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Change in Markers of Bone Metabolism with Chemotherapy for Advanced Prostate Cancer: Interleukin-6 Response Is a Potential Early Indicator of Response to Therapy

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Men with androgen-independent prostate cancer (AIPC) frequently have bone metastasis. The effects of chemotherapy on markers of bone metabolism have not been well characterized. We conducted a prospective study of patients with AIPC randomized in the first cycle to receive either docetaxel/estramustine or zoledronic acid, a bisphosphonate, to inhibit osteoclastic activity. Here we report the effects of therapy on markers of bone metabolism in these patients following the first cycle of therapy. Serum levels of several indices of bone remodeling were evaluated using commercial enzyme-linked immunosorbent assays. Changes in markers of bone metabolism were compared in patients receiving initial chemotherapy versus bisphosphonate. There was no significant difference in median change in any of the measured bone markers in patients given zoledronic acid when compared to chemotherapy. When comparing responders to nonresponders, overall interleukin-6 (IL-6) decreased by 35% in prostate-specific antigen responders; whereas, IL-6 levels increased by 76% in nonresponders (p = 0.03). Elevated IL-6 levels and reductions in IL-6 levels early in treatment may reflect ultimate clinical response to docetaxel-based regimens.

Introduction

Metastatic, androgen-independent prostate cancer (AIPC) is a bone predominant disease, and therapy directed specifically at bone metastases has shown significant clinical benefit. Zoledronic acid (Zometa™), one of the most potent of the new generation bisphosphonates, is effective in reducing skeletal related events and bone pain in metastatic AIPC (Saad and others 2004a). Zoledronic acid's primary mechanism of action involves inhibition of osteoclast activity. Although characterized primarily by osteoblastic bone lesions, it is clear that AIPC has an osteolytic component (Keller 2002) which probably accounts for the clinical benefit of bisphosphonates in AIPC. In addition to its anti-osteoclastic effect, there are controversial data suggesting that bisphosphonates have a direct antitumor effect (Berenson 2005; Brubaker and others 2006). Unlike bisphosphonates, the effects of chemotherapy alone on markers of bone metabolism have not been well characterized. Docetaxel-based regimens are frequently used in patients with AIPC and have proven clinical and survival benefits. Because AIPC metastasizes to bone, we hypothesized that therapy induced changes in levels of bone metabolism markers could serve as an early biomarker of therapeutic clinical response.

For this study, we obtained levels of several markers of bone metabolism from men with AIPC that had bone metastases who were enrolled on a study of docetaxel-based chemotherapy. We studied markers of bone resorption, including receptor activator of nuclear-κ B ligand (RANKL), tartrate-resistant acid phosphatase (TRAPC), and urinary deoxypyridinolines (DpDs). Soluble or cell membrane attached RANKL activates its receptor, RANK, which is a member of the tumor necrosis factor receptor family and is critical in driving osteoclast activation and bone resorption (Wittrant and others 2004). In human PCa cell lines and primary samples, RANKL/RANK expression correlates with more aggressive and advanced metastatic PCa (Brown and others 2001; Chen and others 2006). TRAPC is secreted by active osteoclasts and correlates with resorptive biology (Rummukainen and others 2001; Kirstein and others 2006). DpD is a validated biomarker of bone resorption in

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multiple types of malignancies. The process of bone resorption results in the production of collagen breakdown products that are excreted in the urine (Pecherstorfer and others 1997; Garnero and others 2000a; Saad and others 2004b). Bisphosphonates have been shown to decrease DpD levels in patients with skeletal metastases (Coleman and others 1999). We studied markers of bone production including alkaline phosphatase [bone-specific alkaline phosphatase (BAP)] and intact osteocalcin (OCN); both are products of bone production that have been studied as markers of bone metastases in advanced AIPC (Coleman and others 1988; Francini and others 1988; Marcellini and others 1992; Jung and others 2004; Cook and others 2006). We also studied serum interleukin 6 (IL-6) and osteoprotegerin (OPG). IL-6 has been implicated in prostate cancer progression, chemotherapeutic resistance, and androgen independence and bone remodeling (Ershler and Keller 2000; Smith and others 2001; Domingo-Domenech and others 2006; Dattoli and others 2007). OPG is a soluble decoy receptor for RANKL and, therefore, interferes with osteoclast activation (Wittrant and others 2004). Here, we report on the effects of a docetaxel-based regimen compared to zoledronic acid on markers of bone metabolism following the first cycle of therapy.

Materials and Methods

This prospective study was reviewed and approved by the Institutional Review Board of the University of Michigan and was monitored under the National Cancer Institute–approved Data and Safety Monitoring Plan of the University of Michigan Comprehensive Cancer Center. After providing signed, written informed consent, participants were registered and randomized to treatment. Patients were eligible if they had histologically confirmed adenocarcinoma of the prostate that was metastatic to bone based on a bone scan and had become androgen independent. Androgen independence was demonstrated by the appearance of new lesions on bone scan or computerized tomography and/or an increase in prostate-specific antigen (PSA) of 50% over nadir value on hormone therapy measured on two successive occasions at least 2 weeks apart, and the second PSA value must have been ≥5.0ng/dL. Patients on androgen suppression therapy underwent nonsteroidal antiandrogen withdrawal (4 weeks after withdrawal of flutamide and 6 weeks for biclutamide) with evidence of progression. A Zubrod performance status of 2 or more was required. A ≥50% decline in PSA without evidence of progression by bone or CT scan indicated a responder. Progression was defined as the appearance of new lesions in bone or soft tissue. Worsening of existing bone lesions was not considered criteria for progression. The assessment of response and progression used the consensus criteria described by Bubley and others (1999). There was no progression in measurable disease in those coded as responders.

No concurrent chemotherapy, biologic therapy, or other investigational anticancer therapy was allowed. No prior taxane-based cytotoxic chemotherapy for androgen-independent disease was allowed. Prior to initiation of chemotherapy, a granulocyte count ≥1,500 cells/mm^3, a platelet count ≥100,000 cells/mm^3, and hemoglobin ≥8 gm/dL was required. Patients with a bilirubin greater than the upper limit of normal (ULN) or aspartate transaminase and alanine transaminase ≥1.5× ULN, or serum creatinine ≥1.2× ULN were excluded.

Treatment

Patients were randomized to treatment with either zoledronic acid alone or docetaxel and estramustine for the first cycle of therapy. All subsequent cycles consisted of all three drugs. Docetaxel 70 mg/m^2 was given on day 2 of a 21-day cycle, and estramustine 280 mg was given orally three times per day on days 1–3. Premedication with oral dexamethasone 8 mg by mouth was given every 12 h, beginning prior to each planned dose of docetaxel and continuing for a total of 4 doses over 3 days, Zoledronic acid 4 mg was given intravenously on day 2 for over 15 min and continued monthly. All patients remained on primary androgen ablation during chemotherapy. All patients initially received three courses of therapy.

Bone marker assays

Measurement of bone markers occurred prior to initiating treatment on trial, on day 2 of cycle 2 (prior to initiation of zoledronic acid and docetaxel/estramustine together), and at intervals during treatment and observation. Urinary DpD was measured using an enzyme-linked immunosorbent assay (ELISA) as recommended by the manufacturer (Pyrilinks-D, Metra Biosystems, Mountain View, CA). Urinary creatinine was measured using a chemical assay kit (Metra Biosystems). DpD was then corrected for variations in the urine concentration by normalizing to urine creatinine values. Results are reported as DpD (nmol/mmol creatinine). BAP was measured by an enzyme immunoassay (EIA) (Alkphase-B, Metra Biosystems). Intact OCN was measured by competitive EIA (NovoCalcin, Metra Biosystems). IL6, RANK, OPG, and TRAPC were measured by commercially available ELISAs (Quantikine HS human IL-6, R&D Systems; sRANKL, ALPCO Diagnostics; OPG, ALPCO Diagnostics; Bone TRAP Assay, IDS).

Statistics

The relative difference in each bone marker (IL-6, DpD, TRAPC, BAP, OCN, OCN, and OPG) was the unit of analysis. This adjusts for the baseline differences with negative numbers representing the percent decrease from baseline and positive numbers representing the percent increase from baseline. The actual difference from baseline was used to analyze RANKL due to a majority of baseline values being zero. The median and range for each marker is reported by initial treatment group, zoledronic acid versus docetaxel/estramustine, and by responder versus nonresponder. Medians were used as the data were skewed and a mean value would not be representative of the data. Comparisons were tested using the Wilcoxon Rank test. In the same manner, baseline values of each bone marker were analyzed by responder versus nonresponder. All analyses were performed using SAS 9.1 (SAS Institute, Cary, NC) statistical software.

Results

Patient characteristics

The average patient was 68 years old (range 50–81) and Caucasian (96%). The majority of patients had bone metastases without visceral or lymphatic metastases detected (68%).

Additionally, most patients had attempted at least two different hormone manipulations prior to enrollment (range 1–4). Fifty percent of the patients had received a radical prostatectomy.

Fourteen of the 28 enrolled patients (50%) demonstrated a ≥50% decline in PSA levels following a total of three cycles of therapy and were therefore classified as responders to chemotherapy. The median PSA level was 89 ng/mL (range 0.4–2620) and the median Gleason score was 8 (range 6–10). Fourteen patients were given zoledronic acid and 14 patients were given estramustine/docetaxel plus dexamethasone for the first cycle. After the first cycle, all patients received both treatments.

Bone marker data are available for 26 of the 28 patients at baseline and after the first treatment cycle. After treatment cycle 3, only 17 patients remained on the trial, most of which were responders. Therefore, any data after cycle 3 cannot be used to draw conclusions about response due to withdrawal from the study of mostly the nonresponsive patients.

Biomarkers

To determine whether therapy had an effect on bone markers, the change in each bone marker between baseline and after the first cycle of therapy was examined for each treatment group. Both zoledronic acid (Z) alone and the combination of docetaxel/estramustine (DE) alone resulted in a decline of OCN and TRAPC (Table 1). However, there was no significant difference in the median change in any of the measured bone markers in patients that received Z versus DE. Z alone had no impact on PSA levels; whereas DE did decrease PSA levels (Table 1). This resulted in a significant difference in PSA response between the Z versus DE groups.

Response was determined as a decline in PSA levels by >50% after three cycles. The number of responders and nonresponders was similar in each treatment group, 8 responders and 6 nonresponders, indicating that the initial treatment alone did not account for response.

To determine if pretreatment levels of biomarkers could be used to predict response, values at baseline were compared between responders and nonresponders. There was no significant difference for any bone marker or PSA level between baseline values for responders versus nonresponders (Table 2).

To determine whether therapy-induced changes of bone marker levels could predict response to chemotherapy, we compared the changes in bone marker levels from baseline levels to after the initial cycle of therapy between responders and nonresponders. In patients who ultimately responded to therapy, IL-6 levels decreased by 35% compared to the nonresponders, whose IL-6 levels increased by 76% (p value = 0.03) (Fig. 1). Additionally, there was a trend (p value = 0.09) for OCN levels to decrease (40% decline) in responders with no change in the value observed in the nonresponders (not shown). There were no significant changes for any of the other bone remodeling markers.

A previous study had shown that IL-6 levels correlated with the extent of disease (EOD) based on bone scan (Akimoto and others 1998).

To determine if IL-6 correlated with EOD on bone scan in this study, we classified the patients based on a modification of Soloway's classification system (Soloway and others 1988). In the original system, there were 5 grades as follows: 0, normal; 1, number of bony metastases less than six, each of which is <50% the size of a vertebral body (one lesion about the size of a vertebral body would be counted as two lesions); 2, number of bone metastases between 6 and 20, size of lesions as described above; 3, number of metastases more than 20 but less than a "super scan"; and 4, "superscan" or its equivalent, i.e., >75% of the ribs, vertebrae, and pelvic bones. We modified this as we felt the difference between grade 3 and 4 was rather arbitrary and could not be consistently decided. Thus, we classified the patients on 4 grades as follows: 0, normal; 1, ≤5 lesions; 2, 6–20 lesion; 3, >20 lesions. As the selection criteria for this study was the presence of a positive bone scan, there were no grade 0 scans. The patients were distributes as follows: Grade 1 = 11 patients; Grade 2 = 9 patients; Grade 3 = 8 patients. There was no correlation with response and EOD. In contrast, IL-6 levels were elevated in the Grade 3 patients compared to Grade 1 and 2 patients (Fig. 2).

Table 1. Median Change Relative to Pretreatment in Bone Markers after Cycle 1 of Zoledronic Acid or Docetaxel/Estramustine

	Zoledronic acid		*Docetaxel/estramustine*		
Bone marker[a]	*Number*	*Median (range)*	*Number*	*Median (range)*	*p value*
BAP	13	−0.04 (−0.33, 1.31)	13	−0.15 (−0.59, 0.47)	0.58
DpD	12	0.40 (−0.97, 35.4)	13	0.0 (−0.98, 20.7)	0.81
IL-6	13	−0.12 (−12.2, 4.2)	13	0.15 (−0.83, 3.5)	0.51
OCN	13	−0.16 (−0.43, 63.4)	13	−0.43 (−0.68, 1.05)	0.11
OPG	13	0.10 (−0.38, 0.64)	12	0.06 (−0.48, 1.44)	0.73
TRAPC	13	−0.46 (−1.0, 0.25)	13	−0.39 (−1.1, 2.0)	0.27
RANKL*	13	−0.004 (−0.21, 8.0)	13	−0.01 (−0.19, 1.31)	0.76
PSA	13	0.0 (−0.84, 2.1)	13	−0.28 (−0.77, 0.09)	0.01

[a]Interleukin-6 (IL-6), urinary deoxypyridinoline to serum creatinine ratio (DpD), tartrate-resistant acid phosphatase (TRAPC), bone-specific alkaline phosphatase (BAP), intact osteocalcin (OCN), and osteoprotegerin (OPG), ligand for receptor activator of nuclear-factor κB (RANKL), prostate-specific antigen (PSA). *Values represent actual difference rather than relative difference.

Table 2. Baseline Values Between Responders and Non-responders

	Responders		*Nonresponders*		
Bone marker[a]	*Number*	*Median (range)*	*Number*	*Median (range)*	*p value*
BAP	14	46.7 (16.4, 359.0)	7	52.6 (29.8, 378.0)	0.63
DpD	14	6.8 (1.3, 86.3)	7	51.1 (2.2, 422.8)	0.18
IL-6	14	3.0 (−0.86, 9.7)	7	1.8 (−0.84, 17.2)	0.26
OCN	14	15.1 (6.1, 37.4)	7	12.4 (7.6, 45.7)	0.58
OPG	14	4.1 (0, 10.6)	7	6.93 (2.7, 9.5)	0.09
TRAPC	14	4.5 (0.88, 18.9)	7	5.4 (2.0, 17.6)	0.69
RANKL	14	0.03 (−0.14, 0.23)	7	0.07 (−0.05, 0.09)	0.88
PSA	14	99.3 (8.1, 2620)	8	75.7 (0.4, 815)	0.61

[a]Same abbreviations as in Table 1.

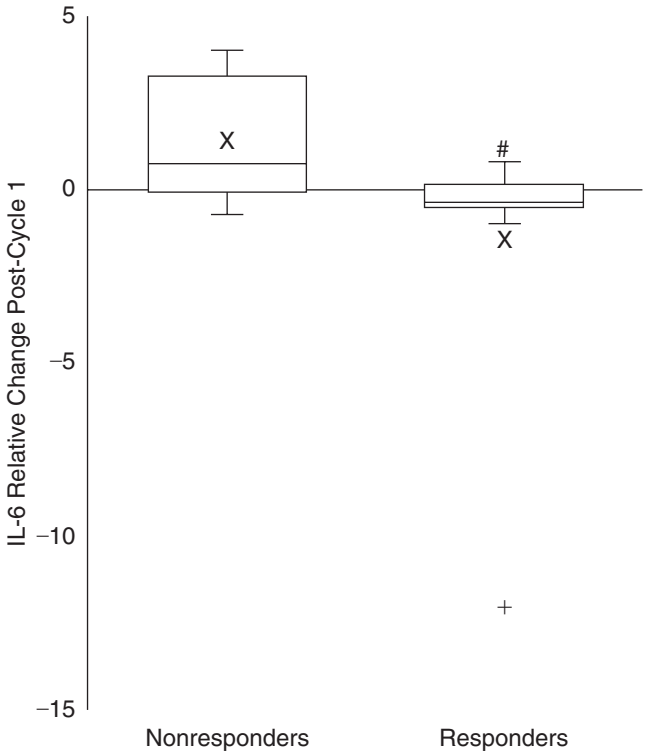

FIG. 1. Changes in serum IL-6 levels predict response to therapy. Bone scan positive patients with prostate cancer were given either zoledronic acid (Z) alone or the combination of docetaxel and estramustine (DE) for an initial cycle followed by two additional treatment cycles of all three drugs. Serum IL-6 levels were measured pretreatment and postinitial cycle. Prostate-specific antigen (PSA) was measured pretreatment and at each cycle. A ≥50% decline in PSA without evidence of progression by bone or CT scan was considered a responder. Results are reported as relative change from the baseline (pretreatment) value. Data are shown as a whisker-box plots based on response. The bottom of the box is the 25th percentile, the line through the box is the median, the top of the box is the 75th percentile, the top whisker cross bar is the maximum value in the upper quartile range, the bottom whisker cross bar is the minimum value in the lower quartile range, x = mean, + = outlier and $^{\#}p = 0.03$ versus nonresponders.

Discussion

Indices of bone remodeling are upregulated in men with AIPC as most of these men have bone metastasis. Zoledronic acid is used to slow progression of bone disease associated with AIPC; however, evaluation of the activity of zoledronic acid versus an AIPC chemotherapy regimen has not been studied. Therefore, we determined bone marker levels in patients that were randomized to zoledronic acid infusion or docetaxel/estramustine for one cycle of treatment. There were no differences in the change in any marker of bone metabolism when comparing the two groups. These data suggest that chemotherapy with docetaxel and estramustine may result in the stabilization of bone lesions to a degree similar to that seen with zoledronic acid. This raises the question of whether there is any benefit to using both approaches in this population. Alternatively, there may be an additive effect. These questions will need to be resolved in subsequent studies.

In our study, the median DpD level was slightly elevated at baseline (data not shown); however, we also found that urinary DpD levels did not significantly decrease in patients given zoledronic acid alone for the first cycle. These data suggest that measures of bone resorption may not be the appropriate way to follow PCa progression. Our data are in contrast to the decrease in type I collagen C-telopeptides (urine assay of bone resorption) observed after administration of bisphosphonates in patients with metastatic PCa(Garnero and others 2000a). The contrasting data could be due to the time point we used, being too early in treatment, to see a difference in NTx, a component of the DpD assay.

We also compared the median change in bone markers in patients who responded to combination chemotherapy to those who did not. Changes in two serum markers differed in this comparison. IL-6, an inflammatory cytokine, has been implicated by our group in PCa growth, progression, and androgen independence (Dattoli and others 2007). IL-6 is a target gene of nuclear factor κB (NF-κB), a transcription factor implicated in PCa progression and biochemical relapse (Karin and others 2004; Ross and others 2004; Le Page and others 2005; Domingo-Domenech and others 2006). Docetaxel induces NF-κB and IL-6 in PCa cell lines (Domingo-Domenech and others 2006); however, we observed that IL-6 significantly decreased in responders and

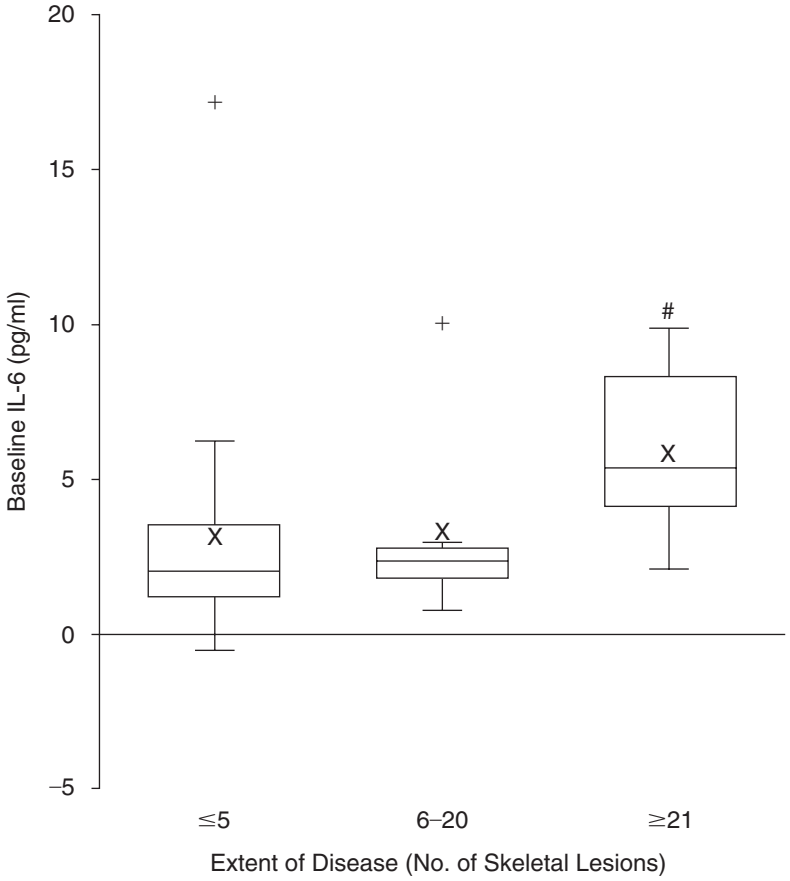

FIG. 2. The extent of bone metastatic disease correlates with serum IL-6 expression. Patients were classified into the categories indicated for their extent of disease based on bone scans. Baseline (pretreatment) serum IL-6 levels were measured. Results are reported as median IL-6 level (pg/mL). Data are shown as a whisker-box plots based on response. The bottom of the box is the 25th percentile, the line through the box is the median, the top of the box is the 75th percentile, the top whisker cross bar is the maximum value in the upper quartile range, the bottom whisker cross bar is the minimum value in the lower quartile range, x = mean, + = outlier and $^{\#}p = 0.04$ versus ≤5.

increased in nonresponders regardless of the initial treatment (Fig. 1). The76 % increase observed in IL-6 levels seen in those patients who are ultimately nonresponders may be indicative of increased disease burden, taxane-resistance, or more aggressive PCa biology. The increase in IL-6 levels in nonresponders, and decrease in responders was also evident in those who were given docetaxel/estramustine alone for the first cycle (data not shown). These data indicate that following IL-6 levels in patients on taxane-based regimens may be a useful way to predict emerging chemotherapy resistance.

One potential confounding factor is that dexamethasone has been shown to decrease IL-6 (Ray and others 1990). However, because there were the same number of responders and nonresponders in both the Z only and the DE treatment groups, the addition of dexamethasone in the DE treatment arm for the first cycle did not account for the decline of IL-6 observed in these patients.

We also compared the baseline IL-6 values between responders and nonresponders, but they were not significantly different. Although not statistically significant, these data indicate that responders typically started with high IL-6 levels that dropped dramatically with one cycle of treatment; whereas, nonresponders started with lower baseline IL-6 levels that didn't drop as much or increased. However, due to the small number of patients in our study, we could not pinpoint a cutoff value for "high" and "low" baseline IL-6. Additionally, we found that the IL-6 levels post-cycle 3 were similar to those post-cycle 1. Specifically, there was not a significant difference in IL-6 at cycle 1 versus cycle 3 ($p = 0.14$), thus indicating the results are consistent over the duration of the study.

One small study obtained IL-6 levels before and after one cycle of docetaxel treatment (Domingo-Domenech and others 2006). In that study, it was shown that high levels of baseline IL-6 were associated with poor response to docetaxel treatment and that 16 pg/mL of IL-6 was the transition point between high and low IL-6 levels. In our study, we have only one patient with a baseline IL-6 level higher than 16 pg/mL. Therefore, our cohort of patients all had serum IL-6 levels in the range of the responders in that previous report except for one. Our data suggests that within the group of patients with IL-6 levels below 16 pg/mL there are subsets of patients that are responders and nonresponders and that the change in IL-6 levels in response to therapy can predict which group these patients are in.

Intact OCN, a marker of bone formation, may also be an early indicator of response. OCN decreased by 40% after the first cycle of therapy in those who ultimately were determined to be responders to at least three cycles of chemotherapy. When compared to the nonresponders, who had no reduction in OCN, the change in OCN between baseline and the end of the first cycle was near statistical significance. ($p = 0.09$). As skeletal metastases in PCa are osteoblastic, or bone forming, this observed decrease may reflect a reduction in bone disease in responders to chemotherapy. If confirmed in a larger sample size then measurement of intact OCN may offer a more reliable way to assess bone turnover in osteoblastic bone disease.

A number of other studies have been published studying bone turnover markers as a sign of disease (Garnero and others 2000b; Jung and others 2004) or progression (Tamada and others 2001; Pectasides and others 2005; Chen and others 2006; Cook and others 2006; Johansen and others 2007; Scher and others 2007). Studies on the presence of disease show that BAP or NTx, another collagen breakdown product, correlate with survival. And studies on disease progression suggest that NTx correlates with type and bulk of bone disease. Taken together, these data indicate that bone markers are a sign of progression. A recent paper by Lein and others (2007) studied six bone markers after patients started zoledronic acid treatment for metastatic bone disease. The data presented in that paper (Lein and others 2007) did not show a significant correlation between baseline values of the bone markers and progression, but did show a significant association of increased bone markers (not including CTx) over the course of the study to disease progression. Our study adds another dimension to the use of bone markers to predict outcome.

We would like to note that multiple comparisons adjustments were not used for analysis of the bone markers by response as this was a secondary end point of this study. We were looking to detect a signal that would allow us to potentially detect patients who are likely to respond. The study was not powered to examine bone markers as an index of response as a primary end point.

The data we present here are the first to describe the changes in bone markers with chemotherapy for prostate cancer and compare them to those seen with bisphosphonates. These are also the first data to show an association between a change in serum IL-6 and response. By measuring multiple markers of bone turnover in response to chemotherapy, we may be able to validate a novel way to follow disease progression or chemotherapy resistance in future chemotherapy trials. These studies provide a strong rationale to validate changes in IL-6 levels as an early indicator of response in a larger prospective clinical trial. If these results are validated in a larger trial, then IL-6 response could serve as an early indicator of response to therapy and rises in IL-6 during early therapy would suggest that chemotherapy is not working and an alternative therapy should be used.

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