

# Expression of Receptor Activator of Nuclear Factor $\kappa$ B Ligand on Bone Marrow Plasma Cells Correlates with Osteolytic Bone Disease in Patients with Multiple Myeloma

Ulrike Heider, Corinna Langelotz,  
Christian Jakob, Ivana Zavrski,  
Claudia Fleissner, Jan Eucker, Kurt Possinger,  
Lorenz C. Hofbauer, and Orhan Sezer<sup>1</sup>

Department of Oncology and Hematology, Universitätsklinikum Charité, Berlin 10098 [U. H., C. L., C. J., I. Z., C. F., J. E., K. P., O. S.], and Department of Gastroenterology, Endocrinology, and Metabolism, Philipps Universität Marburg, Marburg [L. C. H.], Germany

## ABSTRACT

**Purpose:** Increased bone resorption is a hallmark of multiple myeloma and is attributable to osteoclast activation. Recent studies showed that the receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) is the key mediator of osteoclastogenesis and plays a crucial role in bone destruction in malignant bone disease. We found that human myeloma cells express RANKL and analyzed the association of the RANKL expression with the presence of osteolytic bone disease in patients with multiple myeloma.

**Experimental Design:** Flow cytometry was performed on bone marrow samples derived from controls and multiple myeloma patients with or without osteolytic bone lesions on conventional radiography. Plasma cells were identified as CD38+ /CD138+ cells. The level of RANKL expression on the surface of bone marrow plasma cells was correlated with the bone status of the patients.

**Results:** The bone marrow plasma cells from controls showed no or only a weak surface expression of RANKL, and the median mean fluorescence index (MFI) was 6. In contrast, expression of RANKL could be detected on bone marrow plasma cells from all of the patients with multiple myeloma, and median MFI was 47. The difference in MFI for RANKL expression of bone marrow plasma cells from controls and myeloma patients was highly significant ( $P < 0.0005$ ). Myeloma patients with osteolytic bone lesions showed a significantly higher expression of RANKL (median MFI = 60; range, 16–2494) compared with patients without osteolysis (median MFI = 16; range, 6–229;  $P < 0.0005$ ).

**Conclusions:** These results show for the first time that the level of RANKL expression by myeloma cells correlates significantly with osteolytic bone disease.

## INTRODUCTION

Multiple myeloma is characterized by clonal expansion of malignant plasma cells in the bone marrow. Osteolytic bone disease is a hallmark of multiple myeloma, and is caused by excessive osteoclast differentiation and activation. Examination of bone marrow biopsy specimens from patients with multiple myeloma showed accumulation of osteoclasts on bone resorbing surfaces adjacent to myeloma cells (1). Therefore, it has been suggested that osteoclast activity is regulated by local cytokines, which are produced by myeloma or other cells in the local bone marrow microenvironment (2). However, the mechanism(s) and factors leading to bone resorption in multiple myeloma remain to be clarified.

As reviewed by Teitelbaum (3), a novel system of cytokines was identified as key mediators in osteoclastogenesis, including the RANKL,<sup>2</sup> receptor activator of nuclear factor  $\kappa$ B, and OPG. RANKL is a member of the TNF superfamily and plays a crucial role in osteoclast development. It exists as a cell membrane-bound isoform, a secondary soluble variant that is cleaved from the cellular form by metalloproteases, and a primary secreted isoform. RANKL binds to its specific receptor, receptor activator of nuclear factor  $\kappa$ B, which is located on osteoclastic precursors. RANKL induces changes in patterns of preosteoclast gene expression, resulting in differentiation, formation, survival, and fusion of preosteoclasts (4). Furthermore, it has direct effects on mature osteoclasts inducing actin ring formation, a marked cytoskeletal rearrangement that necessarily precedes bone resorption, and activating mature osteoclasts to resorb bone (5). OPG is a member of the TNF receptor superfamily and is secreted as a soluble protein by various tissues (6). OPG acts as a decoy receptor for RANKL, and inhibits osteoclast differentiation and activation by neutralizing the biological effects of RANKL (7).

Recent studies showed that RANKL is a potent factor for bone destruction in benign and malignant bone diseases (8). Several studies demonstrated RANKL to be an essential cytokine that regulates tumor-bone interactions. Recent reports suggested that an enhanced RANKL:OPG ratio is the cause of osteolytic bone disease in multiple myeloma (9). Several investigators detected RANKL mRNA and protein in human my-

Received 8/12/02; revised 12/9/02; accepted 12/10/02.

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

<sup>1</sup> To whom requests for reprints should be addressed, at Universitätsklinikum Charité, Department of Oncology and Hematology, 10098 Berlin, Germany. Phone: 49-30-450-513105; Fax: 49-30-450-527907; E-mail: sezer@charite.de.

<sup>2</sup> The abbreviations used are: RANKL, receptor activator of nuclear factor  $\kappa$ B ligand; OPG, osteoprotegerin; TNF, tumor necrosis factor; MFI, mean fluorescence index; IL, interleukin; MMP, matrix metalloproteinase.

eloma cell lines (10, 11) and murine myeloma cells (12). Other authors described RANKL expression in stromal cells (13). Oyajobi *et al.* (14) demonstrated in a murine myeloma model that interactions between myeloma and stromal cells lead to an increased RANKL mRNA expression in both cell types. Given these results in human and murine models, we investigated recently the RANKL expression of human bone marrow myeloma cells of 10 patients with multiple myeloma and osteolytic bone lesions. Of note, RANKL expression was detected in all of the cases studied (15). Immunocytochemistry also revealed RANKL expression in bone marrow myeloma cells (16). Furthermore, we could detect an expression of RANKL mRNA in plasma cells from multiple myeloma patients.<sup>3</sup> Therefore, we focused in the present study on the clinical relevance of the RANKL expression on bone marrow myeloma cells. We analyzed the surface expression of RANKL protein on plasma cells obtained by bone marrow aspiration using flow cytometry, and compared the level of RANKL expression on plasma cells in controls and multiple myeloma patients with or without osteolytic lesions.

## PATIENTS AND METHODS

**Patients.** Fifty patients with multiple myeloma were included into this study. Diagnosis and stage classification was done according to the criteria of Durie and Salmon. In all of the patients, conventional radiography was performed including X-ray scans of the skull, cervical, thoracic and lumbar spine, ribs, humeri and femora, and pelvis. Fifteen patients (group I) showed no osteolytic bone lesions, whereas one or more osteolytic lesions were found in 35 patients (group II). Bone marrow aspirates from 10 patients with nonmalignant hematological disorders served as controls. Bone marrow aspiration was performed for routine diagnostic purposes after informed consent had been obtained. Samples were collected in EDTA anticoagulant and analyzed within 4 h.

**Flow Cytometry.** Three-color flow cytometry was performed in bone marrow aspirates from 50 patients with multiple myeloma. The following monoclonal antibodies were used: CD38 PC5 (Immunotech, Marseille, France), CD138 FITC (B-B4; Serotec, Oxford, United Kingdom), monoclonal antihuman TRANCE/TNFSF11 (RANKL) antibody (clone 70525.11; R&D Systems, Inc., Minneapolis, MN), IgG2b isotypic control and phycoerythrin-conjugated F(ab')<sub>2</sub> fragment goat antimouse IgG (Immunotech). Cells were consecutively incubated with the monoclonal mouse antihuman RANKL antibody or the isotypic control (mouse immunoglobulin G2b) and the secondary antibody for 30 min at 4°C. Cells were finally stained with the anti-CD38 and anti-CD138 antibodies to allow the identification of plasma cells. Erythrocytes were lysed using ammonium chloride lysis Ortho-mune (Ortho-Clinical Diagnostics, Neckargmund, Germany). Cells (50,000) of each sample were analyzed on a FACSsort flow cytometer (Becton Dickinson, Heidelberg, Germany) using the Cellquest software. Plasma cells were identified as CD38 strongly positive and CD138 (B-B4) positive

Table 1 Patient characteristics in group I without osteolytic bone lesions and group II with one or more osteolytic lesions

Parameter	MM <sup>a</sup> Group I (n = 15)	MM Group II (n = 35)
Age, median (range)	65 (56–80)	66 (41–87)
Male/female	1.1/1	1.1/1
Immunoglobulin type		
IgG	80%	57%
IgA	20%	11%
Bence-Jones	0%	26%
Nonsecretory	0%	6%
Plasma cell infiltration in bone marrow (%), median (range)	30 (5–90)	70 (5–99)
RANKL MFI, median (range)	16 (6–229)	60 (16–2494)

<sup>a</sup> MM, multiple myeloma.

cells as described previously (17). RANKL expression was analyzed by calculating the MFI of the anti-RANKL antibody on gated plasma cells. As a control, the surface expression of RANKL on plasma cells was studied in 10 patients with non-malignant hematological disorders.

**Statistical Analysis.** The Mann-Whitney U test was applied to compare two independent groups. For all of the tests  $P < 0.05$  were considered as statistically significant.

## RESULTS

In all of the bone marrow samples derived from controls, CD38+ /CD138+ cells could be detected. These plasma cells showed no or only weak surface expression of RANKL (median MFI, 6; range, 4–22). By contrast, CD38+ /CD138+ cells of all of the multiple myeloma patients expressed RANKL. The median MFI of plasma cells for RANKL expression was 47 (range, 6–2494) in multiple myeloma. The difference in the intensity of RANKL expression of bone marrow plasma cells was highly significant between controls and myeloma patients ( $P < 0.0005$ ).

According to the bone status determined by radiography, multiple myeloma patients were divided into two groups, one without osteolytic bone lesions (group I) and one with osteolytic lesions (group II). The characteristics of the patients are shown in Table 1. Bone marrow plasma cells derived from patients with osteolytic bone lesions showed a significantly higher level of surface RANKL expression (median MFI = 60; range, 16–2494) compared with plasma cells from patients without osteolysis (median MFI = 16; range, 6–229;  $P < 0.0005$ ). Interestingly, patients with advanced myeloma and high bone marrow infiltration do not necessarily show a high RANKL expression on plasma cells if no osteolytic lesions are present. Two patients with stage III myeloma with extensive bone marrow infiltration by plasma cells (90% in both cases) but no osteolysis expressed RANKL at a very low level on bone marrow plasma cells (MFI = 9 and 16, respectively). On the other hand, 2 myeloma patients with low plasma cell infiltration (25% and 30%, respectively) and high level of RANKL expression on their plasma cells had osteolytic bone disease.

Fig. 1 demonstrates representative flow cytometric histograms showing the RANKL expression on plasma cells compared with isotypic control in the two groups of patients with

<sup>3</sup> Unpublished observations.

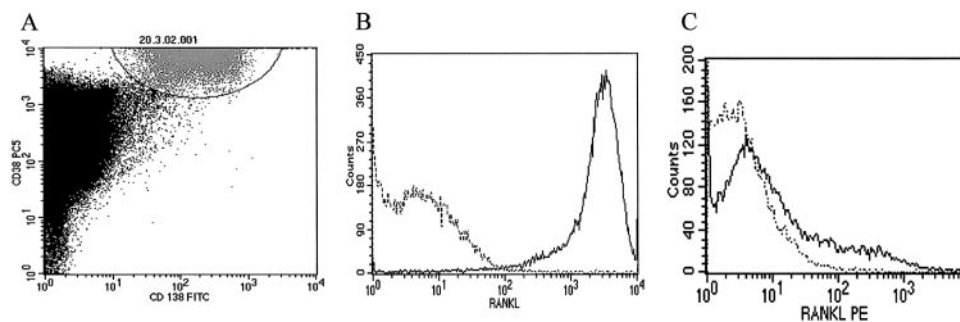


Fig. 1 Surface labeling for RANKL in bone marrow aspirates from multiple myeloma patients. A, identification of plasma cells according to their CD38<sup>+</sup>/B-B4 expression. B and C, RANKL expression (—) of the gated plasma cells compared with isotypic control (····). B, patient with osteolytic lesions and (C) patient without osteolytic lesions.

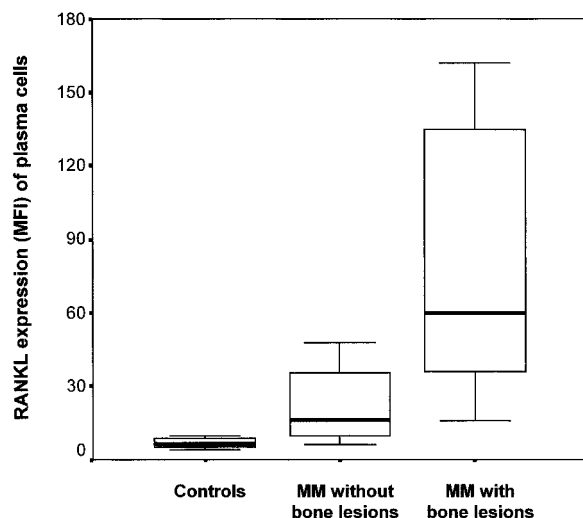


Fig. 2 Box plots of MFI of RANKL expression on bone marrow plasma cells from controls, and multiple myeloma (MM) patients with or without osteolytic lesions.

multiple myeloma, with and without osteolytic lesions, respectively. Boxplots of the MFI of the RANKL expression in different groups of patients are shown in Fig. 2.

## DISCUSSION

Excessive bone destruction is a hallmark of multiple myeloma. Because osteoclasts accumulate only on bone-resorbing surfaces adjacent to myeloma cells, but not unaffected areas, it has been proposed that local production of cytokines regulate osteoclast activity (1). Local factors were supposed to activate osteoclasts in multiple myeloma but the entire mechanism leading to bone resorption remains to be clarified. A number of potentially osteoclast-activating factors, like IL-1 $\beta$ , IL-6, TNF- $\alpha$ , hepatocyte growth factor, and metalloproteinases MMP1, MMP2, and MMP9, were discussed in multiple myeloma, but none of these factors were either present at high levels in the majority of the patients or correlated with the activity of their bone disease (1). For example, IL-1 mRNA (18) but not high levels of IL-1 protein (19, 20) could be detected in bone marrow samples of myeloma patients. In addition, no correlation between IL-6 levels and osteolytic bone disease was found (19, 21). Moreover, TNF- $\alpha$  was detected in clonal plasma

cells both from patients with myeloma and monoclonal gammopathy of unknown significance without osteolytic lesions. Thus, these factors cannot be regarded as main inducers of osteoclast activation in multiple myeloma. On the other hand, a novel factor, macrophage inflammatory protein 1- $\alpha$ , seems to play a role in the induction of bone resorption in this disease (22).

RANKL was identified recently as the key mediator of osteoclastogenesis, and several studies indicated that a dysbalance in the RANKL-OPG axis leads to excessive bone resorption. In murine models, altered expression of these cytokines led to extreme skeletal phenotypes, *i.e.*, severe osteopetrosis in RANKL knockout mice (23) and osteoporosis in OPG-deficient mice (24). In humans, an abnormal RANKL:OPG ratio was found in benign and malignant bone diseases (6, 8).

Myeloma cells have been demonstrated to produce and to shed syndecan-1, which inactivates OPG produced within the confines of bone (25). In multiple myeloma, the RANKL:OPG ratio was found to be increased (13, 26); however, it remained unclear whether RANKL is expressed by either myeloma cells themselves or by stromal cells in response to myeloma cell contact, or by both cell types. Croucher *et al.* (12) detected mRNA and protein expression of RANKL in 5T2MM myeloma cells. Furthermore, numerous investigators reported direct expression of RANKL by human myeloma cell lines or by human bone marrow myeloma cells (10, 11, 15, 16, 27, 28). Other studies unambiguously showed that myeloma leads to an increased RANKL expression by bone marrow stromal cells, as described by Pearse *et al.* (26). Some authors could not detect RANKL on plasma cells (13, 26). The discrepancies might be because of methodological differences, as different isoforms of RANKL exist (29), and different primers were used by different groups. The immunohistochemical evaluation of formalin-fixed, decalcified, and paraffin-embedded tissue sections may be associated with some limitations, because it is known that decalcification of formalin-fixed tissue samples reduces its antigenicity (16, 30).

In the current study, RANKL protein was detected on the surface of plasma cells obtained by bone marrow aspirates from all of the patients with multiple myeloma. These results suggest that myeloma cells are promoting osteoclast differentiation through direct cell-cell contact. The level of RANKL expression on plasma cells was significantly higher in patients with multiple myeloma than in healthy controls. Furthermore, myeloma cells in patients with one or more osteolytic bone lesions ex-

pressed RANKL significantly higher compared with myeloma cells in patients without osteolysis. In the group of patients with osteolytic lesions, the mean percentage of plasma cell infiltration in the bone marrow was also increased. Therefore, it appears possible that increased bone destruction may be because of increased bone marrow infiltration in addition to enhanced RANKL expression. Nevertheless, there were patients in this study with advanced myeloma and high bone marrow infiltration who expressed RANKL at a low level and did not have osteolytic lesions. On the other hand, some myeloma patients with low plasma cell infiltration and high level of RANKL expression on their plasma cells had osteolytic bone disease. These findings show that the level of expression of RANKL on bone marrow plasma cells correlates with the bone status in multiple myeloma and underscores the clinical relevance of the RANKL expression by myeloma cells. Similar findings have also been reported in patients with adult T-cell leukemia, which is characterized frequently by episodes of severe hypercalcemia as a result of excessive osteoclastic bone resorption (31). In this study, RANKL mRNA was overexpressed in adult T-cell leukemia cells of patients with hypercalcemia but not without hypercalcemia, and the level of RANKL gene expression was positively correlated with the capacity to induce osteoclasts and the severity of hypercalcemia.

Our data are of clinical relevance, because OPG may be used to correct the imbalance in the RANKL-OPG system to treat myeloma bone disease. In murine models of multiple myeloma, a soluble RANK fusion protein was able to inhibit bone resorption (32). Animal models showed that OPG treatment was able to mitigate bone destruction in malignant bone disease (33) and in a murine model of myeloma (12). In clinical studies, postmenopausal women showed a decrease of urinary *N*-telopeptide levels, a biochemical marker of bone resorption, after receiving a single dose of OPG, indicating that OPG therapy is feasible in humans (34). Given the excessive RANKL expression by myeloma cells and increased bone resorption in multiple myeloma, strategies aimed at targeting the RANKL-OPG imbalance may have a therapeutic potential for patients suffering from this disease.

## REFERENCES

- Roodman, G. D. Biology of osteoclast activation in cancer. *J. Clin. Oncol.*, 19: 3562–3571, 2001.
- Callander, N. S., and Roodman, G. D. Myeloma bone disease. *Semin. Hematol.*, 38: 276–285, 2001.
- Teitelbaum, S. L. Bone resorption by osteoclasts. *Science (Wash. DC)*, 289: 1504–1508, 2000.
- Hsu, H., Lacey, D. L., Dunstan, C. R., Solovyev, I., Colombero, A., Timms, E., Tan, H. L., Elliott, G., Kelley, M. J., Sarosi, I., Wang, L., Xia, X. Z., Elliott, R., Chiu, L., Black, T., Scully, S., Capparelli, C., Morony, S., Shimamoto, G., Bass, M. B., and Boyle, W. J. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. *Proc. Natl. Acad. Sci. USA*, 96: 3540–3545, 1999.
- Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y. X., Kaufman, S., Sarosi, I., Shalhoub, V., Senaldi, G., Guo, J., Delaney, J., and Boyle, W. J. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell*, 93: 165–176, 1998.
- Khosla, S. Minireview: The OPG/RANKL/RANK system. *Endocrinology*, 142: 5050–5055, 2001.
- Simonet, W. S., Lacey, D. C., Dunstan, C. R., Kelley, M. J., Chang, M.-S., Lüthy, R., Nguyen, H. Q., Wooden, S., Bennett, L., Boone, T., Shimamoto, G., DeRose, M., Elliott, R., Colombero, A., Tan, H.-L., Trail, G., Sullivan, J., Davy, E., Bucay, N., Renshaw-Gegg, L., Hughes, T. M., Hill, D., Pattison, W., Campbell, P., Sander, S., Van, G., Tarpley, J., Derby, P., Lee, R., and Boyle, W. J. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*, 89: 309–319, 1997.
- Hofbauer, L. C., Neubauer, A., and Heufelder, A. E. Receptor activator of nuclear factor- $\kappa$ B ligand and osteoprotegerin. *Cancer (Phila.)*, 92: 460–470, 2001.
- Sezer, O., Heider, U., Zavrski, I., Kühne, C. A., and Hofbauer, L. C. RANK ligand and osteoprotegerin in myeloma bone disease. *Blood*, 101:2094–2098, 2003.
- Westin, J. S., Ljunghall, S., Nilsson, K., and Ljunggren, O. Human multiple myeloma cell lines express mRNA for osteoclast differentiation factor. *J. Bone Miner. Res.*, 14(Suppl. 1): S225, 1999.
- Altamirano, C. V., Ma, H. J., Parker, K. M., Glowalla, A., Mikail, A. E., Sjak-Shie, N., Vescio, R., and Berenson, J. R. RANKL is expressed in malignant multiple myeloma cell lines. *Blood*, 96(Suppl. 1): 1578, 2000.
- Croucher, P. I., Shipman, C. M., Lippitt, J., Perry, M., Asosingh, K., Hijzen, A., Brabbs, A. C., van Beek, E. J., Holen, I., Skerry, T. M., Dunstan, C. R., Russell, G. R., Van Camp, B., and Vanderkerken, K. Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma. *Blood*, 98: 3534–3540, 2001.
- Giuliani, N., Bataille, R., Mancini, C., Lazzaretti, M., and Barille, S. Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow microenvironment. *Blood*, 98: 3527–3533, 2001.
- Oyajobi, B. O., Traianedes, K., Yoneda, T., and Mundy, G. R. Expression of Rank ligand (RANKL) by myeloma cells requires binding to bone marrow stromal cells via an a4b1-VCAM-1 interaction. *Bone*, 23: S180, 1998.
- Sezer, O., Heider, U., Jakob, C., Eucker, J., and Possinger, K. Human bone marrow myeloma cells express RANKL. *J. Clin. Oncol.*, 20: 353–354, 2002.
- Sezer, O., Heider, U., Jakob, C., Zavrski, I., Eucker, J., Possinger, K., Sers, C., and Krenn, V. Immunocytochemistry reveals RANKL expression of myeloma cells. *Blood*, 99: 4646–4647, 2002.
- Sezer, O., Heider, U., Zavrski, I., and Possinger, K. Differentiation of MGUS and multiple myeloma using flow cytometric characteristics of plasma cells. *Haematologica*, 86: 837–843, 2001.
- Lacy, M. Q., Donovan, K. A., Heimbach, J. K., Ahmann, G. J., and Lust, J. A. Comparison of interleukin-1  $\beta$  expression by *in situ* hybridization in monoclonal gammopathy of undetermined significance and multiple myeloma. *Blood*, 93: 300–305, 1999.
- Sati, H. I., Greaves, M., Apperley, J. F., Russell, R. G., and Croucher, P. I. Expression of interleukin-1  $\beta$  and tumour necrosis factor- $\alpha$  in plasma cells from patients with multiple myeloma. *Br. J. Haematol.*, 104: 350–357, 1999.
- Choi, S. J., Cruz, J. C., Craig, F., Chung, H., Devlin, R. D., Roodman, G. D., and Alsina, M. Macrophage inflammatory protein 1- $\alpha$  (MIP-1  $\alpha$ ) is a potential osteoclast stimulatory factor in multiple myeloma. *Blood*, 96: 671–675, 2000.
- Ballester, O. F., Moscinski, L. C., Lyman, G. H., Chaney, J. V., Saba, H. I., Spiers, A. S., and Klein, C. High levels of interleukin-6 are associated with low tumor burden and low growth fraction in multiple myeloma. *Blood*, 83: 1903–1908, 1994.
- Han, J. H., Choi, S. J., Kurihara, N., Koide, M., Oba, Y., and Roodman, G. D. Macrophage inflammatory protein 1- $\alpha$  (MIP-1  $\alpha$ ): a potential osteoclastogenic factor in myeloma. *Blood*, 97: 3349–3353, 2001.
- Kong, Y. Y., Yoshida, H., Sarosi, I., Tan, H. L., Timms, E., Capparelli, C., Morony, S., Oliveira-dos-Santos, A. J., Van, G., Itie, A., Khoo, W., Wakeham, A., Dunstan, C. R., Lacey, D. L., Mak, T. W.,

- Boyle, W. J., and Penninger, J. M. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature (Lond.)*, *397*: 315–323, 1999.
24. Bucay, N., Sarosi, I., Dunstan, C. R., Morony, S., Tarpley, J., Capparelli, C., Scully, S., Tan, H. L., Xu, W., Lacey, D. L., Boyle, W. J., and Simonet, W. S. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev.*, *12*: 1260–1268, 1998.
25. Tricot, G. New insights into role of microenvironment in multiple myeloma. *Lancet*, *355*: 248–250, 2000.
26. Pearce, R. N., Sordillo, E. M., Yaccoby, S., Wong, B. R., Liao, D. F., Colman, N., Michaeli, J., Epstein, J., and Choi, Y. Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. *Proc. Natl. Acad. Sci. USA*, *98*: 11581–11586, 2001.
27. Plesner, T., Boissy, P., Dartell, M., Norrild, K., Hastrup, N., Rasmussen, M., Gaarsdal, E., Delaissé, J.-M., and Anderson, D. Myeloma cells express and release Rank-ligand that promotes the generation of osteoclast-like cells. VIII<sup>th</sup> International Myeloma Workshop, P204, 2001.
28. Mitsiades, C. S., Mitsiades, N., Poulaki, V., Hayashi, T., Hideshima, T., Podar, K., LeBlanc, R., Catley, L., Tai, Y., Chauhan, D., Lin, B., Schlossman, R., Richardson, P. G., Munshi, N., Treon, S. P., and Anderson, K. C. Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand expression in human multiple myeloma cells. *Blood*, *98*(Suppl. 1): 2675, 2001.
29. Ikeda, T., Kasai, M., Utsuyama, M., and Hirokawa, K. Determination of three isoforms of the receptor activator of nuclear factor- $\kappa$ B ligand and their differential expression in bone and thymus. *Endocrinology*, *142*: 1419–1414, 2001.
30. Shi, S. R., Tandon, A. K., Haussmann, R. R., Kalra, K. L., and Taylor, C. R. Immunohistochemical study of intermediate filament proteins on routinely processed, celloidin-embedded human temporal bone sections by using a new technique for antigen retrieval. *Acta Otolaryngol.*, *113*: 48–54, 1993.
31. Nosaka, K., Miyamoto, T., Sakai, T., Mitsuya, H., Suda, T., and Matsuoka, M. Mechanism of hypercalcemia in adult T-cell leukemia: Overexpression of receptor activator of nuclear factor  $\kappa$ B ligand on adult T-cell leukemia cells. *Blood*, *99*: 634–640, 2002.
32. Oyajobi, B. O., Garrett, I. R., Williams, P. J., Yoneda, T., Anderson, D. M., and Mundy, G. R. A soluble murine receptor activator of NF- $\kappa$ B-human immunoglobulin fusion protein (RANK. Fc) inhibits bone resorption in a murine model of human multiple myeloma bone disease. *J. Bone Miner. Res.*, *15*(Suppl. 1): 1151, 2000.
33. Luger, N. M., Honore, P., Sabino, M. A., Schwei, M. J., Rogers, S. D., Mach, D. B., Clohisey, D. R., and Mantyh, P. W. Osteoprotegerin diminishes advanced bone cancer pain. *Cancer Res.*, *61*: 4038–4047, 2001.
34. Bekker, P. J., Holloway, D., Nakanishi, A., Arrighi, M., Leese, P. T., and Dunstan, C. R. The effect of a single dose of osteoprotegerin in postmenopausal women. *J. Bone Miner. Res.*, *16*: 348–360, 2001.

# Clinical Cancer Research

## Expression of Receptor Activator of Nuclear Factor $\kappa$ B Ligand on Bone Marrow Plasma Cells Correlates with Osteolytic Bone Disease in Patients with Multiple Myeloma

Ulrike Heider, Corinna Langelotz, Christian Jakob, et al.

*Clin Cancer Res* 2003;9:1436-1440.

**Updated version** Access the most recent version of this article at:  
<http://clincancerres.aacrjournals.org/content/9/4/1436>

**Cited articles** This article cites 32 articles, 16 of which you can access for free at:  
<http://clincancerres.aacrjournals.org/content/9/4/1436.full#ref-list-1>

**Citing articles** This article has been cited by 19 HighWire-hosted articles. Access the articles at:  
<http://clincancerres.aacrjournals.org/content/9/4/1436.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, contact the AACR Publications Department at [permissions@aacr.org](mailto:permissions@aacr.org).