Review

Failure to Suppress Markers of Bone Turnover on First-Line Hormone Therapy for Metastatic Prostate Cancer Is Associated With Shorter Time to Skeletal-Related Event

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Abstract

Biomarkers to identify patients with metastatic prostate cancer who are destined to have a shorter response to testosterone suppression are limited. In a cohort of 63 patients, failure to suppress markers of bone turnover while receiving therapy was associated with a shorter time to progression. This suggests that more durable anti−prostate cancer activity is associated with less cancer-associated bone turnover.

Background: Elevated markers of bone turnover are prognostic for shorter survival in castration-resistant prostate cancer. We aimed to determine the prognostic value of bone turnover markers in metastatic hormone-sensitive prostate cancer. **Patients and Methods:** Markers of bone turnover (urine deoxypyridinoline [DPD] and N-telopeptide, serum bone alkaline phosphatase (AP), and osteocalcin [OC]) from baseline and after 6 months of study were assessed in men enrolled in a prospective metastatic prostate cancer trial with androgen deprivation therapy (ADT) with or without risedronate (ClinicalTrials.gov, NCT00216060). **Results:** Serum samples were collected from 63 patients with bone involvement and a median follow-up of 39.7 months. A multivariate model using Cox regression—which included prostate-specific antigen (PSA) nadir, bisphosphonate treatment, and extent of metastases—showed that suppression of bone turnover markers after 6 months of therapy compared with baseline was significantly associated with longer skeletal-related event (SRE)-free survival. ADT without bisphosphonate therapy was also associated with a decline in markers of bone turnover, presumably resulting from direct anticancer activity. Elevated baseline bone turnover markers were not prognostic. **Conclusion:** Failure to suppress bone turnover while receiving ADT, even when otherwise responding to therapy, may identify patients with hormone-sensitive metastatic prostate cancer who are destined for a shorter time to SREs and progression.

Introduction

Prostate cancer afflicts approximately 240,000 men per year in the United States and causes about 33,000 deaths.[1] Bone metastases occur in about 90% of patients with metastatic prostate cancer and cause significant morbidity from pain, pathologic fractures, and spinal cord compression.[2] Although effective for palliation of symptoms and control of prostate cancer growth, androgen deprivation therapy (ADT) as medical or surgical castration with or without androgen receptor inhibition increases bone turnover and reduces bone mineral density.[3] In patients with metastatic castration-resistant prostate cancer (CRPC), intravenous administration of the bisphosphonate zoledronic acid and the receptor activator of nuclear factor-κB ligand inhibitor denosumab decrease the rate of cancer-related SREs compared with placebo,[4,5] whereas pamidronate did not confer a benefit in CRPC.[6]

Regarding patients with metastatic prostate cancer who are beginning ADT, long-term follow-up of a study by Dearnaley et al

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demonstrated that oral clodronate, when compared with placebo, prolonged time to progression and overall survival (5-year overall survival, 30% vs. 21%; $P = .03$).[7] Data on SREs were not presented in this study; however, an improvement in serum total alkaline phosphatase (AP) levels was noted after treatment in the clodronate arm.[8] Follow-up phase III studies in the same patient population are ongoing to assess the ability of zoledronic to delay SREs (Cancer and Leukemia Group B [NCT00079001] and MRC [NCT002684760]. In addition to prostate cancer increasing bone turnover, such turnover is also increased by ADT itself. Numerous studies have shown that bisphosphonates prevent ADT-induced bone loss (measured by dual-energy x-ray absorptiometry) and decrease ADT-induced increase of markers of bone turnover (alkaline phosphatase [AP], osteocalcin [OC], deoxypyridinoline [DPD], N-telopeptide [NTX]).[9-11]

The benefit that is seen with zoledronic acid and denosumab in CRPC, but is not seen with pamidronate, is presumed to result from the lower potency of the latter; zoledronic acid is 150 to 850 times more potent than pamidronate.[12] The notion that greater suppression of bone turnover would be beneficial is supported by observations that robust bone turnover (elevated markers of bone turnover) is associated with more SREs and a shorter time to progression in patients with bone metastases emanating from CRPC, non—small-cell lung cancer, breast cancer, and myeloma.[13,14] Conversely, normalization of bone markers within 3 months of bisphosphonate therapy for patients with breast cancer or multiple myeloma correlated with decreased risks of a first SRE, disease progression in bone, and death.[15]

The impact of bone turnover in patients beginning ADT for metastatic disease is not well characterized. The net effect of ADT on bone turnover is speculated to be complicated by competing effects. Namely, although ADT itself increases bone turnover, the anticancer effect of ADT will also decrease tumor burden, which may in turn decrease bone turnover and delay progression and SREs when compared with no therapy. Effective direct anticancer hormone therapy with abiraterone in CRPC has been shown to decrease SREs.[16] In this article, we detail the association between time to SREs and changes of markers of bone turnover after commencing ADT for metastatic prostate cancer.

Patients and Methods

Patients

Samples were collected from patients enrolled in a prospective clinical trial (ClinicalTrials.gov, NCT00216060) after approval by the institutional review boards of participating centers. All patients were ≥ 18 years of age, had provided written informed consent before study registration, and were required to have histologically or cytologically documented prostate adenocarcinoma with radiographic (computed tomographic [CT] scan or bone scan) evidence of bone metastases, an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 2, and adequate organ function. Patients were excluded if they had diseases of abnormal bone metabolism (including Paget disease, untreated hyperthyroidism, untreated hyperprolactinemia, untreated Cushing disease), or had undergone > 4 months of adjuvant hormone therapy, previous hormone therapy for biochemical-only disease, or hormone therapy within 12 months of study except when used

within 1 month for the management of metastatic disease. Patients in this study were allowed to receive only the bisphosphonate risedronate 30 mg daily or matching placebo. Randomization stratification variables included age ≥ 70 years vs. < 70 years, ECOG PS 0 to 1 vs. 2, and minimal vs. extensive metastatic disease. Minimal disease was defined as metastases limited to the pelvic and axial skeleton, and extensive disease was defined as metastases involving the appendicular skeleton or visceral metastases. The trial was slow to accrue and closed after 63 of a planned 360 patients were enrolled.

All patients were treated with ADT in the form of luteinizing hormone—releasing hormone agonist therapy or surgical castration. Combined androgen blockade with the use of antiandrogens in addition to ADT was at the discretion of the treating physician. Antiandrogen therapy as monotherapy was not allowed, nor was intermittent ADT. Treatment with risedronate/placebo on both arms was continued until the occurrence of SREs, serologic progression without symptoms, symptomatic progression of bone disease, unacceptable toxicity, or death. In addition to risedronate/placebo, all patients were treated with oral calcium carbonate 500 mg per day with at least 400 IU of vitamin D per day.

Patient and Disease Evaluations

Baseline CT scan of the abdomen and pelvis, chest radiograph or CT scan, bone scan, lumbosacral spine radiograph, and tumor measurements were recorded within 28 days of registration. History and physical examination, ECOG PS, laboratory measurements (including serum prostate-specific antigen [PSA] and baseline toxicity grading per National Cancer Institute NCI Common Terminology Criteria for Adverse Events, version 3.0) were collected within 14 days before registration and every 12 weeks thereafter while in the study. Imaging studies were repeated every 12 weeks. A 10-mL urine sample (second specimen in the morning between 5 AM and 8 AM after an overnight fast, collected at home, and stored at 4°C until clinic visit) and 2 separate 10-mL whole blood samples processed for serum were collected before therapy initiation, after 24 weeks of treatment, and at the occurrence of an SRE. Markers of bone turnover included DPD, urine NTX, urine creatinine, serum bone alkaline phosphatase (BAP), and serum OC. Details of the assay methods are provided in the Supplementary Data.

All tumor responses were assessed per Response Evaluation Criteria in Solid Tumors. An SRE was defined as the occurrence of any of the following: a pathologic fracture, spinal cord compression, palliative radiation or surgery to bone, an increase in pain medication dose by 20 mg intramuscular morphine equivalence or a doubling of morphine equivalence from baseline, or an asymptomatic vertebral compression fracture. Treatment failure with hormone therapy was defined as the occurrence of either disease progression per Response Evaluation Criteria in Solid Tumors or the occurrence of an SRE. Serologic response and progression was assessed per the Prostate Cancer Working Group.[17]

CRPC was defined as (1) progressive serologic disease, progressive measurable disease, or progressive nonmeasurable disease (eg, symptomatic progression of bone disease or new pleural effusion resulting from progressive cancer) and (2) development of a cancer-related SRE, which must have been documented by an appropriate

Table 1 Patient Demographics

Variable	Placebo n = 31	Risedronate n = 32	Total
	Treatment Arm		
Median Age, years (interquartile range)	71.0 (63.8-76.0)	70.5 (62.5-78.5)	—
Median Baseline PSA (ng/mL) (interquartile range)	35.1 (17.7-274.9)	32.2 (11.4-88.7)	—
	n (%)	n (%)	—
Race			
White	29 (93.6)	30 (93.8)	59 (93.7)
African American	2 (6.4)	2 (6.2)	4 (6.3)
Previous Adjuvant Hormone Therapy			
Yes	5 (16.1)	2 (6.2)	7 (11.1)
No	26 (83.9)	30 (93.8)	56 (88.9)
ECOG PS			
0	19 (61.3)	26 (81.3)	45 (71.4)
1	10 (32.3)	4 (12.4)	14 (22.2)
2	2 (6.4)	2 (6.3)	4 (6.4)
Disease Extent			
Minimal	11 (35.5)	11 (34.4)	22 (34.9)
Extensive	20 (64.5)	21 (65.6)	41 (65.1)
Comorbidity Score			
None	3 (9.7)	6 (18.9)	9 (14.3)
Mild	11 (35.5)	7 (21.9)	18 (28.6)
Moderate	11 (35.5)	12 (37.5)	23 (36.5)
Severe	5 (16.2)	3 (9.5)	8 (12.6)

ECOG PS = Eastern Cooperative Oncology Group performance status; PSA = prostate-specific antigen.

radiographic study, and included any radiation to bone including radiopharmaceuticals (eg, samarium) to palliate painful lesions, radiation to treat or prevent fractures or spinal cord compression (each portal of radiation was considered a separate SRE), or surgical procedures to treat or prevent pathologic fractures or spinal cord compression.

Statistical Considerations

An association between an individual factor with time to SRE or CRPC was assessed using a univariate analysis. Individual factors considered were bisphosphonate treatment, ECOG PS, PSA nadir as a continuous variable, extent of metastases, and markers of bone turnover. Having identified the variables associated with time to SRE, a multivariable Cox regression analysis was performed. Comparisons of hazard ratios resulting from the univariable (unadjusted) or multivariable (adjusted) models were compared by Wald tests. Changes in bone turnover from baseline to 6 months on therapy were assessed by the Wilcoxon signed rank test.

Results

Patients

From December 8, 2003 through August 26, 2005, 63 patients were enrolled (Fig. S1) and had a median follow-up of 39.7 months. The median age was 71 years, with a median PSA of 33.2 ng/mL. The majority of patients were white, had no previous history of adjuvant hormone therapy, had extensive metastatic disease, and were not symptomatic at the time of registration. Patient demographic characteristics were well balanced between patients receiving bisphosphonate and those receiving placebo (Table 1).

SREs and Survival

Confirmed SREs occurred in 16 of 63 patients (25.4%), 8 each of patients receiving a bisphosphonate and those receiving a placebo. Cumulatively, SRE or death occurred in 24 (38%.1) patients—11 patients taking risedronate and 13 patients taking placebo (Fig. S2A). There were 7 deaths on both arms during the study (Fig. S3A), and overall survival was significantly worse in patients with moderate or severe comorbidity indices at baseline as assessed by the Adult Comorbidity Evaluation (ACE)-27 index[18] compared with those with mild or no comorbidities (3-year overall survival was 62.3%; 95% CI, 44.9-86.3%) vs. 77.5% (95% CI, 56.7-100%; $P = .04$) (Fig. S3B).

Changes in Bone Turnover Markers

Results of bone turnover marker changes in response to treatment on both study arms are summarized in Table 2A. Baseline urine

Table 2A Bone Turnover Markers by Exposure to Bisphosphonate

Marker	Placebo n = 31	Residronate n = 32	P Value[a]
Baseline Median			
Urine total DPD (nmol/mmol creatinine)	10.11 (n = 26)	8.88 (n = 24)	.67
Urine NTX (nmol BCE/mmol creatinine)	48.08 (n = 26)	41.33 (n = 24)	.48
Serum BAP (ng/mL)	12.8 (n = 26)	13.04 (n = 25)	.87
Serum OC (μg/L)	18.24 (n = 25)	20.08 (n = 24)	.48
24-wk Median			
Urine total DPD (nmol/mmol creatinine)	12.62 (n = 22)	6.91 (n = 22)	< .001
Urine NTX (nmol BCE/mmol creatinine)	12.8 (n = 19)	6.51 (n = 20)	< .001
Serum BAP (ng/mL)	13.16 (n = 17)	9.5 (n = 19)	.010
Serum OC (μg/L)	27.35 (n = 18)	11.88 (n = 19)	< .001

Abbreviations: BAP = bone alkaline phosphatase; BCE = bone collagen equivalents; DPD = deoxypyridinoline; NTX = N-telopeptide; OC = osteocalcin.
[a]Kruskal-Wallis test.

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DPD, urine NTX, serum BAP, and serum OC levels were similar in both the bisphosphonate and placebo arms. After adjusting for baseline levels, week 24 markers of bone turnover were significantly lower on the risedronate arm (DPD, $P = .002$; NTX, $P < .001$; BAP, $P = .010$; OC, $P < .001$). Intraindividual changes in markers of bone turnover over time (Fig. 1) show that some patients on ADT alone also had declines in markers of bone turnover (Table 2B). This is presumably because of regression of the cancer and a decrease of overall bone turnover. In the patients treated with ADT alone, serum BAP was the only marker to decrease significantly by week 24 ($P = .0002$).

Bone Turnover Markers as Prognostic Markers of Time To SRE and CRPC

Three analyses of the markers of bone turnover were performed: (1) dichotomizing bone turnover markers as above or below the median at baseline (51 patients), (2) dichotomizing bone turnover markers as above or below the median at week 24 (41 patients), and (3) dichotomizing bone turnover markers at week 24 as above or below baseline median bone turnover levels (41 patients). Patients were considered to have "low bone turnover" when all 4 markers were less than the target median. On the multivariate analysis model (Table 3), patients with all 4 markers of bone turnover below the baseline median at 24 weeks had a statistically significantly longer SRE-free survival ($P = .038$; adjusted hazard ratio, 0.22; 95% CI, 0.05- 0.923) as did patients with a lower PSA nadir. Figure 2, A and

Table 2B	Changes in Bone Turnover in Patients With Bone Metastases Treated With Androgen Deprivation Therapy Alone		
Bone Turnover Marker	**Median Baseline**	**Median Responding**	**Wilcoxon Signed Rank Test**
Serum BAP n = 16	28.8 Range, 8.91-1194.38	13.05 Range, 7.35-197.93	.0002
Serum OC n = 17	23.95 Range, 10.12-149.40	23.91 Range, 8.91-177.40	.854
Urine DPD n = 19	10.12 Range, 2.98-111.11	12.77 Range, 6.03-59.53	.515
Urine NTX n = 17	48.08 Range, 17.05-3031.00	62.95 Range, 17.80-494.55	.208

Abbreviations: BAP = bone alkaline phosphatase; DPD = deoxypyridinoline; NTX = N-telopeptide; OC = osteocalcin.

B show the results of univariate analyses of time to SRE and CRPC by failure to suppress all 4 markers of bone turnover below the baseline median at 24 weeks. This also shows that suppression of all 4 markers below the baseline median at 24 weeks was associated with longer time to CRPC. Notably, elevation of baseline markers of bone turnover above the median at baseline and week 24 levels

| Figure 1 | Intraindividual Changes of Markers of Bone Turnover Over Time. Levels of Markers of Bone Turnover at Baseline and After 6 Months (and When Available, at Time of Progression to Castration-Resistant Prostate Cancer [CRPC]). Each Paired Sample Represents 1 Unique Patient and Demonstrates Change in Marker of Bone Turnover on Androgen Deprivation Therapy (ADT) Alone or ADT Plus Risedronate. Markers of Bone Turnover Included Urine Deoxypyridinoline (DPD), Urine N-Telopeptide (NTX), Serum Bone Alkaline Phosphatase (BAP), and Serum Osteocalcin |
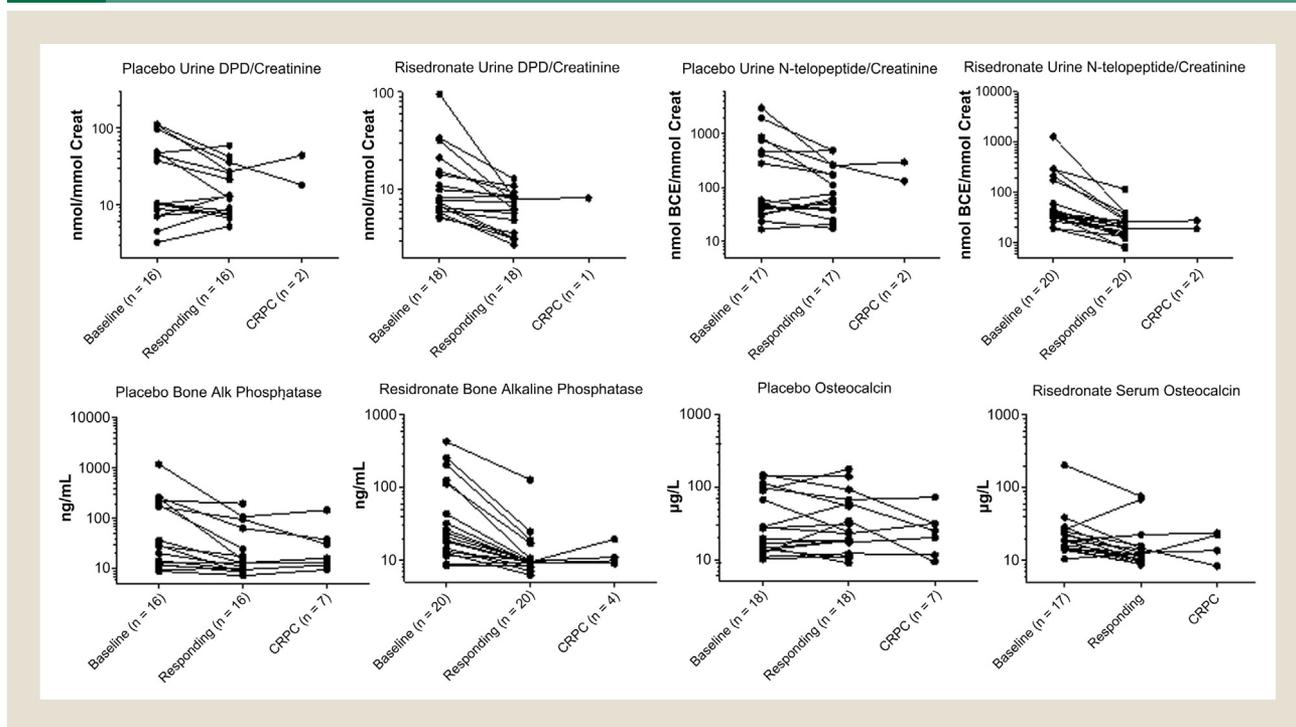
Abbreviation: Alk = alkaline.

Table 3	Mutlivariate Analysis and Time to SRE	
Variable	**Hazard Ratio (95% CI)**	**P Value[a]**
Time to SRE		
Bone Turnover Markers at Week 24	0.22 (95% CI, 0.05-0.923)	
Above/below baseline median bone turnover levels	4-fold decrease risk of SRE if bone turnover is suppressed	.038
PSA Nadir	1.05 (95% CI, 1.00-1.10)	
Continuous variable	(better outcome with lower nadir)	.04
Treatment Arm		
Residronate vs. placebo	2.08 (95% CI, 0.51-8.40)	.3
Extent of Bone Metastases		
Extensive vs. minimal	1.33 (95% CI, 0.31-5.57)	.69
ECOG Performance Status		
0 vs. 1	0.71 (95% CI, 0.15-3.22)	.95

Abbreviations: CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; SRE = skeletal-related event.
[a]Wald test.

above the 24-week median were not associated with SRE-free or CRPC-free survival (Fig. 2, A and B).

The analyses were then restricted to urine and serum markers of bone turnover separately, using only a univariate method. Low bone turnover for urine markers was defined as both urine markers below the respective median. Low bone turnover for serum markers was defined as both serum markers suppressed below the respective median. In both cases, low serum and low urine markers at the week 24 mark relative to the appropriate baseline median were also associated with a longer time to SRE and CRPC (Fig. 3 A-D, left panels). Low baseline serum markers of bone turnover relative to baseline median level and low 24-week levels relative to 24-week median levels were not associated with SRE-free or CRPC-free survival (Fig. 3, A and B, middle and right panels). Low baseline urine markers of bone turnover relative to baseline median were not associated with longer time to SREs but were associated with longer time to CRPC (Fig. 3, C and D, middle panels). Low levels at 24 weeks relative to the median at 24 weeks were not associated with SRE-free or CRPC-free survival (Fig 3, C and D, right panels).

PSA Nadir and Time to Cancer Event Outcomes

Fifty percent of patients on the bisphosphonate arm achieved a PSA nadir < 0.2 ng/mL compared with 29% of patients on the placebo arm (50% vs. 29%; $P = .12$); median time to CRPC was 32.2 months in the risedronate arm vs. 17.4 months in the placebo arm ($P = .15$), and median time to serologic progression was 23.4 months in the risedronate arm vs. 14.2 months in the placebo arm ($P = .42$). Three-year survival was 72.5% (95% CI, 55.1%-89.9%) and 71.5% (95% CI, 51.5%-91.5%) in the risedronate and placebo arms, respectively (Fig. S3A).

Discussion

Although new-generation hormone therapies, immunotherapy, and chemotherapy have led to improvements in overall survival for patients with metastatic CRPC, more work is to be done.[19-23] As detailed earlier, in CRPC it is well characterized that inhibition of osteoclast bone resorption with potent bisphosphonates and receptor activator of nuclear factor-κB ligand inhibitors reduces SRE

morbidity, whereas elevated markers of bone turnover at baseline are associated with a greater rate of SREs.[4,5,13,14] Moreover, the suppression of markers of bone turnover is associated with better cancer outcomes.[15] In contrast, the impact of markers of bone turnover in men beginning ADT for metastatic disease is poorly characterized.

The current study presents data from a uniform patient population with standardized follow-up in a prospective trial. Notable findings are the following: Unlike patients treated with ADT in the absence of bone metastases, markers of bone turnover do not increase with ADT alone in patients with bone metastases, and in the case of bone-specific AP, they go down significantly with ADT alone. This is presumably because of a decrease of total bone turnover, with cancer regression caused by the direct anticancer activity of ADT. Also, it is noted that suppression of bone turnover markers after 6 months of therapy compared with baseline markers appears to be prognostic for better cancer control (time to CRPC) and a longer time to SREs. Notably, this was independent of bisphosphonate use (treatment arm). As such, suppression of markers of bone turnover on therapy may be an early biomarker for assessing long-term efficacy of anti−prostate cancer therapy in patients with metastatic disease to bone, regardless of whether or not it is a bone-targeted therapy. It is also of note that suppression of serum markers of bone turnover, easier to obtain than fasting urine specimens, was prognostic. If confirmed in other studies, this analysis may indicate that lower bone turnover is a marker of less cancer growth/activity at the subclinical level (ie, not appreciated by scans, PSA, and other measures). Moreover, it could be used as an early biomarker of treatment efficacy and aid drug development. These data are supported by a study of 30 men with metastatic disease (either CRPC or hormone-sensitive disease) who were treated with zoledronic acid; it showed that failure to suppress type 1 collagen or BAP, compared with baseline levels, was associated with a shorter time to SRE.[24] The potential of these markers of bone turnover to be surrogates of therapeutic activity is also underscored by the observation that improvements in levels of NTX and BAP have been associated with reductions in SREs in a pooled analysis in 2 phase III randomized zoledronic acid trials totaling

Figure 2 (A and B) Time to Skeletal-Related Event (SRE) and Castration-Resistant Prostate Cancer (CRPC) and Relationship to Markers of Bone Turnover. Three Analyses of the Markers of Bone Turnover Were Performed: Left Panels, Dichotomizing Bone Turnover Markers at Week 24 as to Whether They Were Above or Below Baseline Median Bone Turnover Levels (41 Patients); Middle Panels, Dichotomizing Bone Turnover Markers as Above or Below the Median at Baseline (51 Patients); Right Panels, Dichotomizing Bone Turnover Markers as Above or Below the Median at Week 24 (41 Patients). Patients Were Considered to Have "Low Bone Turnover" When all 4 Markers Were Below the Target Median
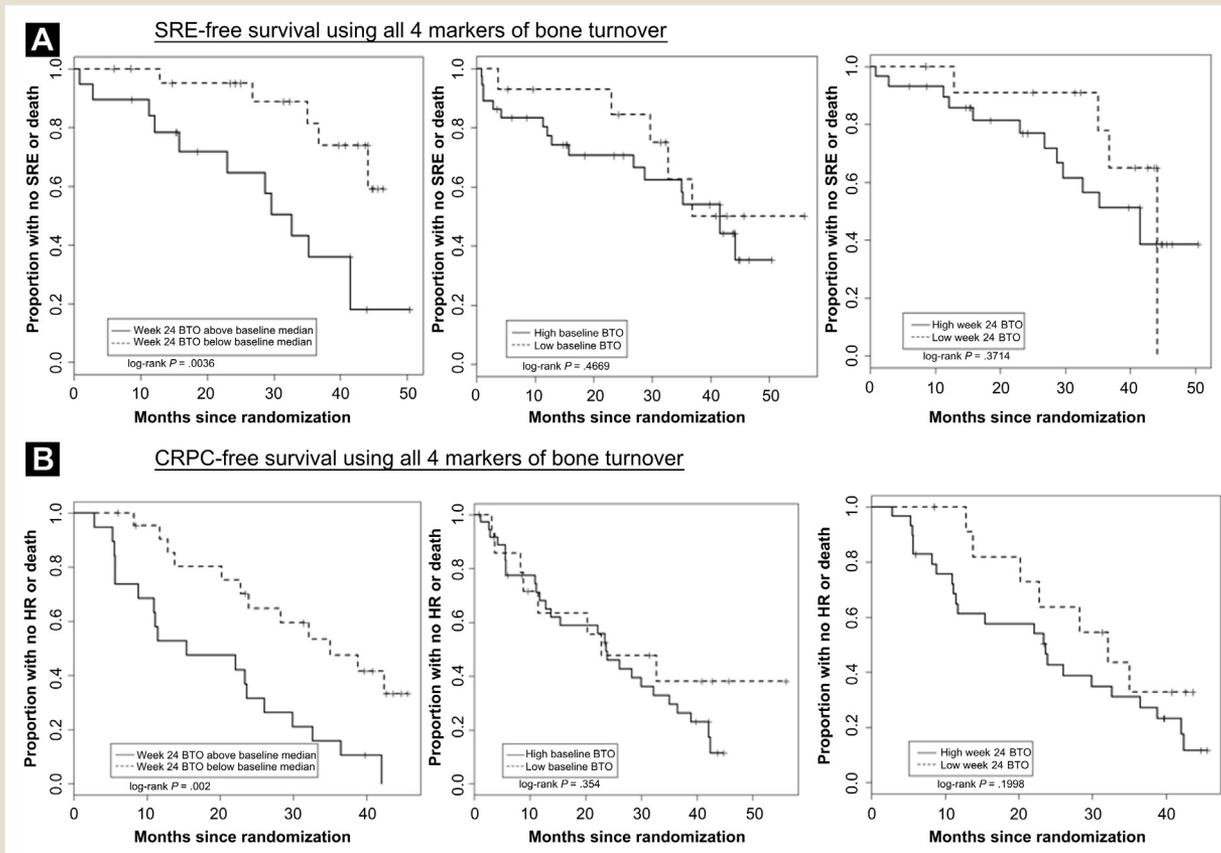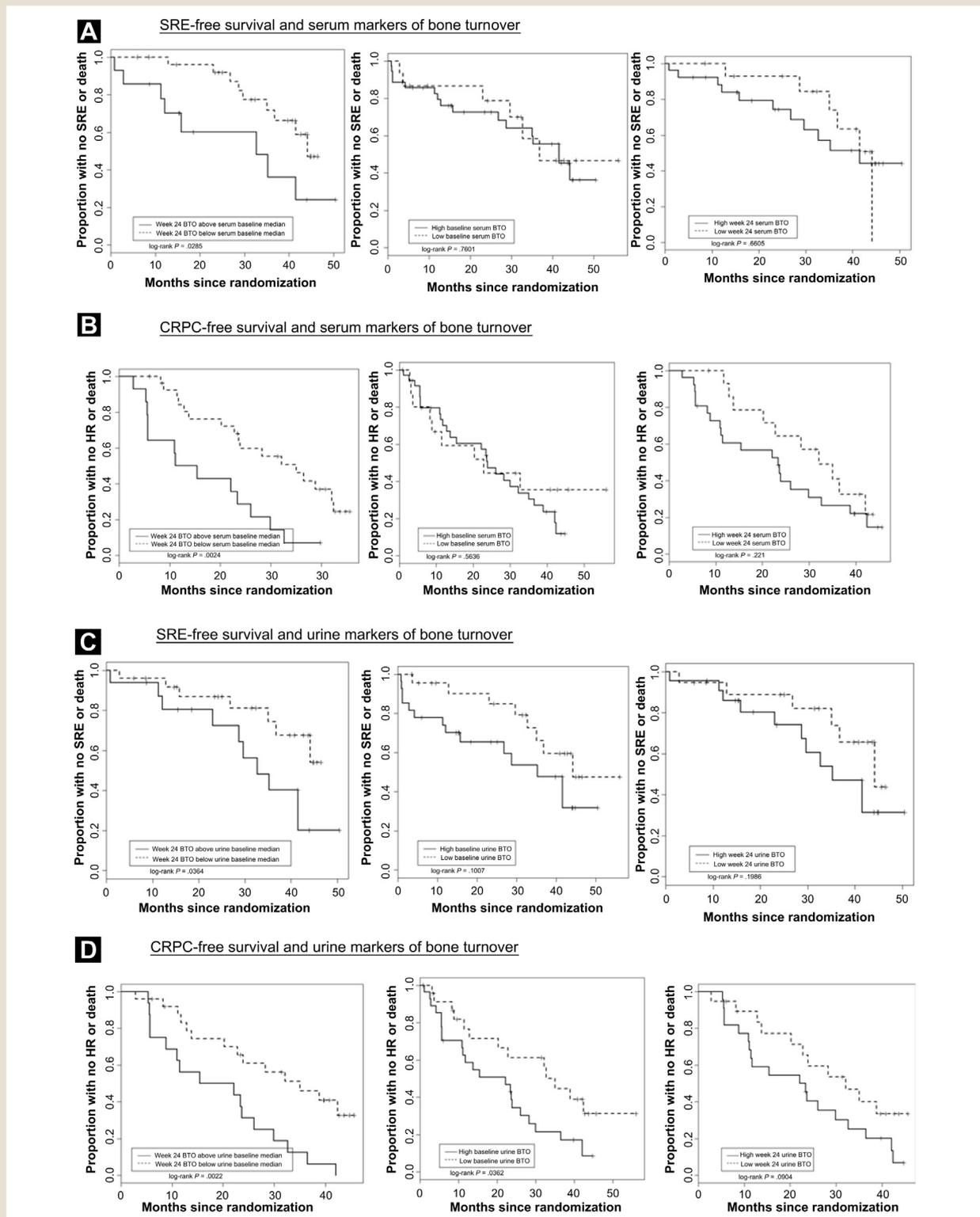

Abbreviation: BTO = bone turnover.

441 patients with bone metastases, including 203 patients with prostate cancer.[13]

It is interesting to note that markers of bone turnover above the median at baseline or at 6 months was not associated with poorer outcomes. Presumably this is because the effect of absolute elevation of baseline bone turnover markers is lost if a patient has a good response to therapy, and their markers are suppressed below the baseline median. The 6-month median level of bone turnover markers was below the baseline median, and it is presumed that failure to achieve suppression below the higher benchmark (baseline median) is able to better identify those having a poorer response to therapy.

It was noted that significant improvements in bone turnover markers (DPD, NTX, BAP, and OC) were observed in association with ADT plus risedronate therapy over ADT alone. The finding is in agreement with those reported with other bisphosphonates in patients with prostate cancer.[5,25-28] In our study, we investigated the efficacy of oral risedronate to reduce SRE rates in patients with metastatic prostate cancer initiating ADT. Baseline patient characteristics were well balanced and reflective of patients beginning ADT for metastatic disease. No significant differences in SRE rates were observed between arms. However, the study data do not allow a definitive conclusion on the ability of risedronate to delay or prevent SREs, because of lack of power related to the small sample size (early termination from slow accrual). Although not statistically significant, it is worth noting that every secondary measure of efficacy (frequency of PSA nadir < 0.2 ng/mL, median time to CRPC, and 1-year objective progression rate) was numerically in favor of the risedronate-treated patients, suggesting that the additional agent targeting the bone microenvironment may augment the anticancer activity of ADT. The Cancer and Leukemia Group B (NCT00079001) and Medical Research Council trials (NCT002684760)] assessing zoledronic acid in men beginning ADT will address this question with well-powered phase III trials. The other finding of note is that patients with moderate or severe baseline comorbidities have an impaired overall survival. This suggests that comorbidities should be a stratification factor or a factor included in multivariate analyses when overall survival is the end point.

Figure 3 Time to skeletal-Related Event (SRE) and Castration-Resistant Prostate Cancer (CRPC) and Relationship to Serum-Only (A and B) and Urine-Only (C and D) Markers of Bone Turnover. Three Analyses of the Markers of Bone Turnover Were Performed: Left Panels, Dichotomizing Bone Turnover Markers at Week 24 as to Whether They Were Above or Below Baseline Median Bone Turnover Levels (41 Patients); Middle Panels, Dichotomizing Bone Turnover Markers as Above or Below the Median at Baseline (51 Patients); Right Panels, Dichotomizing Bone Turnover Markers as Above or Below the Median at Week 24 (41 Patients). Patients Were Considered to Have "Low Bone Turnover" When Both Markers Were Below the Target Median

Abbreviations: BTO = bone turnover; HR = hazard ratio.

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Clinical Practice Points

- Elevation of markers of bone turnover when a patient has CRPC is known to be a poor prognostic marker.

- This article reports that the inability to suppress markers of bone turnover after 6 months of first-line testosterone suppression is associated with a shorter time to progression to castration-resistant disease and skeletal complications of metastatic prostate cancer.

- Declines in markers of bone turnover occurred in patients with metastatic prostate cancer who responded to therapy on testosterone suppression alone. This is in contrast to patients receiving testosterone suppression therapy in which bone turnover is increased and can lead to osteoporosis. This indicates that the more prominent cause of bone turnover in men with metastatic prostate cancer is from cancer-associated bone turnover in the microenvironment.

- If these findings are validated, measurement of bone turnover may be able to be used as a prognostic marker. It may also be useful as a biomarker that is independent of PSA levels that can assess the effect of a new drug with direct anticancer activity in prostate cancer with bony metastatic disease being treated with testosterone suppression.

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Details of Assays of Markers of Bone Turnover

Serum Bone Alkaline Phosphatase

Method

The Ostase assays are performed with an access immunoassay system, which is an assay of serum samples that provides a quantitative measurement of bone alkaline phosphatase (BAP). The Access Ostase Assay (Beckman Coulter, Brea, CA) is a 1-step immunoenzymatic assay. A mouse monoclonal antibody specific to BAP is added to a reaction vessel with paramagnetic particles coated with goat antimouse polyclonal antibody. Calibrators, controls, and samples containing BAP are added to the coated particles and bind to the anti-BAP monoclonal antibody. After the formation of a solid phase/capture antibody/BAP complex, separation in a magnetic field and washing remove materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos 530 (Lumigen/Beckman Coulter, Brea, CA), is added to the reaction vessel, and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of BAP in the sample. The amount of analyte in the sample is determined from a stored multipoint calibration curve.

Performance Data

The performance data refers to the Synarc Lyon validation report "Measurements of Bone Alkaline Phosphatase in Serum Samples," version 08, data on file.

Precision

Intraassay variations were determined using 8 different control samples (at least 15 times).
Intraassay variations (coefficient of variation - CVs) range from **2.2% to 5.3%.**
Interassay variations were determined using 4 different control samples (15 times).
Interassay variations (CVs) range from **5.0 to 6.6%.**

Lower Limit of Quantification

The lower limit of quantification (LLOQ) has been assessed as being **0.28 ng/mL**.

Serum Total Osteocalcin

Method

The assays are performed with the Elecsys 2010 automated analyzer (Roche Diagnostics, Indianapolis, IN), which uses an electrochemiluminescence immunoassay technique for the in vitro quantitative determination of serum total osteocalcin in human serum. The Elecsys S total osteocalcin assay uses a sandwich test principle, in which a first biotinylated monoclonal antibody recognizing N-MID osteocalcin and a second monoclonal antibody against N-MID osteocalcin labeled with ruthenium are incubated with 20 μL of serum. After a first incubation, streptavidin-coated microparticles are added for a second incubation, and the complex becomes bound to the solid phase by interaction of biotin and streptavidin. These microparticles are then magnetically captured onto the surface

of an electrode. Application of a voltage on this electrode induces chemiluminescent emission, which is measured by a photomultiplier and compared with a calibration curve that is generated in an instrument-specific manner by 2-point calibration.

Performance Data

The performance data refers to the Synarc Lyon validation report "Measurement of Total Osteocalcin Levels in Serum Sample," version 06.

Precision

Intraassay variations were determined using 8 different serums (15 times each).
Intraassay variations (CVs) range from **0.8% to 2.2%.**
Interassay variations were determined using 6 different serums (18 runs).
Interassay variations (CVs) range from **2.8% to 4.2%.**

Lower Limit of Quantification

The LLOQ has been assessed as being **5.9 ng/mL.**

Urinary N-Telopeptide of Type I Collagen

Method

The assays are performed with the NTx Reagent Pack kit from Ortho-Clinical Diagnostics (Ortho-Clinical Diagnostics/Johnson & Johnson, Amersham, UK), which is a kit designed for the quantitative determination of N-terminal telopeptide (NTx) in human urine on the automated Vitros Immunodiagnostic System ECi (Ortho-Clinical Diagnostics/Johnson & Johnson, Amersham, UK). A competitive immunoassay technique is used. This depends on competition between NTx present in the sample and a synthetic NTx peptide coated on the wells for binding by a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-NTx). The conjugate is captured by the peptide coated on the wells; unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the Vitros Immunodiagnostic System. The amount of HRP conjugate bound is indirectly proportional to the concentration of NTx present. The results are expressed in nanomolar of bone collagen equivalent.

Performance Data

The performance data refers to the validation report "Measurement of Cross-Linked N-Telopeptides of Type I Collagen Levels in Urine Samples," version 08.

Bone Turnover in Metastatic Prostate Cancer

Precision

Intraassay variations were determined using 4 different urine samples (15 times each).

Intraassay variations (CVs) range from **2.0% to 9.6**%.

Interassay variation were determined using 7 different urines (10 times each).

Interassay variations (CVs) range from **3.5% to 7.8**%.

Lower Limit of Quantification

The LLOQ has been assessed as being **22 nM bone collagen equivalent**.

Urinary Total Deoxypyridinoline

Method

Deoxypyridinoline (DPD) is measured in hydrolyzed urine samples using high-performance liquid chromatography technique. After extraction of the cross-links and elimination of the urine impurities by a Bio-Rad SPE cartridge (Bio-Rad Laboratories, Hercules, CA), total DPD is eluted from reverse-phase high-performance liquid chromatography by ion pair chromatography with isocratic elution. The compounds are detected as a result of their natural fluorescence with a fluorescence detector.

Performance Data

The performance data refers to the validation report "Measurement of Total Deoxypyridinoline in Urine Samples," version 01.

Precision

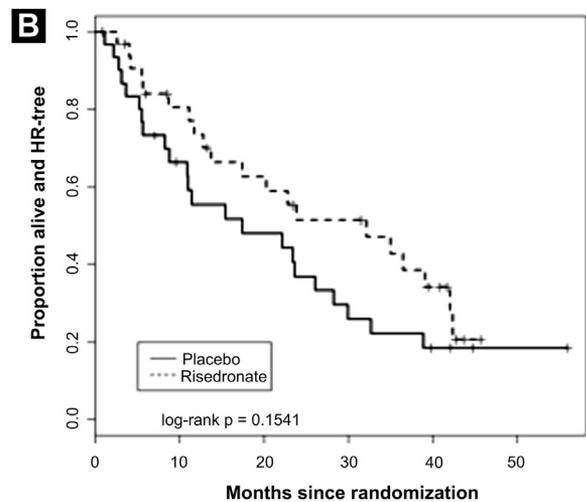Intraassay variations were determined by using 5 urine samples (20 times each).
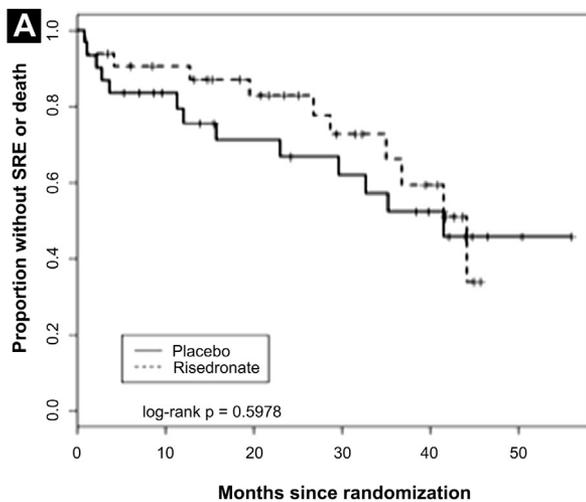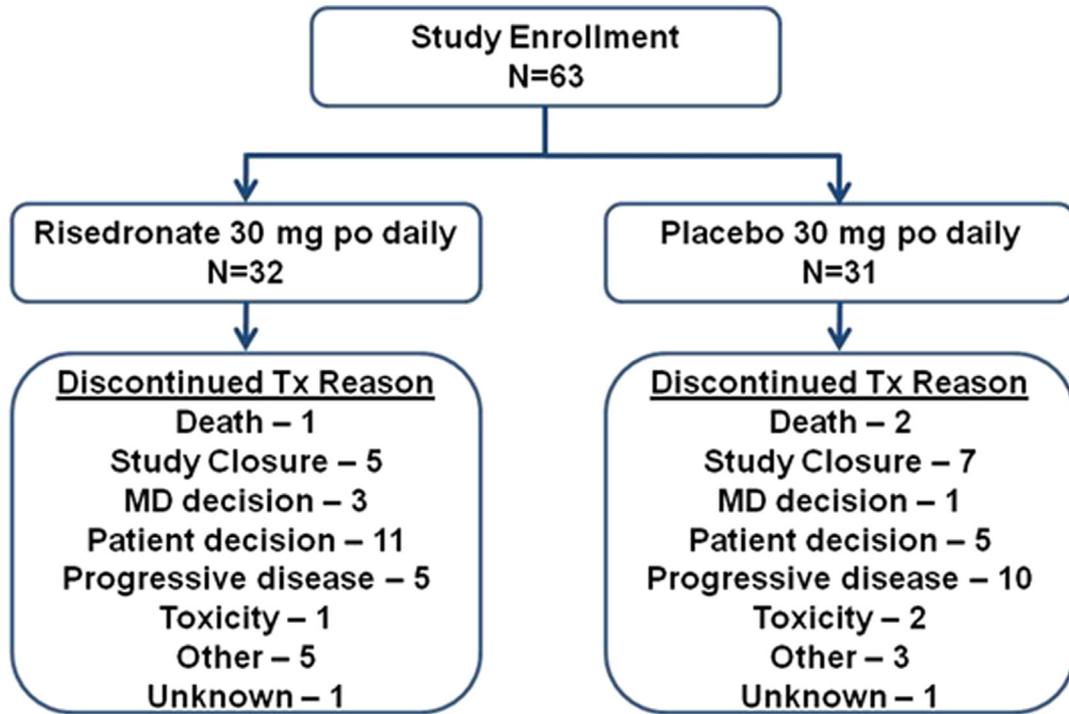
Intraassay variations (CVs) range from **3.2% to 9.4%**.

Interassay variations were determined by using 3 urine samples (16 times each).

Interassay variations (CVs) range from **6.3% to 7.4%.**

Lower Limit of Quantification

The LLOQ has been assessed as being **30 nmol/L** and is the lower concentration, which can be quantified at 30 nmol/L; the ratio signal-noise is ≥ 10.

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                          ┌─────────────────────┐
                          │   Study Enrollment  │
                          │       N=63          │
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Risedronate 30 mg po daily N=32	Placebo 30 mg po daily N=31
Discontinued Tx Reason Death – 1 Study Closure – 5 MD decision – 3 Patient decision – 11 Progressive disease – 5 Toxicity – 1 Other – 5 Unknown – 1	**Discontinued Tx Reason** Death – 2 Study Closure – 7 MD decision – 1 Patient decision – 5 Progressive disease – 10 Toxicity – 2 Other – 3 Unknown – 1

A — Proportion without SRE or death vs. Months since randomization. Placebo; Risedronate. log-rank p = 0.5978

B — Proportion alive and HR-tree vs. Months since randomization. Placebo; Risedronate. log-rank p = 0.1541