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

Chirayu G. Patel1, Andrew J. Yee1,3, Tyler A. Scullen1, Neerhika Nemani1,3, Loredana Santo1,3, Paul G. Richardson2,3, Jacob P. Laubach2,3, Irene M. Ghobrial2,3, Robert L. Schlossman2,3, Nikhil C. Munshi2,3, Kenneth C. Anderson2,3, and Noopur S. Raje1,3

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; 20(15); 3955–61. ©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
**Translational Relevance**

Aminobisphosphonates (aBP), such as zoledronate or pamidronate, are highly effective in preventing skeletal-related events in patients with multiple myeloma and solid tumors with bone metastases. aBPs are dosed monthly based on the suppression of urinary N-telopeptide (NTX), a marker of bone resorption correlated with bone-related morbidity and mortality in patients with cancer. NTX levels are suppressed for 4 to 8 weeks following a single dose of aBP. However, the long-term use of aBPs has highlighted osteonecrosis of the jaw and stress fractures as possible consequences of aBP-related oversuppression of bone remodeling. Our study found that NTX levels remained stable in the 6 months following cessation of aBPs in 28 of 29 patients with multiple myeloma previously treated with aBPs for at least 8 to 12 doses, suggesting that less frequent dosing of aBPs is feasible.

For long-term aBP therapy, we sought to examine bone remodeling after cessation of long-term aBP therapy for a period of 6 months in patients with multiple myeloma in complete or partial remission with 8 to 12 prior doses of aBPs. A 6-month duration of follow-up was chosen as it is reasonably greater than the current monthly aBP dosing interval to be able to document the clinical effects of aBP cessation, yet short enough to minimize the previously uncertain risk of aBP cessation to enrolled patients. We also evaluated whether NTX or other potential surrogate markers of aBP activity are predictive of skeletal-related events (SRE) in these patients.

**Materials and Methods**

**Patients and study design**

Patients were enrolled at Massachusetts General Hospital and Dana-Farber Cancer Institute after study approval by the Institutional Review Board and informed consent. This trial is registered at www.clinicaltrials.gov as NCT00577642. The inclusion criteria were age at least 18 years, confirmed diagnosis of multiple myeloma by International Myeloma Working Group (IMWG) criteria, intravenous bisphosphonate therapy with either pamidronate or zoledronate for 8 to 12 months, complete response or partial response by European Blood and Marrow Transplantation (EBMT) criteria, and Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2. Patients were excluded if they were on active anti–multiple myeloma therapy, renal dysfunction with serum creatinine >2 mg/dl and/or creatinine clearance <30 mL/min, or had relapsed, refractory, or progressive disease. Maintenance therapy for multiple myeloma was allowed on study.

At entry into study, patients underwent measurement of urinary NTX (Quest Diagnostics), banking of serum, bone marrow aspiration and core biopsy, and skeletal survey.

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 (RayBiotech, 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 1 collagen (CTX1), amino-terminal propeptide of type 1 collagen (P1NP), sclerostin, soluble receptor activator of NF-κB 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 significant 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 osteoprotegerin 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 relevance, we selected GDF15, decorin, and activin A for validation by ELISA. Activin A showed no significant difference \( P = 0.980 \), 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 multiple 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 \((<50 \text{ nmol/mmol Cr})\). This observation extends on previous studies showing that patients who respond to antmyeloma therapy have significant reduction in bone resorption markers \( (18–20) \). In a recent study of patients who were
of long-term aBP therapy last well beyond 1 month, 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.

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 osteoblast recovery may be possible with cessation of long-term aBP therapy. There is some evidence that there is increased in osteopontin and osteoprotegerin, which increased in about half of patients, although the results 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 predictive of freedom from SRE in patients who are in remission, but not necessarily freedom from multiple myeloma disease progression, as 1 patient with suppressed NTX was removed from the study for disease progression based on monoclonal protein elevation. Although our findings are based on a small sample size, they suggest that the effects of long-term aBP therapy last well beyond 1 month, the current aBP dosing interval.

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.

Table 2. Cytokine array: change at end-of-study relative to baseline

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Baseline</th>
<th>End-of-study</th>
<th>% Change</th>
<th>P</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activin A</td>
<td>96 ± 17</td>
<td>135 ± 36</td>
<td>41.2</td>
<td>0.0384</td>
<td>0.345</td>
</tr>
<tr>
<td>BDNF</td>
<td>605 ± 88</td>
<td>684 ± 81</td>
<td>13</td>
<td>0.184</td>
<td>0.402</td>
</tr>
<tr>
<td>BMP7</td>
<td>440 ± 33</td>
<td>471 ± 24</td>
<td>6.9</td>
<td>0.0791</td>
<td>0.402</td>
</tr>
<tr>
<td>Decorin</td>
<td>4,113 ± 240</td>
<td>3,669 ± 239</td>
<td>−10.8</td>
<td>0.276</td>
<td>0.439</td>
</tr>
<tr>
<td>DKK1</td>
<td>451 ± 33</td>
<td>430 ± 32</td>
<td>−4.7</td>
<td>0.382</td>
<td>0.573</td>
</tr>
<tr>
<td>GDF15</td>
<td>3,928 ± 427</td>
<td>3,127 ± 322</td>
<td>−20.4</td>
<td>0.00183</td>
<td>0.0493</td>
</tr>
<tr>
<td>HGF</td>
<td>313 ± 132</td>
<td>304 ± 84</td>
<td>−3.1</td>
<td>0.109</td>
<td>0.402</td>
</tr>
<tr>
<td>ICAM1</td>
<td>2,522 ± 1,359</td>
<td>2,222 ± 981</td>
<td>−11.8</td>
<td>0.074</td>
<td>0.402</td>
</tr>
<tr>
<td>IGFI</td>
<td>152 ± 10</td>
<td>157 ± 7</td>
<td>3.6</td>
<td>0.483</td>
<td>0.653</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>336 ± 34</td>
<td>318 ± 25</td>
<td>−5.4</td>
<td>0.889</td>
<td>0.923</td>
</tr>
<tr>
<td>IL3</td>
<td>435 ± 30</td>
<td>478 ± 27</td>
<td>10</td>
<td>0.136</td>
<td>0.402</td>
</tr>
<tr>
<td>IL1α</td>
<td>1,049 ± 82</td>
<td>1,038 ± 77</td>
<td>−1.1</td>
<td>0.799</td>
<td>0.899</td>
</tr>
<tr>
<td>IL1β</td>
<td>215 ± 15</td>
<td>236 ± 16</td>
<td>9.6</td>
<td>0.12</td>
<td>0.402</td>
</tr>
<tr>
<td>IL6</td>
<td>1,103 ± 85</td>
<td>1,098 ± 83</td>
<td>−0.5</td>
<td>0.862</td>
<td>0.923</td>
</tr>
<tr>
<td>IL7</td>
<td>1,076 ± 77</td>
<td>1,124 ± 71</td>
<td>4.5</td>
<td>0.238</td>
<td>0.431</td>
</tr>
<tr>
<td>IL8</td>
<td>1,190 ± 235</td>
<td>1,036 ± 70</td>
<td>−13</td>
<td>0.963</td>
<td>0.963</td>
</tr>
<tr>
<td>MCSF</td>
<td>207 ± 18</td>
<td>191 ± 14</td>
<td>−8</td>
<td>0.547</td>
<td>0.703</td>
</tr>
<tr>
<td>MIP1α</td>
<td>175 ± 17</td>
<td>188 ± 20</td>
<td>7.4</td>
<td>0.643</td>
<td>0.789</td>
</tr>
<tr>
<td>MMP9</td>
<td>10,346 ± 2,171</td>
<td>8,644 ± 2,525</td>
<td>−16.5</td>
<td>0.403</td>
<td>0.573</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>1,466 ± 238</td>
<td>1,507 ± 157</td>
<td>2.8</td>
<td>0.267</td>
<td>0.439</td>
</tr>
<tr>
<td>Osteoprotegerin</td>
<td>142 ± 10</td>
<td>157 ± 12</td>
<td>10.9</td>
<td>0.193</td>
<td>0.402</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>443 ± 45</td>
<td>384 ± 35</td>
<td>−13.3</td>
<td>0.0254</td>
<td>0.343</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>1,079 ± 67</td>
<td>1,185 ± 83</td>
<td>9.8</td>
<td>0.173</td>
<td>0.402</td>
</tr>
<tr>
<td>VCAM1</td>
<td>2,389 ± 189</td>
<td>2,562 ± 179</td>
<td>7.3</td>
<td>0.24</td>
<td>0.431</td>
</tr>
<tr>
<td>VEGF</td>
<td>216 ± 22</td>
<td>201 ± 14</td>
<td>−7</td>
<td>0.742</td>
<td>0.871</td>
</tr>
<tr>
<td>VEGFC</td>
<td>352 ± 39</td>
<td>302 ± 23</td>
<td>−14.2</td>
<td>0.185</td>
<td>0.402</td>
</tr>
<tr>
<td>VEGFΔ</td>
<td>196 ± 11</td>
<td>179 ± 12</td>
<td>−8.6</td>
<td>0.182</td>
<td>0.402</td>
</tr>
</tbody>
</table>

NOTE: Values reported are mean relative fluorescence units ± SE in 21 out of 28 patients. P values are from paired t tests of log-transformed measurements. BMP4, MIP3α, and SDF1α are not reported; measurements were not above baseline. Of note, comparisons of cytokine measurements may not necessarily be directly possible from this platform.
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 αvβ3 integrin, is secreted by osteoclasts and may enhance endothelial cell–mediated osteoclastic bone resorption and angiogenesis (27).

GDF15, a member of the TGFβ 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.

Figure 2. Change at end-of-study compared with baseline for selected biomarkers. Selected biomarkers were measured by ELISA at baseline and end-of-study. Data for GDF15, decorin, and BSALP are shown as waterfall plots. Horizontal line, mean difference from baseline to end-of-study. P values are from paired t tests.
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.

Authors’ Contributions

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 Fellow Award.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby markedadvertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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


