/mL) over baseline; (*c*) unequivocal progression of nonmeasurable disease in the opinion of the treating physician; (*d*) appearance of any new lesion/site; or (*e*) death due to disease without prior documentation of progression and without symptomatic deterioration. Symptomatic deterioration was defined as global deterioration of health status requiring discontinuation of treatment without objective evidence of progression.

Statistical considerations

The phase II trial aimed to determine the efficacy and toxicity of two doses of BMS 275291 in HRPC patients with bony metastatic disease. The proportion of patients alive and progression free at 4 months after the start of protocol therapy was used as the primary design criterion. The proportion of patients alive and progression free at 6 months was used as a secondary criterion. The two-stage samples sizes were based on the desire to pursue BMS-275291 if progression-free survival (PFS) was at least as good as that for patients treated with hydrocortisone + mitoxantrone in a phase III trial (13). In that trial, time to progression was 2.3 months in the hydrocortisone arm versus 3.7 months in the mitoxantrone/hydrocortisone arm. These correspond to approximately 30% and 50% PFS at 4 months, and the present trial was designed to detect this difference.

The initial stage of accrual consisted of 24 patients, 12 assigned to each dose level. Accrual continued without interruption (beyond the first 24 patients) until all of the first 24 patients either progressed, died, or had been followed for 4 months. If ≤6 of the first 24 patients had 4 months of PFS, then the trial was to be stopped. If ≥7 of the first 24 patients survived 4 months progression free, the trial continued to its full sample size of 68 patients. The two arms were to be combined for the primary estimate of 4 month PFS. If ≥27 of the total of 68 patients survive 4 months progression-free, the 4-month PFS rate on BMS-275291 would be regarded as significantly better than the reference rate of 30% cited above. This two-stage inference had a 0.05 probability of endorsing the regimen when the true 4-month PFS rate was 30%, and a 0.054 probability of failing to endorse the regimen when the true 4-month PFS rate was 50%. Due to rapid accrual during the course of the study, and the expected delays in evaluating PFS in the first stage of accrual, 80 patients were enrolled before the protocol officially closed.

The prognostic significance of the laboratory assays was assessed using log-rank testing, with the assay results dichotomized at the median, an analysis that was prospectively planned. The relative hazard estimates were based on these dichotomizations. Proportional hazards regression was also used to test the association of assays, individually and in combination, with survival and PFS. Logarithms of assay results were used to reduce skewness. Significance was defined as $P < 0.05$.

Correlative studies: markers of bone metabolism

Serial serum specimens were collected from all patients before treatment start, after completion of two cycles of therapy, and if feasible, after every two cycles thereafter. Specimens were batched and stored at −70°C. The following markers of bone metabolism were analyzed. All samples were run in duplicate.

N-telopeptide. Serum *N*-telopeptide levels was measured using the *N*-telopeptide assay (OSTEX, Seattle, WA). This assay employed a competitive enzyme immunoassay in a microassay stripwell format, using a monoclonal antibody ELISA with a 96-well strippable plate; incubation was at room temperature and was not photosensitive. Values were expressed in nmol/L BCE. Two hundred microliters of serum (sufficient for duplicates) were required. Intra-assay variability was 4.6%, and interassay variability was 6.9%.

Deoxypyridinoline. Total deoxypyridinoline was measured using Metra total deoxypyridinoline reagents and controls and the Metra deoxypyridinoline immunoassay (Quidel Corp., Mountain View, CA). Deoxypyridinoline is a competitive enzyme immunoassay in a microassay stripwell format using a monoclonal anti-deoxypyridinoline antibody coated on the strip to capture deoxypyridinoline. Deoxypyridinoline in the sample competes with conjugated deoxypyridinoline-alkaline phosphatase for the antibody, and the reaction is detected with the substrate pNPP. Color developed during the incubation of captured enzyme conjugate and substrate is measured at 405 nm in a 96-well microassay plate reader. Total deoxypyridinoline values are expressed in nmol/L.

Pyridinoline. This assay used a Metra enzyme linked immunoassay kit (Quidel). Similar to deoxypridinoline, pyridinoline was measured with a competitive enzyme immunoassay in a microassay stripwell format using a monoclonal anti-pyridinoline coat on the strip to capture the pyridinoline. The reaction was detected with the substrate pNPP. Pyridinoline values were expressed in nmol/L.

Osteocalcin. Serum intact osteocalcin was measured with the Metra Osteocalcin assay (Quidel), an enzyme immunoassay in a microassay stripwell format using a murine monoclonal anti-osteocalcin antibody. Osteocalcin in the sample competes for antibody binding sites with osteocalcin coated on the stripwell. A rabbit anti-mouse IgG antibody conjugated to alkaline phosphatase is added, and the reaction is detected with the substrate, *p*-nitrophenyl phosphate. Color developed during the incubation of captured enzyme conjugate and substrate was measured at 405 nm in a 96-well microassay plate reader. Osteocalcin values of unknown specimens were calculated from a calibration curve fit with a four-variable logistic equation. Values were expressed in ng/mL.

Procollagen I amino-terminal propeptide. Serum procollagen I NH_2-terminal propeptide (PINP) was measured with the DiaSorin (Saluggia, IT) PINP assay. This technique employed a RIA double-antibody procedure. The radiolabel isotope used was ^{125}I. The required sample size was 50 μL; a 2-hour incubation period at 37°C was required. Seven standards ranging from 0 to 250 μg/L were included. Assay sensitivity was 2 μg/L.

Procollagen III amino-terminal propeptide. Serum procollagen III NH_2-terminal propeptide (PIIINP) was measured using the DiaSorin (Saluggia, IT) PIIINP assay. The method is a RIA double-antibody procedure. The radiolabel used was ^{125}I. The required sample size was 200 μL with a 2-hour incubation at 37°C. Seven standards ranging from 0 to 50 μg/L were included. Assay sensitivity was 0.2 μg/L.

Results

Patient characteristics. A total of 80 patients with HRPC and bone metastases were enrolled between May 2002 and July 2003. Thirty-nine patients were randomized to the 1,200 mg once-daily arm (arm A), whereas 41 were randomized to the 1,200 mg twice-daily arm (arm B). Patient characteristics are summarized in Table 1. Median age was 69 years, with a range of 48 to 87 years. The majority of patients (56%) had a Karnofsky performance score of 90% to 100%, whereas 16% had a Karnofsky performance score of 60% to 70%. Only 25% of patients had prior chemotherapy. Gleason's scoring was available from 67 patients: 34 patients had a score of 5 to 7,

Table 1. Patient characteristics

	All patients	Arm A (1,200 mg once daily)	Arm B (1,200 mg twice daily)
No. patients	80	39	41
Age, median (y)	69.8	69.3	70.2
KPS ≥ 80%	62	28	34
No. courses (median)	2	2	2
No. courses (range)	1-10	1-10	1-6
Bisphosphonate use	23	13	10

Abbreviation: KPS, Karnofsky performance status.

whereas 33 patients had a score of 8 to 10. Twenty-three patients were on concurrent bisphosphonates on study entry. Mean PSA level was 147 ng/dL, whereas the median value was 57 ng/dL. The mean number of cycles delivered was 2 for both arms. Median follow-up time was 15 months (range, 2-28 months). Sixty-nine patients had baseline serum available for bone marker analysis; of these, only 34 had post-registration sera collected serially due to the early progression in many patients. Thus, due to the limited number of post-registration sera and the corresponding limitations in correlating these results with outcome, we did correlations only with the baseline serum data.

Efficacy and toxicity. There were no responders by PSA or measurable disease to treatment. Stable disease was noted at 8 weeks in 39% of patients in arm A and 17% of patients in arm B. Progression of disease at 8 weeks was seen in 61% of patients in arm A versus 83% of patients in arm B. For all enrolled patients, the median survival time was 21.6 months (95% confidence interval, 17.5; not reached), whereas median PFS time was 1.8 months (95% confidence interval 1.74; 2). Patients in arm A had a median survival time that was not reached, whereas patients on arm B had a median survival time of 21 months ($P = 0.2$). PFS at 4 months favored arm A: 22% versus 10% (log-rank $P = 0.008$). Treatment was generally well tolerated. Grade 3 toxicities occurred in 5 (13%) patients in arm A and in 9 (22%) patients in arm B. Grade 4 toxicities were uncommon (only 4% of patients): one each of thrombosis, fatigue, and motor neuropathy was seen in the arm B.

Serum markers of bone metabolism, hemoglobin and PSA levels. Table 2 summarizes the baseline serum levels, expressed as median values and ranges, of the assayed bone markers from 69 patients. Thirty-five patients had *N*-telopeptide levels less than or equal to the median of 14.4 nmol/L, whereas 34 patients had values above the median. Thirty-four patients had osteocalcin levels above the median of 0.89 ng/mL, whereas 34 were at or below that level (one patient had insufficient serum for osteocalcin analysis).

Figure 1 summarizes the overall survival and PFS estimates from these patients based on the baseline values of each of the markers, dichotomized at the median. Patients with *N*-telopeptide levels ≤ 14.4 nmol/L (Fig. 1A) had a higher median survival time (not reached versus 10.7 months; $P < 0.001$) and a higher PFS rate at 4 months (22% versus 10%, $P = 0.04$) compared with patients with levels > 14.4 nmol/L. Patients with deoxypyridinoline levels ≤ 57 nmol/dL (Fig. 1B) had a better median survival time (not reached versus 12 months; $P < 0.001$) and 4-month PFS rate (25% versus 9%; $P = 0.04$). Similar results were observed for pyridinoline (Fig. 1C). Patients with osteocalcin levels > 0.89 ng/mL had better 4-month PFS rate (20% versus 12%; $P = 0.01$; Fig. 2A). Patients with PINP and PIIINP levels below their medians (86.5 and 5.6 ng/mL, respectively) had better median survival times (not reached versus 12 months; $P < 0.001$ and 0.005, respectively) and 4-month PFS rates (9% versus 22%; $P = 0.02$ and $P = 0.01$, respectively; Fig. 2B). Patients with hemoglobin levels below the median of 12.9 g/dL had worse median survival than those with levels above the median (12 months versus not reached; $P < 0.001$; Fig. 3). Patients with alkaline phosphatase values above the median had better survival than those below ($P < 0.001$). Similar results were seen for patients with baseline PSA levels above the median compared with those below (12 months versus not reached; $P < 0.001$).

Because of the lack of efficacy of BMS275291 in this tumor type as manifested by the high rate of disease progression in either arm, only 39 patients were able to provide serial serum samples for analysis. In these 39 patients, 9 had a decrease in *N*-telopeptide levels following at least cycle of treatment, whereas 30 patients had an increase in levels. Survival was numerically better in patients with decreasing *N*-telopeptide levels (estimated survival at 18 months was 100%) versus those with increasing levels (78% survival rate at 18 months), but the log-rank *P* was not significant ($P = 0.2$). Similar trends were seen with serial levels of PINP, deoxypyridinoline, and pyridinoline. Specifically, in the 16 patients who had decreasing PINP levels, survival seemed to be better (95% at 18 months) compared with the 24 patients with increasing levels

Table 2. Baseline serum levels of bone metabolism markers

Marker	Unit of measure	Median (range)	Mean (SD)	No. patients with levels above median
Bone resorption				
N-telopeptide	NM	14.4 (2.7-300)	24 (39)	35
Deoxypyridinoline	nM/L	57.1 (5.2-712)	102 (123)	34
Pyridinoline	nM/L	297 (4.3-5,010)	590 (966)	34
Bone formation				
Osteocalcin	ng/mL	0.89 (0.1-25.2)	2.4 (3.8)	34
PINP	μg/L	86.5 (10-489)	130 (115)	34
PIIINP	μg/L	5.6 (2.8-37.6)	7.2 (5.4)	34

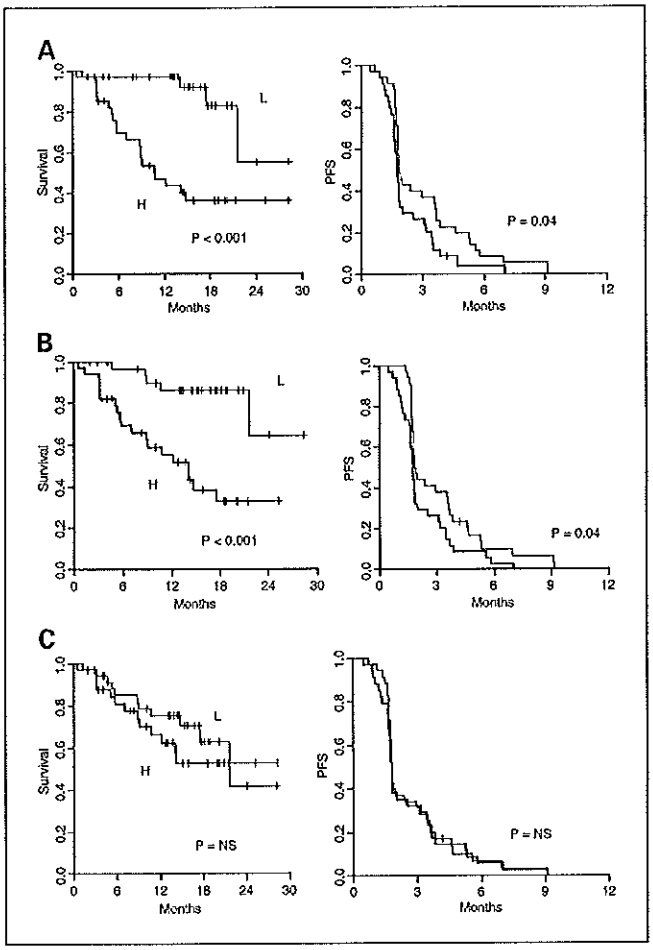

Fig. 1. Overall survival and PFS curves according to bone resorption markers. *A*, *N*-telopeptide. *B*, deoxypyridinoline. *C*, pyridinoline. L, lower values than median; H, higher values than median; NS, not significant.

(68% at 18 months), where the log-rank P was 0.09. Patients with decreasing deoxypyridinoline levels (n = 10) also had longer survival compared with those with increasing levels (n = 29; 18-month estimate = 100% versus 75%; P = 0.1). Finally, decreasing pyridinoline levels (n = 13) was associated with better survival when compared with increasing levels (n = 27; 92% versus 70% at 18 months; log-rank, P = 0.2). These data are summarized in Table 3.

In a multivariate analysis of assays with clinical variables that included bisphosphonate use, baseline PSA level, Gleason score, prior chemotherapy, performance status, hemoglobin level, and total alkaline phosphatase, no significant interactions between the clinical variables and assay results were seen, except for an equivocal association of PINP levels with bisphosphonate therapy (P = 0.04, rank sum).

Table 4 summarizes the individual variables used in the multivariate analysis and the associated relative hazards for death for each variable. In this analysis, baseline levels of PSA, hemoglobin, total alkaline phosphatase, *N*-telopeptide, PINP, PIIINP, and deoxypyridinoline had a significant association with increased risk of death when dichotomized at the median.

Multiple-predictor modeling was done using Cox regression on log-transformed values to adjust for skewness. The final model included log PSA, log alkaline phosphatase, hemoglobin level, log *N*-telopeptide, log PINP, and log DPD. Using these six predictors, all possible two-predictor Cox models were fitted. Only two models retained both variables at the 0.05 level of significance: one involving log PSA plus long PINP, and the other involving hemoglobin with long PINP. Two others (i.e., log PSA/hemoglobin and log PSA/log DPD) included both variables at the 0.06 level of significance. All three of the two-variable predictor models involving hemoglobin, log PSA, and log PINP were highly predictive of survival (P < 0.05).

Discussion

We conducted a randomized phase II trial of the MMPI BMS275291 in HRPC patients with skeletal metastases and found it to be well tolerated, although with limited efficacy. It seems that the once daily schedule (at 1,200 mg orally) is the optimal dose-schedule for future studies of this regimen in HRPC, due to its tolerability and better overall PFS results.

The correlative studies associated with this phase II study also indicated that serum levels of bone metabolism markers obtained before initiation of investigational therapy in these

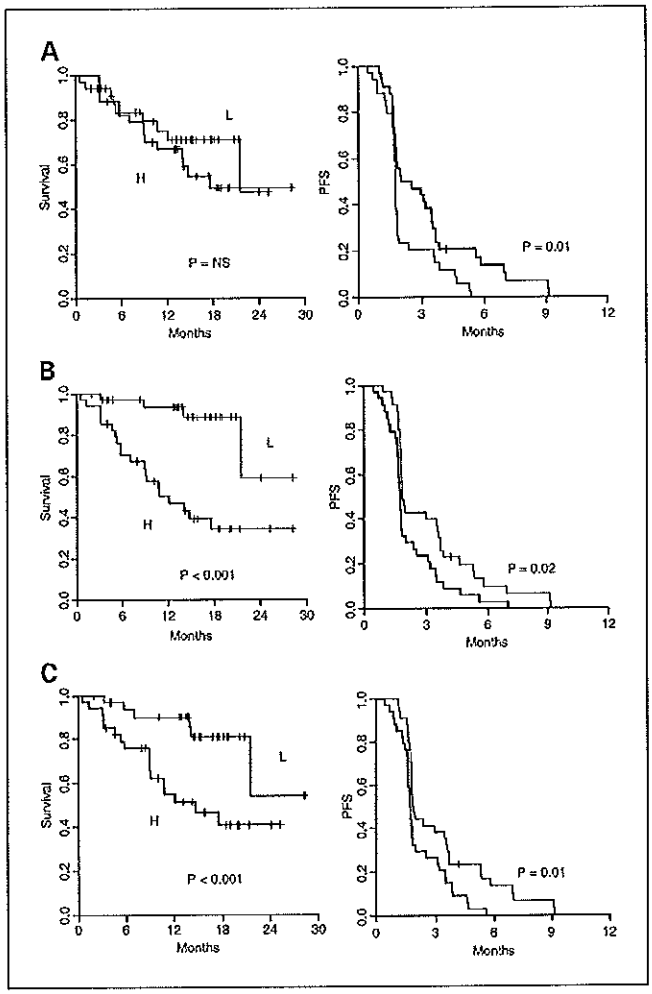

Fig. 2. Overall survival and PFS curves according to bone resorption markers. *A*, osteocalcin. *B*, PINP. *C*, PIIINP. L, lower values than median; H, higher values than median; NS, not significant.

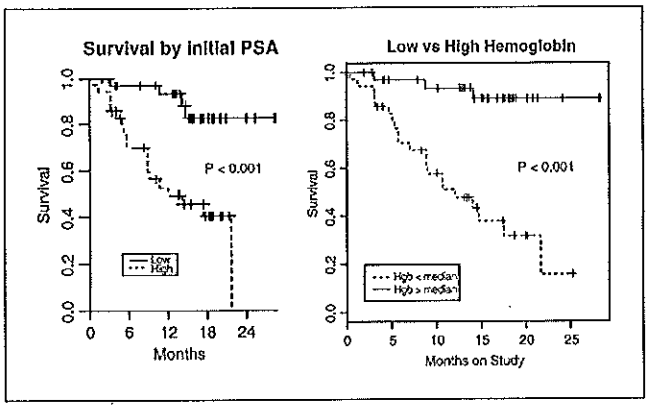

Fig. 3. Overall survival and PFS curves according to PSA and hemoglobin.

patients were predictors of PFS and overall survival. Specifically, elevated baseline levels of the bone resorption markers *N*-telopeptide and deoxypyridinoline, as well as the bone formation markers PINP and PIIINP, were associated with an increased risk of death during the course of this prospective trial. In addition, baseline nonfractionated total alkaline phosphatase and PSA levels above the median values and pretreatment hemoglobin levels below the median were associated with worse outcome.

Many processes and molecules have been implicated in the development of bone metastases from prostate cancer (14, 15). One of these processes is the ability of malignant cells to invade surrounding tissues and metastasize, mediated by enzymes called MMPs, a group of proteinases that have physiologic roles in degrading and remodeling the extracellular membrane (16). Tumor cells metastasizing to bone stimulate osteoclasts to release bone-resorbing MMPs, often mediated by parathyroid hormone-like peptide (9, 17). Furthermore, MMPs are overexpressed in a variety of malignant tumor types, including prostate cancer, and are associated with increased tumor aggressiveness and metastatic potential (18). The critical role of MMPs in the biology of metastasis have understandably made them attractive agents for anticancer therapy. One such agent targeting this process is BMS 275291, a nonhydroxamate MMPI that was designed to inhibit a broad spectrum of MMPs, including MMP-2 and MMP-9, which have previously been implicated in prostate cancer metastasis (19).

The current study attempted to test the efficacy and toxicity of two doses of BMS 275291 in patients with advanced, androgen-independent (hormone refractory) prostate cancer. Because of the central role played by MMPs in both the metastatic process and bone remodeling, we hypothesized that markers of bone turnover would have predictive value in the evaluation of the activity of BMS 275291. Because of the lack of efficacy of BMS275291 in this tumor type as manifested by the high rate of disease progression in either arm, only a handful of patients were able to provide serial serum samples for analysis. However, baseline (or pretreatment) serum samples were obtained from 69 patients and were found to be evaluable for analysis. Instead of finding predictive markers for response to an MMPI, we found that many of these markers had prognostic value. In the past, these markers were primarily used in investigational studies as quantitative markers of bone metastases to monitor response to therapy and to supplement qualitative modalities, such as bone scintigraphy or radiologic imaging (20–23).

Our study adds to these observations by providing evidence that associates levels of these markers with overall survival and PFS in prostate cancer. Interestingly, a similar study in breast cancer patients with bone-only metastases has shown that elevated *N*-telopeptide levels are significantly associated with shorter duration of clinical benefit, time to progression, and overall survival (11). Similarly, serum and urine samples from patients entered onto the placebo arms of two phase III trials of zoledronic acid in prostate cancer, non–small cell lung cancer, and other solid tumors showed that *N*-telopeptide levels provided more prognostic information than bone-specific alkaline phosphatase (24). These results are consistent with those reported herein.

The observation that elevated baseline values markers for osteoblast activity and bone formation, such as PINP and PIIINP, correlated with poorer outcome initially seems to be counterintuitive. After all, it was expected that patients with increased bone formation, and correspondingly decreased bone resorption, would have the most optimal outcomes. This "paradoxical" observation may have been due, in part, to a breakdown in homeostatic mechanisms in metastatic prostate cancer, wherein the tight coupling of resorption and formation has been disrupted. In addition, other yet unidentified epigenetic molecular pathways may also have been responsible for this uncoupling.

In patients with low osteocalcin levels, a marker of osteoblast activity and bone formation, significantly worse PFS was seen when compared with those with higher osteocalcin levels. What could account for this disparity with PINP and PIIINP, similar markers of osteoblastic activity? It is possible that osteocalcin is not as reliable a predictor of metastatic bone disease activity because it is a vitamin K–dependent GLA protein, in contrast to the greater specificity of PINP and PIIINP, which are

Table 3. Serial evaluation of bone markers: effect on overall survival rates (at 18 months)

Marker	Decreasing levels		Increasing levels	
	No. patients	Survival rate (%)	No. patients	Survival rate (%)
N-telopeptide	9	100	30	78
PINP	16	95	24	68
Deoxypyridinoline	10	100	29	75
Pyridinoline	13	92	27	70

Table 4. Individual markers or clinical variables as predictors of survival

Variable	Relative hazard for death (95% confidence interval)	P (log-rank)
Treatment arm	1.65 (0.73, 3.8)	0.22
Bisphosphonate use (yes/no)	0.78 (0.31, 2.0)	0.59
Performance status (≥90/<80)	0.66 (0.29, 1.5)	0.31
Prior chemotherapy (yes/no)	2.38 (0.9, 5.8)	0.06
Gleason score (>8/≤8)	1.03 (0.4, 2.4)	0.95
Hemoglobin (>/≤ median)	0.11 (0.03, 0.67)	<0.001
PSA level*,†	6.47 (2.2, 19.2)	<0.001
Total alkaline phosphatase*,†	5.94 (1.9, 17.9)	<0.001
N-Telopeptide*,†	6.50 (2.2, 19.1)	<0.001
Deoxypyridinoline*,†	5.43 (2.0, 14.7)	<0.001
Pyridinoline*	1.43 (0.6, 3.2)	0.38
Osteocalcin*	1.48 (0.6, 3.4)	0.34
PINP*,†	6.30 (2.2, 18.5)	<0.001
PIIINP*,†	3.51 (1.4, 8.9)	0.0045

*Baseline values dichotomized at the median (i.e., ≤ vs > calculated median value).
†Baseline values above the median were significantly associated with an increased hazard for death.

collagen-derived markers of bone metabolism. In other words, osteocalcin may not be as discriminatory for osteolytic disease activity in patients with metastatic HRPC than the other markers measured.

Finally, bisphosphonate therapy can potentially confound these observations because of its known effects on bone remodeling (25, 26). Although our study showed no such interaction between bisphosphonate use and bone metabolism markers, the limited sample size may have obscured a smaller but clinically relevant interaction.

Although it is premature to use these assays in immediate clinical practice, the prognostic implications of our findings may potentially be used as stratification factors for patients with advanced prostate cancer who are being entered onto investigational studies. Our study could not address the predictive value of such markers with bone "targeted" or other systemic therapies due to limited number of serial samples and the ineffectiveness of our investigational therapy. This issue is currently being addressed in a Southwest Oncology Group phase III trial of docetaxel with or without the endothelin-A antagonist atrasentan in HRPC (S0421, presently in development).

We therefore conclude that regardless of dose schedule, BMS-275291 was well tolerated in HRPC patients and had no dose-limiting arthritis, likely because of the limited exposure to the compound as many patients progressed early (as opposed to that seen in an adjuvant breast cancer trial where arthralgia was prominent in a population receiving the agent for a longer time period; ref. 27). Toxicities differed modestly according to which dose schedule was employed. As overall survival and PFS favored the once daily schedule, this was recommended for future studies in other tumor types. We also conclude that baseline markers of bone metabolism may have prognostic value in HRPC patients with bone metastases.

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