Lytic bone disease is a frequent complication of multiple myeloma (MM). Lytic lesions rarely heal and X-rays are of limited value in monitoring bone destruction during anti-myeloma or anti-resorptive treatment. Biochemical markers of bone resorption (aminoterminal and carboxy-terminal cross-linking telopeptide of type I collagen (NTX and CTX, respectively) or CTX generated by matrix metalloproteinases (ICTP)) and bone formation provide information on bone dynamics and reflect disease activity in bone. These markers have been investigated as tools for evaluating the extent of bone disease, risk of skeletal morbidity and response to anti-resorptive treatment in MM. Urinary NTX, serum CTX and serum ICTP are elevated in myeloma patients with osteolytic lesions and correlate with advanced disease stage. Furthermore, urinary NTX and serum ICTP correlate with risk for skeletal complications, disease progression and overall survival. Bone markers have also been used for the early diagnosis of bone lesions. This International Myeloma Working Group report summarizes the existing data for the role of bone markers in assessing the extent of MM bone disease and in monitoring bone turnover during anti-myeloma therapies and provides information on novel markers that may be of particular interest in the near future.

**Introduction**

Multiple myeloma (MM) is characterized by the presence of osteolytic bone lesions that result in skeletal-related events (SREs), such as pathologic fractures, need for radiation or surgery to bone, spinal cord compression and hypercalcemia. In the absence of effective bisphosphonate therapy, more than 50% of patients with Durie–Salmon stage III MM will experience at least one SRE over 2 years. The development of lytic bone lesions is not only related to increased osseous breakdown but also to uncoupling of the bone remodeling process, in which osteoclast-mediated bone resorption is normally tightly coupled both temporally and spatially with osteoblast-mediated bone formation. Lytic lesions rarely heal even in patients at complete remission. Further, owing to the marked decrease in osteoblast activity, bone scans are often negative in myeloma patients with extensive lytic lesions and offer very little in the follow-up of bone disease in these patients. Finally, sequential measurement of bone mineral density using Dual-energy X-ray Absorptiometry scans produce heterogeneous local bone mineral density changes; as such the routine use of sequential Dual-energy X-ray Absorptiometry scans is not recommended to assess bone disease in MM.

Although osteolytic lesions are usually assessed by plain radiographs, conventional radiography cannot provide information about ongoing bone remodeling. Accordingly, biochemical markers of bone metabolism have been used in MM to assess the rate of bone turnover (defined as the prevalent rates of both bone formation and bone resorption) and to improve monitoring of bone destruction in MM. Moreover, bone turnover markers have been used to follow myeloma bone disease during specific therapies. However, at present there is no consensus for the use of bone turnover markers in MM. This report of the International Myeloma Working Group summarizes the existing data for the role of markers of bone remodeling in assessing the extent of myeloma bone disease and in monitoring bone turnover during anti-myeloma treatment. It also proposes markers that can be used in clinical practice, and presents novel markers that may be of particular interest in the future.

**Markers of bone remodeling**

Throughout life, bone undergoes continuous remodeling with removal of old bone by osteoclasts and replacement with new bone by osteoblasts; a process that is balanced under normal conditions. However, in MM, there is increased activation of osteoclasts and suppression of osteoblast function. Over the past two decades, the isolation and characterization of cellular and extracellular components of the skeletal matrix have resulted in the development of biochemical markers that reflect
either bone formation or bone resorption. 12–15 Markers of bone resorption and formation are depicted in Tables 1 and 2. Measurement of bone turnover markers is noninvasive, comparatively inexpensive and when applied and interpreted correctly, can be of significant help in the assessment of bone disorders. However, factors that affect bone turnover marker levels, including circadian rhythm, diet, age, gender, renal function and drugs, should be clearly defined and appropriately adjusted for whenever possible. It is also important to recognize that these biochemical measurements reflect whole-body bone turnover and give little information about the function of local changes in skeletal homeostasis. All these issues are discussed below.

Biochemistry of bone markers

**Bone resorption markers**

**Hydroxyproline (Hyp) and hydroxylysine (Hyl).** Hyp is formed in the cell from the post-translational hydroxylation of proline and is the predominant amino acid within all collagens. Hyl is another structural amino acid of collagenous proteins. 11

However, both Hyp and Hyl are also contained in certain serum proteins, such as the C1q component of complement. This disadvantage, in combination with the effect of age and circadian rhythm (both Hyp and Hyl have their peak excretion after midnight) on their circulating levels, makes both Hyp and Hyl less specific indices of bone resorption. As such, both have been largely replaced by newer markers. 17–19

**Pyridinoline (PYD) and deoxypyridinoline (DPD) cross-links of type I collagen.** PYD and DPD are formed by the enzymatic action of lysyl oxidase on lysine and Hyl. PYD and DPD act as mature cross-links in type I collagen of all major connective tissues (Figure 1). 20 The products of bone collagen degradation by osteoclasts include amino- and carboxy-terminal peptide fragments (NTX and CTX, respectively) of various sizes that remain attached to helical portions of nearby collagen molecules by a pyridinium cross-link. These molecules are released into the circulation. Further degradation occurs in the liver and kidney, where the fragments are finally degraded to their constituent amino acids and the pyridiniums, PYD and DPD.

**Table 1** Markers of bone resorption

<table>
<thead>
<tr>
<th>Marker</th>
<th>Abbreviation</th>
<th>Tissue of origin</th>
<th>Analytical method</th>
<th>Analytical specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyproline</td>
<td>Hyp</td>
<td>All tissues and all genetic types of collagen</td>
<td>Colorimetric, assay, HPLC</td>
<td>Urine</td>
</tr>
<tr>
<td>Hydroxylysine</td>
<td>Hyl</td>
<td>All tissues and all genetic types of collagen</td>
<td>Reversed-phase HPLC</td>
<td>Urine</td>
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<tr>
<td>Galactosyl-hydroxylysine</td>
<td>Gal-Hyl</td>
<td>Both Gal-Hyl and Glc-Gal-Hyl appears to be specific for bone collagen degradation</td>
<td>Reversed-phase HPLC</td>
<td>Urine</td>
</tr>
<tr>
<td>Glucosyl-galactosyl-hydroxylysine</td>
<td>Glc-Gal-Hyl</td>
<td>Bone, cartilage, tendon, blood vessels</td>
<td>Reversed-phase HPLC</td>
<td>Urine</td>
</tr>
<tr>
<td>Pyridinoline</td>
<td>PYD</td>
<td>Bone, dentin</td>
<td>HPLC, ELISA</td>
<td>Urine</td>
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<td>Deoxypyridinoline</td>
<td>DPD</td>
<td>All tissues containing type-I collagen</td>
<td>RIA</td>
<td>Urine (free DPD can be also measured in serum or plasma)</td>
</tr>
<tr>
<td>N-terminal cross-linking telopeptide of type-I collagen</td>
<td>NTX</td>
<td>All tissues containing type-I collagen</td>
<td>ELISA, RIA</td>
<td>Urine, serum</td>
</tr>
<tr>
<td>C-terminal cross-linking telopeptide of type-I collagen</td>
<td>CTX</td>
<td>All tissues containing type-I collagen</td>
<td>ELISA, RIA</td>
<td>Urine, serum (β-form only)</td>
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<tr>
<td>C-terminal cross-linking telopeptide of type-I collagen generated by MMPs</td>
<td>CTX-MMP or ICTP</td>
<td>All tissues containing type-I collagen</td>
<td>RIA</td>
<td>Serum</td>
</tr>
<tr>
<td>Tartrate-resistant acid phosphatase isoform 5b</td>
<td>TRACP-5b</td>
<td>Bone (osteoclasts)</td>
<td>Colorimetric RIA, ELISA</td>
<td>Serum, plasma</td>
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<td>Bone sialoprotein</td>
<td>BSP</td>
<td>Bone, dentin, hypertrophic cartilage, cancer cells</td>
<td>RIA, ELISA</td>
<td>Serum</td>
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</table>

**Table 2** Markers of bone formation

<table>
<thead>
<tr>
<th>Marker</th>
<th>Abbreviation</th>
<th>Tissue of origin</th>
<th>Analytical method</th>
<th>Analytical specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin (or bone gla-protein)</td>
<td>OC</td>
<td>Bone, platelets</td>
<td>RIA, ELISA, IRMA</td>
<td>Serum</td>
</tr>
<tr>
<td>Bone-specific alkaline phosphatase</td>
<td>Bone ALP</td>
<td>Bone</td>
<td>ELISA, IRMA, colorimetric assay</td>
<td>Serum</td>
</tr>
<tr>
<td>Procollagen type-I N-propeptide</td>
<td>PINP</td>
<td>Bone, soft tissue, skin</td>
<td>RIA, ELISA</td>
<td>Serum</td>
</tr>
<tr>
<td>Procollagen type I C-propeptide</td>
<td>PICP</td>
<td>Bone, soft tissue, skin</td>
<td>RIA, ELISA</td>
<td>Serum</td>
</tr>
</tbody>
</table>

**Abbreviations:** ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; MMP, matrix metalloproteinase; RIA, radioimmunoassay.

**Table 1** Markers of bone resorption

**Table 2** Markers of bone formation

**Abbreviations:** ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; IRMA, immunoradiometric assay.

**Abbreviations:** ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; IRMA, immunoradiometric assay.

**According to the bone marker nomenclature by the Committee of Scientific Advisors of the International Osteoporosis Foundation.16**

Pyridinoline (PYD) and deoxypyridinoline (DPD) cross-links of type I collagen. PYD and DPD are formed by the enzymatic action of lysyl oxidase on lysine and Hyl. PYD and DPD act as mature cross-links in type I collagen of all major connective tissues (Figure 1).20 The products of bone collagen degradation by osteoclasts include amino- and carboxy-terminal peptide fragments (NTX and CTX, respectively) of various sizes that remain attached to helical portions of nearby collagen molecules by a pyridinium cross-link. These molecules are released into the circulation. Further degradation occurs in the liver and kidney, where the fragments are finally degraded to their constituent amino acids and the pyridiniums, PYD and DPD.
Leukemia

Amino- and Carboxy-terminal cross-linking telopeptide of type I collagen. During bone collagen degradation by osteoclasts, NTX and CTX fragments are released into the circulation. These fragments represent a spectrum of proteins of different molecular weights. The proteolytic activities of cathepsin K and matrix metalloproteinases result in the production of a variety of degradation peptides with different antigenic properties. The majority of these peptides is relatively small and is freely filtered by the glomerulus into the urine. NTX fragments are considered specific for bone tissue breakdown, as other tissues that contain type I collagen, for example, skin, do not undergo osteoclast-mediated metabolism. Thus, different type I collagen fragments are formed during the breakdown of non-skeletal tissues.

An enzyme-linked immunoabsorbent assay method has been developed to recognize a discrete pool of NTX isolated from urine, namely the α2-chain N-telopeptide fragment. Although this fragment contains the pyridinium cross-links, the assay does not recognize the PYD and DPD per se. This confers bone specificity, as the PYD cross-link in bone primarily involves the α2-chain, whereas in other tissues the α1-chain predominates. NTX contains the cross-linked α2 N-telopeptide sequence QYDGKGVG, which is a product of osteoclastic proteolysis, in which lysine (K) is embodied in a trivalent cross-linkage. Collagen must be degraded to small cross-linked peptides that contain this exact sequence before the antibody will recognize the NTX antigen. This ensures that the NTX peptide is a direct product of osteoclastic proteolysis that does not require further metabolism in the liver or kidney for generation, and is rapidly cleared by the kidney. Urinary NTX results are expressed relative to creatinine as nmol of bone collagen equivalent per mmol of creatinine.

Other assays have also been developed for the measurement of epitopes associated with CTX (α2-CTX, β-CTX and CTX generated by matrix metalloproteinases (ICTP)) in both serum and urine. These include a radioimmunoassay that detects ICTP by rabbit polyclonal antibodies directed against a large antigen (10 000 Da) with a preserved trivalent cross-linked structure. This antigen is liberated when type I collagen is degraded by matrix metalloproteinases. In contrast to the approach used in the ICTP assay, the CTX-I (β-CTX) antigen is liberated when type I collagen is degraded by cathepsin K, but not by matrix metalloproteinases. The recognized antigen must be cross-linked, but can be imbedded into fragments of the C-terminal telopeptide of variable size (1000–10 000 Da). As half of the recognized antigens in normal sera are smaller than 3000 Da, it is smaller than the ICTP antigen.

Owing to specificity for type I collagen and their unique characteristics, the bone resorption markers NTX, ICTP and CTX have almost completely replaced the use of older resorption indices in the diagnostic assessment of bone disease.

Tartrate-resistant acid phosphatase isofrom 5b (TRACP-5b). Two forms of TRACP circulate in human serum, macrophage-derived TRACP-5a and osteoclast-derived TRACP-5b. In human serum, TRACP-5b circulates in a large complex that contains z2-microglobulin and calcium. Osteoclasts secrete TRACP-5b into the circulation as a catalytically active enzyme that is inactivated and degraded to fragments in the circulation. Thus, TRACP-5b molecules measured in serum are freshly liberated from osteoclasts, providing a resorpive index that is useful as a surrogate marker for total activated osteoclast numbers.

Bone formation markers

Bone-specific alkaline phosphatase (ALP). ALP is a ubiquitously expressed, cell membrane-associated enzyme. Liver and bone (bALP) isofroms account for almost 95% of the total ALP activity in the serum. bALP is produced by osteoblasts and has been demonstrated in matrix vesicles deposited as ‘buds’ derived from the cell membrane. These deposits have an important role in bone formation. bALP is produced in extremely high amounts during the bone formation phase of bone turnover, and is, therefore, an excellent indicator of total bone formation activity.

Osteocalcin (OC). OC is one of the most abundant non-collagenous proteins within bone. It is produced by osteoblasts, odontoblasts and hypertrophic chondrocytes. Most of the circulating OC is a product of osteoblast activity. OC is incorporated into bone matrix, where it serves to bind calcium; as such, OC is considered a marker of bone formation. OC is a small protein of 49 amino acids. In serum, OC is degraded so that both the intact peptide and fragments coexist in the circulation. Thus, assays that evaluate both intact OC and OC fragments are more accurate for the measurement of serum OC. Serum levels of OC are significantly influenced by gender, age and renal function.

Type I procollagen propeptides. Collagen type I is a 300-kDa protein that comprises 90% of the organic bone matrix. It is synthesized by osteoblasts in the form of procollagen. Extracellular processing of procollagen before collagen fiber assembly includes cleavage of the amino- and carboxy-terminal extension propeptides (termed procollagen type I N-propeptide (PINP) and C-propeptide (PICP)). Circulating PINP is cleared by the scavenger endothelial system in the liver, whereas PICP is removed from the circulation by the mannose receptors on liver endothelial cells. Because PINP and PICP peptides are generated in a stoichiometric 1:1 ratio with newly formed collagen molecules, their serum levels are considered an index of collagen synthesis and thus of bone formation. Most studies suggest that the PINP has a greater diagnostic validity than PICP in metastatic bone disease.
Markers of bone remodeling in myeloma bone disease

Bone turnover markers and extent of myeloma bone disease

Markers of both resorption and formation have been used in attempts to better evaluate the extent of bone disease in MM. Table 3 summarizes the results of the most important studies to date regarding the levels of bone markers in MM patients. As has been shown in multiple studies, urine levels of PYD, DPD and NTX and serum levels of CTX, ICTP and TRACP-5b were elevated in MM patients compared with healthy controls, and correlated with the extent of osteolytic disease. Urinary NTX levels were increased even in myeloma patients who had reached a clinical plateau phase of their disease, whereas PYD, DPD and NTX were also elevated in myeloma patients before autologous transplantation.

A histomorphometric study in bone marrow biopsies of myeloma patients showed that urinary NTX correlated most positively with dynamic histomorphometric indices of bone resorption, followed by serum ICTP and urine DPD: urine PYD did not correlate with the histomorphometric findings. Moreover, comparison between these four markers (PYD, DPD, NTX and ICTP) revealed that serum ICTP and urinary NTX better reflected the extent of myeloma bone disease and could better predict early progression of the bone disease after conventional chemotherapy (CC). However, serum ICTP remained more sensitive than the urinary assays when patients with impaired renal function were excluded from that analysis.

Jakob et al. demonstrated that serum ICTP was elevated in MM patients who did not have detectable osteolytic lesions by plain radiograph, but had abnormal bone magnetic resonance imaging scans. Urinary NTX also correlated with the overall score of skeletal involvement as measured by Tc-99m-MIBI scintigraphy and bone marrow infiltration by plasma cells. Coleman et al. showed in 210 MM patients that high or intermediate urinary NTX correlated with an increased risk for SRE development compared with low NTX values (risk ratio (RR) 2.25, P = 0.032 and RR 1.75, P = 0.016, respectively). High NTX values also correlated with a three-fold increased risk for developing a first SRE (Figure 2), whereas there was also a trend toward increased risk for progression of osteolytic lesions in the high NTX group (P = 0.08). A recent study by Terpos et al. in 282 myeloma patients who participated in a randomized phase III study comparing zoledronic acid or pamidronate. Increased baseline levels of urinary NTX were associated with an increased risk for the development of first SRE (68% increase in risk for SRE development per 100-unit increase of NTX, P = 0.005). In summary, these results suggest that serum ICTP and urinary NTX strongly correlate with the extent of MM bone disease, the risk for the development of SREs, and possibly with risk for MM progression.

Markers of bone turnover have been evaluated in several studies, but the results have been more variable than those found with bone resorption markers. Serum bALP and OC were elevated in myeloma patients compared with controls, whereas in others they were either reduced or within normal limits. In a study by Fonseca et al., which included a large number of myeloma patients (n = 313), serum bALP correlated with bone pain, lesions and fractures, whereas OC levels were lower in myeloma patients than in controls but did not correlate with the extent of bone disease. Furthermore, Coleman et al. showed that myeloma patients with high bALP levels are at increased risk for developing a SRE (RR 3.29; P < 0.001) and for disease progression (RR 2.42; P < 0.001). Terpos et al. showed that OC levels were reduced in myeloma patients and correlated with the extent of bone disease, whereas bALP levels were not. Why differences between the studies exist is not clear, but may reflect different study populations and/or different phases of bone turnover in each population. PICP values do not seem to reflect the extent of myeloma bone disease. As shown in Table 3, although markers of bone formation may be of some value in myeloma, they do not appear to reflect the extent of myeloma bone destruction. Thus at present, their clinical utility is doubtful.

Correlations of bone turnover markers with myeloma activity and survival

In several studies, biochemical markers of bone resorption strongly correlated with stage of MM. Serum ICTP and urinary NTX were higher in myeloma stage II/III than in stage I disease. High UDPIP and urinary NTX levels also correlated with advanced myeloma stage. In 121 newly diagnosed myeloma patients, NTX and TRACP-5b, but not OC or bALP, strongly correlated with disease stage. Two additional studies also failed to show a correlation between OC and bALP with myeloma stage.

Markers of bone remodeling have also correlated with well-characterized markers of disease activity, such as B2-microglobulin and interleukin-6, and also with overall survival (OS). Fonseca et al. showed that the median survival was 4.1 and 3.5 years for patients who received CC and had low or high ICTP levels, respectively (P = 0.02). Jakob et al. also reported that ICTP is a prognostic factor for OS in MM patients treated with CC (P < 0.03), whereas urinary NTX was only of borderline prognostic value (P = 0.05). The same group showed in 100 patients with newly diagnosed symptomatic MM that disease stage, according to International Staging System, del(13q14), high dose therapy and ICTP independently predicted for OS, with ICTP having the most powerful prognostic value (hazard ratio: nine-fold increase; P < 0.001). Incorporation of ICTP in the International Staging System separated four risk groups with a 5-year OS rate of 95, 65, 46 and 22%, respectively.

Abildgaard et al., using sequential measurements of both ICTP and NTX showed that high levels of ICTP and NTX correlated with an increased risk for early progression of bone lesions during CC, suggesting that their measurements are clinically useful for identifying patients with increased risk of early disease progression. In a recent study, Terpos et al. analyzed the effect of urinary NTX on survival in 210 patients participating in a randomized study comparing treatment with either zoledronic acid or pamidronate. Increased baseline levels of urinary NTX (≥ 50 nmol bone collagen equivalent per m M creatinine) correlated with an 88% increased risk of death and a 67% increased risk of first SRE (Figure 3). The update of this study in 282 patients confirmed that high urinary NTX independently predicted for poor survival (RR = 1.60; P = 0.017). These data suggest that the bone resorption markers serum ICTP and urinary NTX have prognostic significance for disease progression and survival in MM under CC, and that their routine measurement in future clinical trials may be of prognostic value in the current era of novel anti-MM agents. In contrast, measurement of bone formation markers seem to be of limited prognostic value.

Bone markers during anti-resorptive therapy

Biochemical markers of bone turnover have been used in MM both to monitor biphosphonate treatment, and to determine
### Table 3  Markers of bone remodeling in myeloma patients and correlations with clinical data

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>No. of patients</th>
<th>Parameter studied</th>
<th>Comparison with controls (symbol refers to MM patients)</th>
<th>Correlation with extent of bone disease</th>
<th>Correlation with survival (in multivariate models)</th>
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<td>Nawawi et al., 1996&lt;sup&gt;43&lt;/sup&gt;</td>
<td>17</td>
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<td>109</td>
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<td>DPD, ICTP, OC, PICP</td>
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<td>62</td>
<td>NTX, OC, bALP</td>
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<td>57</td>
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<td>↑ ↑ ↑</td>
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<td>Terpos et al., 2003&lt;sup&gt;55,56&lt;/sup&gt;</td>
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<td>25</td>
<td>CTX, DPD</td>
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those subjects who would benefit most from bisphosphonate therapy. C Terpos et al. showed that the addition of pamidronate to CC significantly reduced urinary NTX and disease-related pain compared with CC alone, and that pamidronate in combination with interferon-α induced bone formation in MM patients at plateau phase. Ibandronate at a dose of 2 mg showed a substantial reduction of CTX and OC in only one-third of MM patients, whereas in a randomized study, monthly pamidronate (90 mg, IV) produced a greater reduction of NTX and TRACP-5b compared with monthly ibandronate (4 mg, IV). In a large, randomized study comparing 4 mg zoledronic acid with 90 mg pamidronate, given IV every 3–4 weeks in patients with bone metastases from breast cancer or with MM osteolytic disease, urinary NTX was strongly suppressed (up to 64% below baseline in both treatment groups) for the duration of the study. Bone marker data from this and other bisphosphonate studies clearly demonstrate that there is a subset of myeloma patients who do not respond to, or who

Table 3 (Continued)

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>No. of patients</th>
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<th>Comparison with controls</th>
<th>Correlation with extent of bone disease</th>
<th>Correlation with survival (in multivariate models)</th>
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<td>DPD</td>
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</tbody>
</table>

Abbreviations: bALP, bone-specific alkaline phosphatase; BSP, bone sialoprotein; CTX, carboxy-terminal cross-linking telopeptide of type I collagen; DPD, deoxypyridinoline; ICTP, carboxy-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinases; NA, not assessed; NS, non significant; NTX, amino-terminal cross-linking telopeptide of type I collagen; OC, osteocalcin; OPG, osteoprotegerin; PICP, procollagen type I C-propeptide; PIIINP, N-terminal propeptide of procollagen type III; PYD, pyridinoline; sRANKL, soluble receptor activator of nuclear factor-κB ligand; TRACP-5b, tartrate-resistant acid phosphatase isofom 5b.

\(^a\)MM compared with MGUS patients.

\(^c\)Correlation with SREs.

\(^c\)In this study high NTX also independently predicted for high risk for development of first SRE.

Figure 2 Relative risk for experiencing any skeletal-related event (SRE) and a first SRE for multiple myeloma patients with high levels of N-telopeptide of type I collagen (NTX; ≥ 100 nm bone collagen equivalent (BCE)/mM creatinine) or moderate NTX (50–99 nm BCE/mM creatinine) versus patients with low NTX (<50 nm BCE/mM creatinine) treated with zoledronic acid. Length of horizontal lines represents 95% confidence intervals.57,77

Figure 3 Kaplan–Meier Curves for (a) survival and (b) first on-study SRE by baseline NTX levels in 210 myeloma patients treated with conventional chemotherapy and zoledronic acid.70 NTX, N-terminal cross-linking telopeptide of type I collagen; E, elevated; N, normal; RR, risk ratio; CI, confidence interval; SRE, skeletal-related event.
become refractory to bisphosphonate therapy. Patients with persistently elevated bone marker levels are at higher risk for SREs and disease progression compared with patients who respond to bisphosphonate therapy and have normalized bone resorption. Lipton et al. showed that among breast cancer or myeloma patients (n = 170) who had high baseline NTX levels (≥64 nm bone collagen equivalent per mm creatinine), those with persistently elevated NTX levels after 3 months of zoledronic acid therapy (n = 26, 15%) had a significantly increased risk of developing a first SRE (RR = 1.71; P = 0.035) and shorter SRE-free survival (RR = 1.65; P = 0.039) compared with subjects who normalized NTX in response to bisphosphonate treatment (n = 137, 81%). In this study, among patients with high NTX at baseline, 15% treated with zoledronic acid and 30% treated with pamidronate did not normalize NTX levels after 3 months of bisphosphonate therapy. Although unknown, one might speculate that patients who did not have biochemical improvement in their NTX levels may have an osteoclast-independent mechanism of bone resorption and might, therefore, benefit from additional therapies.

Denosumab is a fully human monoclonal antibody against receptor activator of nuclear factor-κB ligand (RANKL), the most potent osteoclast activator to-date (see below). In a recent study, 1776 adult patients with solid tumors or MM (n = 10% of the total) who were naïve to intravenous bisphosphonates were randomized to receive either subcutaneous denosumab 120 mg or intravenous zoledronic acid every 4 weeks. Denosumab produced similar results regarding the delay in time to first on-study SRE or subsequent SREs compared with zoledronic acid, whereas it also rapidly and potently reduced (by more than 80% within the first month) urinary NTX levels.

Novel molecules related to bone remodeling and myeloma bone disease
RANKL and Osteoprotegerin (OPG). RANKL and OPG have crucial roles in the development of myeloma bone disease. Although RANKL increases osteoclastogenesis and osteoclast activity, OPG serves as a soluble inhibitor of RANKL activity. Within the myeloma bone marrow microenvironment, the RANKL/OPG ratio is shifted in favor of RANKL, leading to increased osteoclastogenesis and increased bone resorption.

Multiple studies have now documented that in MM, serum OPG levels are reduced, whereas the soluble RANKL/OPG ratio is increased. This altered ratio correlates with extent of bone disease, markers of bone resorption, such as NTX and TRACP-5b and myeloma stage. The administration of zoledronic acid in patients with asymptomatic myeloma was found to increase serum levels of OPG and thus reduce the RANKL/OPG ratio, likely accounting for the effect of zoledronic acid on osteoblast and/or bone marrow stromal cells together with its direct effect on osteoclasts. The RANKL/OPG ratio has been found to correlate with OS in MM. This result has not been confirmed in all reported studies to date. may be due to differences in patient populations and/or therapies administered. Further studies are needed before measurement of serum RANKL and OPG levels in the everyday clinical setting should be considered.

Other potential molecules reflecting bone destruction in MM. Bone sialoprotein is a phosphorylated 70–80 kDA glycoprotein that accounts for 5–10% of the non-collagenous bone matrix. Bone sialoprotein is involved in the adhesion of bone resorbing cells to the extracellular bone matrix. In one study of MM, bone sialoprotein levels were associated with skeletal involvement and tumor cell burden. However, further evaluation is needed to support its value in MM bone disease.

Dickkopf-1 (Dkk-1) protein is an inhibitor of the Wingless-type and Int (Wnt) pathway, a pathway crucial for stimulation of osteoblast activity. Tian et al. were the first to describe that increased expression of Dkk-1 in plasma cells correlates with the presence of lytic lesions both by plain radiography and magnetic resonance imaging. Bone marrow plasma Dkk-1 levels were increased in MM patients, and associated with Dkk-1 concentrations in peripheral blood, levels of Dkk-1 transcripts in myeloma cells and the presence of osteolytic lesions. Gene expression studies in 171 newly diagnosed MM patients showed that overexpression of Dkk-1 correlated with the degree of osteolytic bone disease. Similarly, serum Dkk-1 levels were elevated in myeloma patients with lytic bone disease compared with those without lytic lesions by conventional radiography, and also correlated with the number of bone lesions. In contrast, patients with mononclonal gammopathy of undetermined significance had lower serum levels of Dkk-1 compared with MM subjects, with levels similar to those found in control subjects. High values of serum Dkk-1 also correlated with advanced ISS stage. It is interesting that, a recent report from Yaccoby and colleagues demonstrated that Dkk-1-negative myeloma cells in trephine biopsies had more aggressive features and plasmablastic morphology, whereas Dkk-1 was rarely expressed by plasma cells of plasma cell leukemia. The current role of Dkk-1 in the clinical assessment of myeloma bone disease, however, remains to be determined.

The effect of novel anti-myeloma agents on markers of bone remodeling

During the last decade, immunomodulatory drugs, including thalidomide and lenalidomide and proteasome inhibitors, such as bortezomib, have been increasingly used for the treatment of MM. Thalidomide, lenalidomide and bortezomib are effective agents for the treatment of both newly-diagnosed and relapsed/refractory MM. The role of these drugs in bone metabolism has been evaluated in several studies.

Immunomodulatory drugs
Two clinical phase II trials have studied the effect of thalidomide on bone metabolism of MM patients (Table 4). In the first study, Terpos et al showed that in relapsed/refractory MM patients, the combination of thalidomide (200 mg per day) with dexamethasone (TD) produced a significant reduction of serum levels of CTX, TRACP-5b and sRANKL/OPG after 6 months of therapy. There was a strong correlation between changes in the sRANKL/OPG ratio and changes in TRACP-5b and CTX, suggesting that the reduction of bone resorption by TD is in part due to the reduction of RANKL. TD showed no effect on bone formation in that study. In the second study, Tosi et al showed in newly diagnosed MM patients that the combination of TD and zoledronic acid for 4 months produced a significant reduction of urinary NTX and serum CTX, but only in patients who responded to therapy. This reduction was accompanied by a reduction in bone pain in 60% of the patients, and also by a reduction of bALP and OC levels in both responding and refractory patients. This negative effect of TD on bone formation may be due to the concomitant use of high-dose dexamethasone in these patients.

Limited data exists for the effects of lenalidomide on bone remodeling in myeloma patients. In a small study, lenalidomide
An increasing number of studies have now reported the beneficial effects of bortezomib on bone formation in the clinical setting, confirming preclinical observations. As described by Heider et al., the combination of bortezomib and dexamethasone produced significant increases in serum bALP and OC in both responders and non-responders. Giuliani et al. found significant increases in the number of osteoblasts per mm² of bone tissue in trephine biopsies of patients responding to bortezomib, but not in subjects who did not respond. Terpos et al. showed that, in 34 patients bortezomib monotherapy reduced serum Dkk-1 and RANKL levels. This was associated with a concomitant reduction in bone resorption (serum TRACP-5b and CTX) and increase in markers of bone formation (serum bALP and OC), changes that occurred irrespective of response to therapy.

However, when bortezomib was combined with other anti-myeloma agents (VMDT regimen, combination of bortezomib, melphalan, dexamethasone and thalidomide), the reduction of Dkk-1, sRANKL and CTX was not accompanied by increases in ICTP and urinary NTX, changes that occurred irrespective of response to therapy. From the available data, it seems that immunomodulatory drugs reduce osteoclast function but have little or no effect on osteoblast activity.

**Bortezomib**

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**Reduced serum RANKL and increased serum OPG levels, leading to a reduction of the RANKL/OPG ratio.** From the available data, it seems that immunomodulatory drugs reduce osteoclast function but have little or no effect on osteoblast activity.
**Table 5** Summary of the role of markers of bone metabolism in multiple myeloma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reflection of the extend of myeloma bone disease</th>
<th>Prediction for SRE</th>
<th>Prediction for OS</th>
<th>Future possible use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone resorption markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary NTX</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>1. Symptomatic patients to drive initial therapy (NTX)</td>
</tr>
<tr>
<td>Serum ICTP</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>2. Asymptomatic patients to drive decision for anti-resorptive therapy (NTX, ICTP, CTX)</td>
</tr>
<tr>
<td>Serum CTX</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>3. Symptomatic patients under bisphosphonates to decide the duration and intervals of therapy (NTX, ICTP, CTX)</td>
</tr>
<tr>
<td>Serum TRACP-5b</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Bone formation markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum bALP</td>
<td>+/-</td>
<td>–</td>
<td>–</td>
<td>1. Use for the evaluation of bone anabolic agents, such as bortezomib, anti-Dkk1, anti-SOST antibodies (bBALP only)</td>
</tr>
<tr>
<td>Serum OC</td>
<td>+/-</td>
<td>–</td>
<td>–</td>
<td>2. No future use is seen for other bone formation markers</td>
</tr>
<tr>
<td>Serum PINP or PICP</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Osteoclast/osteoblast regulators</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum sRANKL or tRANKL</td>
<td>+/-</td>
<td>–</td>
<td>+/-</td>
<td>1. Use for the follow-up of novel therapies (denosumab-anti-RANKL, anti-Dkk1 etc)</td>
</tr>
<tr>
<td>Serum OPGL</td>
<td>+/-</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Serum Dkk-1</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: (–), no evidence; (+/-), conflicting evidence; (+), low evidence; (++), intermediate evidence; (+++), strong evidence; bALP, bone alkaline phosphatase; CTX, carboxy-terminal cross-linking telopeptide of type I collagen; Dkk-1, dickkopf-1; ICTP, carboxy-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinases; NTX, amino-terminal cross-linking telopeptide of type I collagen; OC, osteocalcin; OPGL, osteo-protegerin; PICP, pro-collagen type I carboxy-propeptide; PINP, pro-collagen type I amino-propeptide; SOST, sclerostin; sRANKL, soluble receptor activator of nuclear factor-xB ligand; TRACP-5b, tartrate resistant acid phosphatase isofform 5b; tRANKL, total RANKL.

In the current era of concern about bisphosphonate-associated adverse side-effects (that is, renal impairment, osteonecrosis of the jaw, subtrochanteric femoral fractures), bone turnover markers may be of particular use: that is, low levels of serum ICTP/CTX or urinary NTX may provide impetus for deciding to lengthen bisphosphonate dosing regimens (that is, changing from monthly to 3-month intervals). Clinical studies have started in patients with breast cancer and bone metastases (BISMARK trial) and are eagerly anticipated in myeloma. Further, with the development of novel anti-resorptive agents (denosumab, other anti-RANKL agents) and drugs that enhance osteoblast function (anti-Dkk1 antibodies), the measurement of bone biochemical indices will likely be of particular value. However, additional trials, such as those described above are urgently needed (and indeed should be highly encouraged) before final conclusions are made about the introduction of biochemical markers of bone remodeling into the routine clinical care of MM patients.

**Conflict of interest**

The authors declare no conflict of interest.

**Authors’ Contributions**

ET collected data and wrote paper; MAD collected data and wrote paper; OS contributed comments and edited paper; DR contributed comments and edited paper; RV contributed comments and edited paper; NA contributed comments and edited paper; PT contributed comments and edited paper; RG-S contributed comments and edited paper; FD contributed comments and edited paper; AC-K contributed comments and edited paper; AP contributed comments and edited paper; PS contributed comments and edited paper; MTD contributed comments and edited paper; J-LH contributed comments and edited paper; KCA contributed comments and edited paper; BGMD contributed comments and edited paper

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Appendix

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Leukemia