Expression of CD56/Neural Cell Adhesion Molecule Correlates with the Presence of Lytic Bone Lesions in Multiple Myeloma and Distinguishes Myeloma from Monoclonal Gammopathy of Undetermined Significance and Lymphomas with Plasmacytoid Differentiation

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Unlike monoclonal gammopathy of undetermined significance (MGUS) or non-Hodgkin's lymphomas (NHLs) with plasmacytoid differentiation, multiple myeloma (MM) is commonly associated with lytic bone lesions. Although the mechanisms of increased osteoclast activity are partially understood, comparatively little is known about the mechanisms that lead to the observed decrease in osteoblast function. Studies have shown neural cell adhesion molecule (NCAM) homophilic binding between MM cell lines and osteosarcoma cell lines, and that binding results in decreased osteoid production *in vitro*. Thus, we postulated that the expression of NCAM by MM cells contributes to lytic lesion formation by causing decreased osteoid production *in vivo*. We used immunohistochemistry in bone marrow core biopsies to assess NCAM expression in osteoblasts and plasma cells (PCs) *in vitro*. We found consistent, strong, uniform NCAM expression by the osteoblasts in all bone marrow core biopsies (352 of 352, 100%). Strong expression of NCAM by PCs correlated with the presence of lytic bone lesions (chi-square, 33.39; $P < 0.000$; odds ratio, 16.9). There was also a strong correlation between NCAM expression and the diagnosis of MM in comparison to reactive PCs, MGUS, or NHLs with plasmacytoid differentiation (all P values < 0.000). In conclusion, using immunohistochemistry, we found strong expression of NCAM by osteoblasts and that when equal to the intensity of osteoblast expression, NCAM expression by PCs correlates with the presence of lytic bone lesions and distinguishes MM from reactive plasmacytosis, NHLs with plasmacytoid differentiation, and most cases of MGUS. (*Am J Pathol* 2002, 160:1293-1299)

Many B-cell neoplasms that involve the bone marrow can exhibit plasmacytoid or plasma cell (PC) differentiation. However, among them, only multiple myeloma (MM) is commonly associated with lytic bone lesions.¹ In this regard, little is known about the functional differences between the neoplastic PCs found in non-Hodgkin's lymphomas (NHLs) or monoclonal gammopathy of undetermined significance (MGUS), and those found in MM.

Most of the pathophysiological osteolysis mechanisms elucidated so far have focused on the role of osteoclasts in tipping the deposition/resorption balance in favor of resorption.²⁻⁸ Research into the role of osteoblasts has been limited primarily to their function as mere effectors of osteoclasts.^{4,5} However, studies have shown that osteoclasts and bone resorption are increased not only in MM, but also in MGUS as well as in B-cell NHLs involving the bone marrow.⁹⁻¹² Yet these latter neoplasms very rarely give rise to lytic bone lesions.¹ Moreover, morphometric studies have shown that the number of osteoclasts and the amount of bone resorption in MM patients with lytic lesions does not differ from that seen in MM patients without lytic lesions or between early and late stage disease.^{13,14} The most striking histomorphometric difference between patients with and those without lytic lesions is that osteoblasts and osteoblast function are decreased in patients with lytic lesions.^{9,13-15} In turn, biochemical indicators of osteoid production, such as serum osteocalcin, correlate inversely with the presence of lytic lesions.^{14,16} These studies have shown that although osteoclast number and function are increased in MM, the key difference *in vivo* between the presence and absence of lytic lesions is that osteoblasts are fewer and less active in patients with lytic lesions.

Supported in part by Specialty Center for Oncology Research grant 527975 from the Leukemia and Lymphoma Society.

Accepted for publication December 20, 2001.

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One potential means for an interplay between MM cells and osteoblasts is through homophilic binding by the neural cell adhesion molecule (NCAM)/CD56. All isoforms, both membrane-bound and secreted, have been found to bind each other, and this homophilic binding causes signal transduction.^{17–21} Although NCAM is known to be expressed by myeloma cells, by osteoblasts in animal models, and by osteosarcoma cell lines,^{22–24} expression of NCAM by normal human osteoblasts has not been documented *in vivo*, until now.

Barille and colleagues²⁵ reported that *in vitro*, myeloma cells adhere to osteosarcoma cells through NCAM-NCAM homophilic binding. In this system, co-culturing with myeloma cells causes a decrease in bone matrix production by the osteosarcoma cells. From this *in vitro* data, we postulate that, *in vivo*, NCAM-NCAM homophilic binding between MM cells and osteoblasts may cause a decrease in bone matrix production, and thus, contribute to the formation of lytic bone lesions.

There is conflicting flow cytometry data regarding the usefulness of NCAM expression in making the diagnosis of MM.^{24,26–31} However, taken together, published studies support the idea that NCAM is low/negative in benign PCs and MGUS, is up-regulated in MM, and is then down-regulated in synchrony with evolution to aggressive, plasmablastic or anaplastic myeloma or to extramedullary disease.^{26,32} Until now, the use of NCAM immunohistochemistry (IHC) for the diagnosis of MM has not been studied.

The goals of this study were as follows: first, to determine whether or not there is a correlation between NCAM expression and the presence of lytic bone lesions in MM, and second, to assess whether or not NCAM expression by IHC can be used to distinguish between MM, MGUS, and NHLs with plasmacytoid differentiation.

Materials and Methods

Biopsy Selection

Between August 1, 1998 and December 20, 2000, biopsies were selected and analyzed on a prospective basis according to one or more of the following criteria: 1) the biopsy was performed because of the presence of a monoclonal serum spike or the clinical suspicion of MM, 2) the biopsy contained >5% PCs, or 3) the biopsy was found to contain a neoplasm with plasmacytoid or PC cytological features. These criteria yielded 352 biopsies in that time period.

Histological Samples

Bone marrow biopsies (BMBXs) were fixed in Bouin's and embedded in paraffin, from which 5- μ m histological sections were cut and stained with hematoxylin and eosin. Fifteen BMBXs fixed in 10% formalin were also examined.

IHC

IHC was performed by indirect immunoperoxidase staining of paraffin tissue sections using a TechMate500 BioTek automated immunostainer (Ventana Medical Systems, Inc., Tucson, AZ) and antibodies recognizing IgA, IgD, IgG, IgM, Ig κ , and Ig λ (DAKO, Glostrup, Denmark), CD56/NCAM (1:25; Synbio/Monosan, Uden, Netherlands), and CD138/Syndecan 1 (Serotec, Oxford, UK). In addition, 20 Bouin's-fixed and 20 formalin-fixed BMBXs were stained with a second anti-CD56/NCAM antibody (1:25; Novocastra, Peterborough, UK). In preparation for immunostaining, after the final hydration step with alcohol, the Bouin's-fixed slides were placed in 70% ethyl alcohol with lithium carbonate to remove any remaining fixative. NCAM antigen retrieval was performed by placing the slides in citrate buffer at pH 6.0 in a pressure cooker for 10 minutes.

NCAM Expression

Using the resting osteoblasts as an internal positive control, a case was considered to be positive for CD56/NCAM expression if >50% of the CD138/Syndecan 1-positive PCs expressed NCAM with an intensity on par with that of the osteoblasts.

Reproducibility of Interpretation of CD56/NCAM Expression

In addition to the authors, NCAM IHC from 50 randomly chosen biopsies was interpreted by two other hematopathologists.

Diagnoses

The NHLs were diagnosed according to the World Health Organization classification.¹ A NHL was considered to have plasmacytoid differentiation if it showed plasmacytoid or PC cytology and contained a population of CD138/Syndecan 1-positive cells with monotypic cytoplasmic immunoglobulin expression. Patients with PC dyscrasias were assigned diagnoses according to the World Health Organization classification.¹

Assessment of Lytic Bone Lesions

The presence of lytic bone lesions was assessed via X-ray skeletal surveys performed within 6 months of the time of the biopsy. The radiographs were interpreted by radiologists who did not know that the results were part of a study. Of the 352 biopsies selected by the above criteria, skeletal surveys were performed in 134 patients.

Statistical Analysis

The associations between NCAM, diagnosis, and lytic bone lesions were assessed by chi-square contingency table analysis using SPSS for Windows (release 10.0;

Figure 1. NCAM is strongly expressed by human osteoblasts (arrows) *in vivo* (IHC with anti-CD56/NCAM; Monosan, Uden, Netherlands). Strong expression in spindle-shaped resting osteoblasts surrounding a bony trabeculum in an adult BMBX (A; original magnification, $\times 100$); activated osteoblasts in a patient with hyperparathyroidism (B; original magnification, $\times 400$); and a growing, 3-year-old child (C; original magnification, $\times 1000$, oil immersion).

SPSS, Inc., Chicago, IL). In all cases, P values <0.05 were considered statistically significant.

Results

NCAM Expression in Human Osteoblasts *In Vivo*

We found strong NCAM expression by osteoblasts *in vivo*, both activated, in growing children (3 of 3, 100%) and in patients with hyperparathyroidism (3 of 3, 100%), and resting, in both benign (88 of 88, 100%) and neoplastic (249 of 249, 100%) adult BMBXs (Figures 1 and 2). However, the intensity of expression was greater in resting cells. In areas where the bone sheered away from the hematopoietic marrow in processing, we found that all or part of the osteoblast layer adhered to the cellular marrow and was clearly visible in IHC sections. Thus, we were able to use osteoblast NCAM expression as an internal positive control in all biopsies.

Comparison of NCAM Expression to the Presence of Lytic Bone Lesions in MM

Of the 150 patients with MM, 113 (75%) had X-ray skeletal surveys. Seventy-nine percent (89 of 113) were found to have lytic bone lesions. Of those with lytic lesions, 81 of 89 (91%) had MM cells that expressed NCAM with an intensity on par with that of the osteoblasts used as internal positive controls (Table 1, Figure 2). Of those without lytic lesions, 9 of 24 (38%) were NCAM-positive. The correlation between NCAM expression and the presence of lytic lesions was statistically significant (chi-square, 33.39; P value <0.000 ; odds ratio, 16.9) (Table 2). The positive predictive value of NCAM expression for the presence of lytic lesions in MM was 90%. The sensitivity and specificity were 91% and 63%, respectively.

Comparison of NCAM Expression to Diagnosis

None of the biopsies with polyclonal plasmacytosis (0 of 88, 0%), had PCs that were NCAM-positive by this meth-

odology (Tables 1 and 2, Figure 2). Although a small number of biopsies contained PCs that expressed NCAM, the intensity of expression was very weak and present only in a minor subpopulation of PCs, $<10\%$ in all cases.

In addition, we immunostained 10 benign lymph nodes (results not shown). Although all 10 contained CD138-positive PCs, none displayed significant NCAM expression. Two of the 10 nodes (20%) contained small subpopulations of PCs ($<10\%$) that exhibited faint NCAM expression. Because of the lack of osteoblasts for use as internal positive controls, it is not possible to assess NCAM expression in extramedullary tissues by the method used in this study. However, when compared to the BMBXs, the intensity of NCAM expression in the subpopulation of benign PCs in LNs was considerably weaker than that seen in MM and in osteoblasts.

We also immunostained two extramedullary plasmacytomas (results not shown), both of which were NCAM-negative. Of the 65 NHLs with plasmacytoid differentiation [44 lymphoplasmacytoid lymphoma (LPL) (41 WM and 3 IgG heavy-chain disease), 8 marginal zone lymphomas, 7 chronic lymphocytic leukemia (CLL), 5 diffuse large B cell lymphomas, and 1 follicular lymphoma], none (0 of 65, 0%) were NCAM-positive. Although a small number of biopsies contained PCs that expressed NCAM, the intensity of expression was very weak and present only in a minor subpopulation of PCs, usually $<5\%$. There were 3 patients with primary systemic amyloidosis. All 3 biopsies contained monoclonal PCs. However, none (0 of 3, 0%) were NCAM-positive. Of the 46 biopsies from MGUS patients, 3 of 46 (7%) were NCAM-positive. None of the NCAM-positive MGUS cases had lytic bone lesions.

Strong NCAM expression was seen in 107 of 150 (71%) patients with MM. Of the NCAM-negative MMs, 2 of 43 (5%) were smoldering myeloma, 3 of 43 (7%) were aggressive plasmablastic cases, 7 of 43 (16%) were anaplastic, and 1 patient (2%) had a PC leukemia. A few of the NCAM-negative cases contained PCs that expressed NCAM, but only very weakly and in a minor subpopulation, usually $<5\%$.

Figure 2. IHC for CD138/Syndecan 1 (**left**) shows strong staining by PCs in all biopsies (Poly. PCs, polyclonal plasmacytosis; LPL/WM, lymphoplasmacytoid lymphoma/Waldenstrom's macroglobulinemia; MM no LL, multiple myeloma without lytic lesions; and MM with LL, multiple myeloma with lytic lesions). IHC for CD56/NCAM (**right**) shows strong expression in the linear rows of paratrabeal osteoblasts used as internal positive controls (**arrows**, spindle-shaped cells adjacent to a bony trabeculum in each frame). The PCs are CD56/NCAM-negative in all biopsies except MM with LL (**open arrow**).

Comparison of Fixation Methods and CD56/NCAM Antibodies

The above results were obtained by analysis of Bouin's-fixed BMBXs. To test whether this methodology could be applied to formalin-fixed material, we performed IHC on formalin-fixed and Bouin's-fixed BMBXs taken side-by-side from nine patient's who did not have PC dyscrasias. The results were identical with both fixatives and clearly showed CD138-positive PCs that lacked NCAM expression, whereas the osteoblasts were strongly positive for NCAM. In addition, we immunostained 11 neoplastic, formalin-fixed biopsies (three MGUS, four NHLs with plasmacytoid differentiation, and four MMs). The PCs in all formalin-fixed MGUS and NHL cases expressed

CD138 but were negative for NCAM, whereas all MM cases were positive for both CD138 and NCAM. Also, to test whether this methodology could be used with other antibodies, we randomly selected 30 cases from the study and performed IHC with a second anti-NCAM antibody (Novocastra). The results were identical to those of the first antibody (Synbio/Monosan).

Reproducibility of Interpretation of CD56/NCAM Expression

According to the methodology detailed above, NCAM IHC from 50 randomly chosen biopsies was interpreted by two hematopathologists in addition to the authors of

Table 1. NCAM Expression Compared to Diagnosis and the Presence of Lytic Bone Lesions

Diagnosis	Total	NCAM-positive
Polyclonal PCs	88	0 (0%)
MGUS	46	3 (7%)
Amyloidosis	3	0 (0%)
Plasmacytoid NHLs	65	0 (0%)
MM, all patients	150	107 (71%)
MM with lytic bone lesions	89	81 (91%)
MM without lytic bone lesions	24	9 (38%)
Total number of cases	352	

the paper. All were in agreement in assessment of NCAM expression in 50 of 50 (100%) biopsies.

Discussion

Using IHC, we found strong NCAM expression by both resting and activated human osteoblasts *in vivo*. Using the intensity of osteoblast NCAM expression for comparison, we also found strong NCAM expression in 71% of MM samples but not in any polyclonal plasmacytoses, NHLs with plasmacytoid differentiation, or amyloidoses, and in only 7% of MGUS cases. Although we used expression by 50% of the PCs as the cutoff to be considered positive, in most biopsies the PCs were virtually all positive or all negative. Close calls were rare. We also found a strong correlation between NCAM expression in PCs and the presence of osteolytic bone lesions.

Although lytic lesions in MM are known to be caused by a disruption of the normal balance between osteoblastic bone deposition and osteoclastic resorption, most research into the operative mechanisms of, and treatment for, osteolytic lesions has been directed toward the osteoclast arm of the balance.²⁻⁸ The role of osteoclasts in lytic lesion formation is well documented. However, morphometric analysis and biochemical studies have shown a decrease in osteoblasts and in bone matrix production in MM *in vivo*.^{9,13-16} Moreover, although osteoclast number and function are increased in MM, the most striking difference *in vivo* between the presence and absence of lytic lesions is that osteoblasts are fewer and less active in patients with lytic lesions.

Because *in vitro* studies have shown NCAM-NCAM homophilic binding between osteosarcoma cell lines and MM cell lines and that co-culturing the osteosarcoma cells with MM cells causes a decrease in bone matrix

production,²⁵ we postulated that an NCAM-mediated decrease in osteoblast function may play a role in the formation of lytic lesions. The results of this study strongly support that idea. Also, unlike most MM cells, those in POEMS syndrome, with osteosclerotic rather than lytic bone changes, are consistently negative for NCAM.³³

Barille and colleagues²⁵ showed that myeloma cells effect a decrease in osteoblast bone matrix production, partially through a soluble factor. In addition to the implications regarding lytic lesion formation, the potential role of soluble NCAM in decreasing matrix production is also intriguing in light of the frequency of generalized osteoporosis in myeloma patients.^{3,9,34} Although studies have shown an increase in osteoclasts and bone resorption in MM BMBXs, they have also shown that the effect of the MM on osteoclasts is limited to the immediate vicinity of myeloma cells. Osteoclasts in remote sites uninvolved by MM are not affected.^{9,11,35} Such focal changes in osteoclasts certainly contribute to lytic lesion formation, but could not cause generalized osteoporosis. However, NCAM is present in high levels in the sera of MM patients.³⁶ Thus, secreted NCAM could result in a systemic decrease in bone matrix production that would contribute to generalized osteoporosis.

Although it is tempting to speculate that the lack of lytic lesions in plasmacytoid lymphomas relates to a comparatively smaller PC mass, we have encountered many cases of lymphoplasmacytoid lymphoma with packed marrows, in which the plasmacytoid differentiation, both morphological and immunophenotypic, makes it difficult to distinguish the lymphoma from MM. Yet, in these cases, all of which are NCAM-negative, lytic lesions are not present. We also have studied many NCAM-negative MMs with extensive marrow involvement, yet without osteolytic lesions (results not shown). Strong NCAM expression is one of the key measurable differences between myeloma PCs and the clonotypic plasmacytoid lymphocytes and PCs in lymphomas. This finding lends further evidence to the contention that NCAM contributes to the formation of lytic lesions and helps to explain the lack thereof in lymphomas with plasmacytoid differentiation as well as in MGUS.

Among B-cell NHLs, NCAM expression is exceptional, with reports limited almost exclusively to the rarely encountered microvillous lymphomas.³⁷⁻³⁹ However, it is sometimes difficult to distinguish NHLs from MM because of the morphological overlap, especially in NHLs associ-

Table 2. Significance of Strong NCAM Expression by Immunohistochemistry in Distinguishing MM from Other B-Cell Neoplasms and in Predicting Lytic Bone Lesions

	Chi-square	P value	Odds ratio
MM versus polyclonal plasmacytosis	114.05	<0.0001	inf.
MM versus MGUS	56.47	<0.000	33.2
MM versus plasmacytoid non-Hodgkin's lymphomas	92.30	<0.000	inf.
MM with lytic lesions versus MM without lytic lesions	33.39	<0.000	16.9
PPV for lytic lesions	90%		
NPV for lytic lesions	65%		
Sensitivity for lytic lesions in MM patients	91%		
Specificity for lytic lesions in MM patients	63%		

PPV, positive predictive value; NPV, negative predictive value.

ated with a serum spike. In this regard, the results of this study make it clear that strong NCAM expression by IHC almost certainly rules out the diagnosis of a B-cell NHL. Conversely, in the case of a PC dyscrasia, strong expression of NCAM argues against MGUS and in favor of MM.

We found IHC to be an ideal method for assessing *in vivo* NCAM expression in bone marrow cells. Although we used expression by 50% of the PCs as the cutoff to be considered positive, in most biopsies the PCs were virtually all positive or all negative. The results were clear and easy to interpret, reproducible, and not time consuming. Moreover, because all core biopsies contain an internal positive control, the variability of interpretation that is inherent to flow cytometry is obviated by IHC. NCAM expression is especially useful in MM diagnosis in small biopsies or when clonality of the PCs is difficult to assess because of background staining. In comparison with an aspirate and flow cytometry, using a core biopsy to assess myeloma has the added efficacy of containing a greater percentage of PCs in nearly half the cases, and has been shown to be more representative of the patient's disease.^{40,41} In this study, we found that the core biopsy contained a greater percentage of PCs than the aspirate in 44% of cases and that the average difference exceeded 20% (results not shown). Lastly, the utility of a core biopsy in the diagnosis of a patient with an inaspirable marrow is foregone.

In conclusion, using IHC, we found that human osteoblasts express NCAM *in vivo*. Using the osteoblasts as an internal positive control, there is a significant correlation between strong expression of NCAM by myeloma cells and the presence of lytic bone lesions. We also found NCAM expression to be a reliable diagnostic criterion in distinguishing MM from polyclonal plasmacytosis, MGUS, and from NHLs with plasmacytoid differentiation.

References

- Jaffe ES, Harris NL, Stein H, Vardiman JW (Eds): Tumours of hematopoietic and lymphoid tissues. World Health Organization Classification of Tumours. Lyon, IARC Press, 2001
- Bataille R, Chappard D, Marcelli C, Dessauw P, Sany J, Baldet P, Alexandre C: Mechanisms of bone destruction in multiple myeloma: the importance of an unbalanced process in determining the severity of lytic bone disease. *J Clin Oncol* 1989, 7:1909-1914
- Bataille R, Manolagas SC, Berenson JR: Pathogenesis and management of bone lesions in multiple myeloma. *Hematol Oncol Clin North Am* 1997, 11:349-361
- Tricot G: New insights into the role of microenvironment in multiple myeloma. *Lancet* 2000, 355:248-250
- Hjertner O, Torgersen ML, Seidel C, Hjorth-Hansen H, Waage A, Borset M, Sundan A: Hepatocyte growth factor (HGF) induces interleukin-11 secretion from osteoblasts: a possible role for HGF in myeloma-associated osteolytic bone disease. *Blood* 1999, 94:3883-3888
- Michigami T, Shimizu N, Williams PJ, Niewolna M, Dallas SL, Mundy GR, Yoneda T: Cell-cell contact between marrow stromal cells and myeloma cells via VCAM-1 and alpha(4)beta(1)-integrin enhances production of osteoclast-stimulating activity. *Blood* 2000, 96:1953-1960
- Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD, Alsina M: Macrophage inflammatory protein 1-alpha is a potential osteoclast stimulatory factor in multiple myeloma. *Blood* 2000, 96:671-675
- Raje N, Anderson KC: Introduction: the evolving role of bisphosphonate therapy in multiple myeloma. *Blood* 2000, 96:381-383
- Bataille R, Chappard D, Alexandre C, Dessauw P, Sany J: Importance of quantitative histology of bone changes in monoclonal gammopathy. *Br J Cancer* 1986, 53:805-810
- Marcelli C, Chappard D, Rossi JF, Jaubert J, Alexandre C, Dessauw P, Baldet P, Bataille R: Histologic evidence of an abnormal bone remodeling in B-cell malignancies other than multiple myeloma. *Cancer* 1988, 62:1163-1170
- Rossi JF, Chappard D, Marcelli C, Laplante J, Commes T, Baldet P, Janbon C, Jourdan J, Alexandre C, Bataille R: Micro-osteoclast resorption as a characteristic feature of B-cell malignancies other than multiple myeloma. *Br J Haematol* 1990, 76:469-475
- Bataille R, Chappard D, Basle MF: Quantifiable excess of bone resorption in monoclonal gammopathy is an early symptom of malignancy: a prospective study of 87 bone biopsies. *Blood* 1996, 87:4762-4769
- Bataille R, Chappard D, Marcelli C, Dessauw P, Baldet P, Sany J, Alexandre C: Recruitment of new osteoblasts and osteoclasts is the earliest critical event in the pathogenesis of human multiple myeloma. *J Clin Invest* 1991, 88:62-66
- Bataille R, Chappard D, Marcelli C, Rossi JF, Dessauw P, Baldet P, Sany J, Alexandre C: Osteoblast stimulation in multiple myeloma lacking lytic bone lesions. *Br J Haematol* 1990, 76:484-487
- Taube T, Beneton MN, McCloskey EV, Rogers S, Greaves M, Kanis JA: Abnormal bone remodelling in patients with myelomatosis and normal biochemical indices of bone resorption. *Eur J Haematol* 1992, 49:192-198
- Bataille R, Delmas PD, Chappard D, Sany J: Abnormal serum bone Gla protein levels in multiple myeloma. Crucial role of bone formation and prognostic implications. *Cancer* 1990, 66:167-172
- Goridis C, Brunet J-F: NCAM: structural diversity, function and regulation or expression. *Semin Cell Biol* 1992, 3:189-197
- Ranheim TS, Edelman GM, Cunningham BA: Homophilic adhesion mediated by the neural cell adhesion molecule involves multiple immunoglobulin domains. *Proc Natl Acad Sci USA* 1996, 93:4071-4075
- Krushel LA, Tai M-H, Cunningham BA, Edelman GM, Crossin KL: Neural cell adhesion molecule (N-CAM) domains and intracellular signaling pathways involved in the inhibition of astrocyte proliferation. *Proc Natl Acad Sci USA* 1998, 95:2592-2596
- Crossin KL, Krushel LA: Cellular signaling by neural cell adhesion molecules of the immunoglobulin superfamily. *Dev Dyn* 2000, 218:260-279
- Cunningham BA, Hemperly JJ, Murray BA, Prediger EA, Brackenbury R, Edelman GM: Neural cell adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing. *Science* 1987, 236:799-806
- Lee YS, Chuong CM: Adhesion molecules in skeletogenesis: I. Transient expression of neural cell adhesion molecules (NCAM) in osteoblasts during endochondral and intramembranous ossification. *J Bone Miner Res* 1992, 7:1435-1446
- Nelissen JM, Torensma R, Pluymers M, Adema GJ, Raymakers RA, van Kooyk Y, Figdor CG: Molecular analysis of the hematopoiesis supporting osteoblastic cell line U2-OS. *Exp Hematol* 2000, 28:422-432
- Van Camp G, Durie BGM, Spier C, De Waele M, Van Riet I, Vela E, Frutiger Y, Richter L, Grogan T: Plasma cells in multiple myeloma express a natural killer cell-associated antigen: cd56 (NKH-1; Leu 19). *Blood* 1990, 76:377-382
- Barille S, Collette M, Bataille R, Amiot M: Myeloma cells upregulate interleukin-6 secretion in osteoblastic cells through cell-to-cell contact but downregulate osteocalcin. *Blood* 1995, 86:3151-3159
- Pellat-Deceunynck C, Bataille R, Robillard N, Harousseau JL, Rapp M-J, Morineau NJ, Wijdenes J, Amiot M: Expression of CD28 and CD40 in human myeloma cells: a comparative study with normal plasma cells. *Blood* 1994, 84:2597-2603
- Sonneveld P, Durie BG, Lokhorst HM, Frutiger Y, Schoester M, Vela EE: Analysis of multidrug-resistance (MDR-1) glycoprotein and CD56 expression to separate monoclonal gammopathy from multiple myeloma. *Br J Haematol* 1993, 83:63-67
- Harada H, Kawano MM, Huang N, Harada Y, Iwato K, Tanabe O, Tanaka H, Sakai A, Asaoku H, Kuramoto A: Phenotypic difference of normal plasma cells from mature myeloma cells. *Blood* 1993, 81:2658-2663
- Zandeck M, Facon T, Bernardi F, Izydorczyk V, Dupond L, Francois M, Reade R, Iaru T, Bauters F, Cosson A: CD19 and immunopheno-

- type of bone marrow plasma cells in monoclonal gammopathy of undetermined significance. *J Clin Pathol* 1995, 48:548–552
30. Mathew P, Ahmann GJ, Witzig TE, Roche PC, Kyle RA, Greipp PR: Clinicopathological correlates of CD56 expression in multiple myeloma: a unique entity? *Br J Haematol* 1995, 90:459–461
 31. Ocqueteau M, Orfao A, Almeida J, Blade J, Gonzalez M, Garcia-Sanz R, Lopez-Berges C, Moro MJ, Hernandez J, Escribano L, Caballero D, Rozman M, San Miguel JF: Immunophenotypic characterization of plasma cells from monoclonal gammopathy of undetermined significance patients. Implications for the differential diagnosis between MGUS and multiple myeloma. *Am J Pathol* 1998, 152:1655–1665
 32. Pellat-Deceunynck C, Barille S, Puthier D, Rapp M-J, Harousseau J-L, Bataille R, Amiot M: Adhesion molecules on human myeloma cells: significant changes in expression related to malignancy, tumor spreading and immortalization. *Cancer Res* 1995, 55:3647–3653
 33. Rose C, Zandecki M, Copin MC, Gosset P, Labalette M, Hatron PY, Jauberteau MO, Devulder B, Bauters F, Facon T: POEMS syndrome: report on six patients with unusual clinical signs, elevated levels of cytokines, macrophage involvement and chromosomal aberrations of bone marrow plasma cells. *Leukemia* 1997, 11:1318–1323
 34. Kyle RA: Diagnostic criteria in multiple myeloma. *Hematol Oncol Clin North Am* 1992, 692:347–358
 35. Bataille R, Chappard D, Basle M: Excessive bone resorption in human plasmacytomas: direct induction by tumour cells in vivo. *Br J Haematol* 1995, 90:721–724
 36. Ong F, Kaiser U, Seelen PJ, Hermans J, Wijermans PW, de Kieviet W, Jaques G, Kluin-Nelemans JC: Serum neural cell adhesion molecule differentiates multiple myeloma from paraproteinemias due to other causes. *Blood* 1996, 87:712–716
 37. Hammer RD, Vnencak-Jones CL, Manning SS, Glick AD, Kinney MC: Microvillous lymphomas are B-cell neoplasms that frequently express CD56. *Mod Pathol* 1998, 11:239–246
 38. Sekita T, Tamaru JI, Isobe K, Harigaya K, Masuoka S, Katayama T, Kobayashi M, Mikata A: Diffuse large B cell lymphoma expressing the natural killer cell marker CD56. *Pathol Int* 1999, 49:752–758
 39. Kern WF, Spier CM, Miller TP, Grogan TM: NCAM (CD56)-positive malignant lymphoma. *Leuk Lymphoma* 1993, 12:1–10
 40. Bartl R, Frisch B: Clinical significance of bone marrow biopsy and plasma cell morphology in MM and MGUS. *Pathol Biol* 1999, 47:158–168
 41. Terpstra WE, Lokhorst HM, Blomjous F, Meuwissen OJ, Dekker AW: Comparison of plasma cell infiltration in bone marrow biopsies and aspirates in patients with multiple myeloma. *Br J Haematol* 1992, 82:46–49