Biomarkers of Bone Remodeling in Multiple Myeloma Patients to Tailor Bisphosphonate Therapy

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Abstract

Background: Patients with multiple myeloma may be susceptible to osteonecrosis of the jaw (ONJ) and stress fractures due to long-term aminobisphosphonate (aBP) therapy. However, it is unknown whether urinary N-telopeptide (NTX) or other bone biomarkers are predictive of skeletal-related events (SRE) or the impact of cessation of aBP therapy on bone remodeling.

Methods: We studied markers of bone turnover over a 6-month period after a single dose of zoledronic acid in 29 patients with multiple myeloma in remission who previously received 8 to 12 doses of pamidronate or zoledronate (NCT00577642). Our primary objective was to determine the duration of time urinary NTX levels remain suppressed after a single dose of zoledronate. A secondary objective was to identify and correlate other markers of bone remodeling with NTX changes. Thirty cytokines, based on their possible role in bone remodeling, were tested using cytokine arrays. Candidates were confirmed by ELISA.

Results: All patients had continued suppression of NTX levels, except 1 patient who had an increase in NTX levels associated with an SRE. GDF-15 and decorin were found to decrease, whereas bone-specific alkaline phosphatase (BSALP) increased. Although not significant in aggregate, osteopontin and osteoprotegerin levels increased in at least half of the patients.

Conclusion: Our data show that NTX levels continue to be suppressed after aBP therapy, and suggest that suppressed NTX levels may be predictive of freedom from SRE in this patient population. Furthermore, osteoblast suppression by aBP may be reversible in myeloma. These data provide the basis for less frequent dosing of aBPs. Clin Cancer Res; 1–7. ©2014 AACR.

Introduction

Aminobisphosphonates (aBP) such as zoledronate and pamidronate are a mainstay of the treatment of multiple myeloma and metastatic solid tumors to prevent bone-related morbidity and mortality (1, 2). For multiple myeloma, most recent consensus guidelines recommend monthly aBP treatment for a period of 2 years (3, 4). However, it is unknown whether monthly dosing of aBPs is optimal, as there are consequences of long-term aBP therapy, such as osteonecrosis of the jaw (ONJ) and atypical stress fractures (3–9). Bisphosphonate-related ONJ is characterized by bone that is necrotic and exposed, located in the maxillofacial area, and present for at least 8 weeks in a patient with history of bisphosphonate treatment, but no history of prior radiation to that area (10). Although varying incidences of ONJ between 0.94% to 18.6% have been reported, in a longitudinal cohort study, patients with multiple myeloma were more likely to develop ONJ than patients with breast or prostate cancer (11). It is thought that ONJ results from oversuppression of bone remodeling by aBPs (12). As overall survival of patients with multiple myeloma has improved, they are exposed to aBPs for longer periods of time, making the issue of optimal aBP dosing even more pertinent.

The pharmacodynamic and pharmacokinetic data of aBPs is limited as these compounds incorporate directly into the bone matrix, targeting osteoclast farnesyl pyrophosphate synthetase and inhibiting protein prenylation (13). The half-life of aBPs in bone is long and on the order of 240 days with zoledronate (14). The activity of aBP can be monitored via surrogates such as urinary N-telopeptide (NTX). The monthly dosing of aBPs is based on the suppression of NTX for 4 to 8 weeks following a single dose of aBP (15, 16). In the context of the bone-related sequelae of
Patients were then given a single dose of zoledronate 4 mg or adjusted according to institutional guidelines of creatinine clearance, and then followed over a period of 6 months. No further aBP therapy was allowed during the study. Each month, urinary NTX was followed and serum was banked. In our study, if NTX surpassed a threshold of 50 nmol/mmol Cr, or there was disease progression or an SRE, patients were removed from the study. At the end of the study, the testing done at entry was repeated.

Assay methods
Antibody-based cytokine arrays were used to evaluate serum samples as per the manufacturer’s protocol (Ray-Biotech, Inc.). Thirty cytokines were curated from the literature as being most relevant to the bone microenvironment for inclusion on the array. For bone biomarkers not available on the array, ELISA was used: C-terminal telopeptide of type I collagen (CTX1), amino-terminal propeptide of type I collagen (P1NP), sclerostin, soluble receptor activator of NF-kB ligand (sRANKL), and bone-specific alkaline phosphatase (BSALP). ELISAs were performed according to the manufacturer’s protocols supplied with the kits: CTX1 and P1NP (TSZ ELISA), sclerostin (ALPCO), sRANKL (Antigenix America), decorin (RayBiotech, Inc.), BSALP (Quidel), and activin A and GDF-15 (R&D Systems).

Statistical analysis methods
Paired t tests were used to compare differences of cytokine levels at study entry versus end-of-study. For antibody-based cytokine array, fluorescence intensities were computed by normalization of fluorescence values using software from the array manufacturer and then log transformed. Fluorescence intensities without log transformation are reported. GDF-15, decorin, and activin A were selected for validation by ELISA.

Results
Baseline characteristics are shown in Table 1. Twenty-nine patients were enrolled in the study. Median age at entry into the study was 60 years (range, 46–85 years). Most patients had received a combination of drugs including bortezomib and lenalidomide (14 patients) or bortezomib in combination with other drugs (10 patients). Five patients received regimens that did not include bortezomib. Ten of 29 patients in the trial were on maintenance therapy (lenalidomide, 5; thalidomide, 4; bortezomib, 1). The mean time between diagnosis and enrollment on the trial was 13 months. The majority of patients had more than three lytic lesions. No patients had appreciable increase in bone lesions over the course of the study.

The mean urine NTX level was 22.2 nmol/mmol Cr at baseline (Fig. 1). All patients had an NTX level at baseline less than 50 nmol/mmol Cr except for 1 patient, whose baseline was 82 nmol/mmol Cr. A paired t test comparing NTX levels at earliest and latest available time points in the study for each patient showed a mean increase of only 1.5
nmol/mmol Cr that was not significant ($P = 0.38$). There
was no difference in change in NTX levels in patients on
maintenance treatment versus patients on observation
alone. The 1 patient with baseline NTX level of 82 nmol/
mmol Cr remained on the study until month 3, as NTX first
decreased to 32 nmol/mmol Cr before climbing to 90
nmol/mmol Cr. This patient developed hypercalcemia,
which was categorized as an SRE. Another patient showed
evidence of disease progression by increasing monoclonal
protein, without elevated NTX level or SRE. This patient was
removed from study at month 5.

Given that NTX levels did not significantly change during
the 6-month duration, we studied cytokine levels at baseline
and end-of-study using ELISA and antibody-based cytokine
arrays. CTX1, P1NP, sclerostin, and sRANKL had no signif-
icant differences between baseline and end-of-study when
tested by ELISA. BSALP had a mean increase of 2.26 U/L ($P =
0.003$) from baseline to end of study.

The results of the antibody-based cytokine array are
shown in Table 2. Although osteopontin and osteoprote-
gerin were not significant in aggregate, at least half of
patients had an increase in these cytokines. On the basis
of the cytokine array results and potential biologic rele-
vance, we selected GDF15, decorin, and activin A for val-
dication by ELISA. Activin A showed no significant differ-
ence ($P = 0.980$), whereas mean GDF15 level decreased by 231
pg/mL ($P = 0.00053$) and mean decorin level decreased by
701 pg/mL ($P = 0.00025$). Waterfall plots for the three
significant cytokines measured by ELISA, GDF15, decorin,
and BSALP, are shown in Fig. 2.

Discussion

The impact of aBP cessation among patients with mul-
tiple myeloma who have achieved a clinical response on
urine NTX, the key marker that was used to originally dose
aBPs, heretofore has not been studied. In a study of more
than 1,800 patients with metastatic cancer treated with
aBPs, patients with urinary NTX greater than 99 nmol/
mmol Cr or 50 to 99 nmol/mmol Cr have 4 to 6 and 2
to 4 times increased risk of SRE, respectively, as compared
with patients with NTX less than 50 nmol/mmol Cr (17).
However, it is unclear what happens to the association
between NTX and SREs with cessation of aBP therapy.

Our study reveals that urine NTX remains suppressed in
patients who had a complete or partial response to multiple
myeloma therapy and who had suppressed NTX at baseline
(<50 nmol/mmol Cr). This observation extends on previ-
ous studies showing that patients who respond to antimye-
loma therapy have significant reduction in bone resorption
markers (18–20). In a recent study of patients who were

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**Table 1. Baseline characteristics of enrolled patients with multiple myeloma**

<table>
<thead>
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<th>Characteristics</th>
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<tbody>
<tr>
<td>Age, y</td>
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<td>&gt;65</td>
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<tr>
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<tr>
<td>I</td>
<td>11</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
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<tr>
<td>III</td>
<td>9</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>Bortezomib + other</td>
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</tr>
<tr>
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<td>Yes</td>
<td>13</td>
</tr>
<tr>
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<td>16</td>
</tr>
<tr>
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</tr>
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</tr>
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<td>≤3</td>
<td>9</td>
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Abbreviation: ISS, International Staging System.
consolidated with bortezomib, thalidomide, and dexamethasone and who did not receive subsequent bisphosphonate therapy, SREs were uncommon (21). In our study, almost a third of patients in our study were on maintenance therapy, and this may have played a part in suppression of NTX. The 1 patient who had elevated NTX at baseline later went on to have an SRE. Suppressed NTX may, therefore, be involved in the bone marrow microenvironment, we wished to see whether a select number of these changed to experience a serious adverse event.

Given that numerous cytokines have been postulated to be involved in the bone marrow microenvironment, we wished to see whether a select number of these changed from baseline to end-of-study and if any of these may be suitable surrogates for aBP activity, in addition to urine NTX. BSALP has been shown to increase in patients treated with bortezomib-containing regimens (23, 24), although the effect may be temporary (25). It has been thought that such rise of BSALP may signal possible osteoblast activity with concomitant bone healing (25). In this study, 64% of patients (18 of 28) had an increase in BSALP, indicating that SRE risk was not any greater than reported previously in the literature (22). Patients who remained in the 3-month interval dosing group throughout the study were less likely to discontinue aBP treatment and less likely to experience a serious adverse event.

Of note, the Z-MARK study in patients with multiple myeloma previously treated with 1 to 2 years of aBPs found that changing the dosing interval of zoledronate from 1 to 3 months based on suppressed NTX levels appeared to be safe for patients, and only 3.3% of patients experienced an SRE, indicating that SRE risk was not any greater than reported previously in the literature (22). Patients who remained in the 3-month interval dosing group throughout the study were less likely to discontinue aBP treatment and less likely to experience a serious adverse event.

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were not significant. Osteoblasts secrete osteoprotegerin, a member of the tumor necrosis receptor superfamily, which acts as a negative regulator of osteoclast differentiation by serving as a decoy receptor for RANKL (26). On the other hand, osteopontin, a ligand for endothelial cell \( \alpha \text{v}\beta 3 \) integrin, is secreted by osteoclasts and may enhance endothelial cell–mediated osteoclastic bone resorption and angiogenesis (27).

GDF15, a member of the TGF\( \beta \) superfamily, has been shown to increase during progression of a number of solid malignancies, but there is also evidence that GDF15 is upregulated by a number of tumor-suppressor pathways (28). In multiple myeloma, GDF15 appears to be secreted from bone marrow mesenchymal stem cells (29). Patients with multiple myeloma with higher levels of GDF15 have worse event-free survival compared with patients with lower levels of GDF15 (30). The exact physiologic role of GDF15 in myeloma is yet to be defined. The cessation of long-term aBP therapy led to a decrease in GDF15 in this study, potentially conferring a better prognosis, but further research is needed to elucidate the role of GDF15 in this context.
Decorin, a small leucine-rich proteoglycan secreted by mesenchymal stem cells and osteoblasts, seems to inhibit angiogenesis and osteoclastogenesis (31). Knockdown of decorin has been shown to increase primary myeloma cell survival (31). In monoclonal gammopathy of unknown significance (MGUS) and patients with multiple myeloma, decorin has been shown to be downregulated as compared with healthy volunteers (32). Thus, given that decorin seems to reduce survival of myeloma cells, the decrease in decorin at the end of the study warrants further study. Our group is currently investigating the change in decorin expression with differentiation of osteoblasts and the effect of recombinant decorin on both osteoblasts and primary myeloma cells. These data further underscore the continued use of aBPs, although less frequent dosing is potentially feasible.

While involving a small number of patients, our study suggests that suppressed NTX levels may be predictive of freedom from SRE among patients in response to multiple myeloma therapy, over a period of 6 months. Furthermore, osteoblast suppression by aBPs is reversible in myeloma, as indicated by the significant increase in BSALP. Importantly, the role of other surrogates such as decorin and GDF15 need further validation and are the subject of ongoing studies. Taken together, these findings may provide a rationale for less frequent aBP dosing in patients with multiple myeloma with at least a partial response to primary myeloma therapy to help lower the incidence of long-term toxicities such as ONJ and stress fractures, by allowing limited recovery of bone remodeling without adverse effects on multiple myeloma progression.

Disclosure of Potential Conflicts of Interest

P.G. Richardson is a consultant/advisory board member for Celgene, Millennium Pharmaceuticals, and Johnson & Johnson. R.L. Schlossman is a consultant/advisory board member for Millennium Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.G. Patel, A.J. Yee, T.A. Scullen, P.G. Richardson, I.M. Ghobrial, N.C. Munshi, K.C. Anderson, N.S. Raje

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Acknowledgments

The authors thank the patients and their families, the coinvestigators, and the nursing and research staff involved in the trial.

Grant Support

N.S. Raje was funded by the ASCO Career Development Award. C.G. Patel was funded by an ASH Research Trainee Award.

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Received February 20, 2014; revised May 9, 2014; accepted May 23, 2014; published OnlineFirst June 23, 2014.

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Clin Cancer Res  Published OnlineFirst June 23, 2014.

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