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REVIEW ARTICLE

ANTI-CS1 ANTIBODIES: A NEW THERAPEUTIC APPROACH TO TREAT MULTIPLE MYELOMA

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CONTENTS

ABSTRACT

CS1 (CD319) is a member of the signaling lymphocytic activation molecule (SLAM) family of cell-surface proteins. It is expressed on natural killer cells and subsets of T and B cells. As part of a screening program to identify cell-surface proteins expressed in plasma cells, CS1 was identified as a candidate molecule. In this manuscript, we briefly review the cellular biology of CS1 and recent preclinical data that supported the development of elotuzumab, a humanized monoclonal antibody to CS1, as a potential therapeutic antibody for multiple myeloma. This antibody is currently in early-phase clinical trials.

INTRODUCTION

CS1 is a member of the signaling lymphocytic activation molecule (SLAM) family of cell-surface proteins expressed on natural killer (NK) cells, a subset of $CD8^+$ T cells and activated T- and B-cell subsets. Cloned in 2001 by two groups, the gene for CS1 (also known as CRACC or CD319) encodes a 335-amino-acid protein and is localized to chromosome 1q23-24 (1, 2). Functionally, CS1 mediates homophilic interactions and regulates the cytotoxicity of NK cells. The intracellular domain of the protein contains the novel immunoreceptor tyrosine-based switch motifs, suggesting a function in intracellular signaling. Recent work has identified CS1 as a potential therapeutic target for the treatment of hematological malignancies (3-5). In particular, CS1 is highly and uniformly expressed in multiple myeloma, and may be an attractive target for therapeutic monoclonal antibodies (mAbs) (4, 5). In this manuscript, we will review CS1 structure and function and detail recent efforts to develop a humanized mAb to CS1 for the treatment of multiple myeloma.

STRUCTURE AND PUTATIVE FUNCTION OF CS1

The structural features of CS1 have been well characterized, but relatively little is known about the functional role of CS1 in lymphocyte biology compared with other SLAM members. Immunoprecipitation with an mAb directed to CS1 revealed a 66-kDa protein under reducing conditions that, after deglycosylation, confirmed the predicted molecular weight of 37 kDa (2). CS1 contains an *N*-terminal extracellular domain consisting of one IgV-like and one IgC-like domain and a single transmembrane domain. SLAM family receptors, including CS1, have a cytoplasmic tail that includes an immunoreceptor tyrosine-based switch motif (ITSM) (Fig. 1) (1, 6). With the exception of 2B4 (CD244), the SLAM family receptors are self-ligands and associate with SH2 domain-containing proteins (SAP or the related EAT-2 in humans) via these ITSM domains. These adaptor proteins are able to interact with tyrosine kinases such as Fyn and thus link SLAM receptors to intracellular signaling pathways (4). Loss of SAP results in X-linked lymphoproliferative disease and illustrates the functional importance of this pathway (7).

Several studies have been published that shed some light on a potential role for CS1 in NK cell activation (2, 8-10). CS1 appears to play a role in the activation of cytotoxic activity in vitro in NK cells derived from both normal donors and patients with X-linked lymphoproliferative disorder who are deficient in SAP. Indeed, immunoprecipitation experiments in NK92 cells have shown that, upon CS1 ligation or pervanadate treatment, EAT-2 associates with CS1 (11). Downstream signaling events are still being explored, but EAT-2 appears to mediate tyrosine phosphorylation of CS1, which appears to be Src family kinase-dependent. Whether this is through direct recruitment or through recruitment of phosphatase inhibitors such as SHP-2 is uncertain (12). Regardless, downstream biochemical effects such as activation of phosphatidylinositol 3-kinase (PI3K) with phosphorylation of AKT, phospholipase PLCγ1 and PLCγ2, as well as an increase in intracellular calcium levels, have been demonstrated upon CS1 ligation (11, 13). Two isoforms have been identified, CS1-L (long) and CS1-S (short). The latter lacks the ITSM motif. In NK cells, the long form is capable of enhancing cytotoxicity upon CS1 ligation, while the short form does not (14). Thus, they appear to differentially regulate NK cell function. While both isoforms are expressed in NK cells, B cells express only the CS1-L form (10).

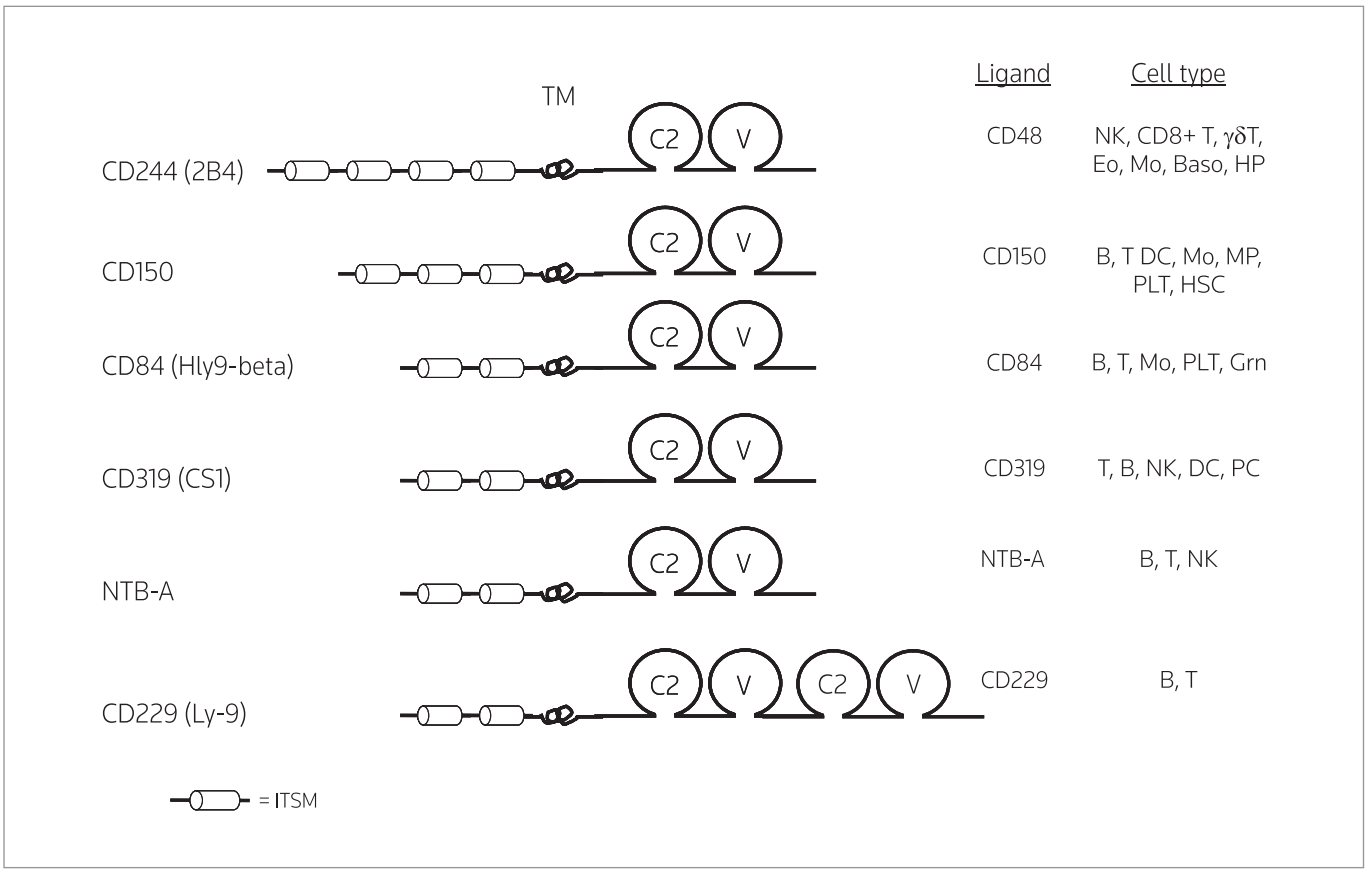

Figure 1. Schematic representation of SLAM family molecules. CS1 contains extracellular immunoglobulin domains, a transmembrane domain and two immunoreceptor tyrosine-based switch motif (ITSM) motifs. TM, transmembrane; B, B cell; T, T cell; Eo, eosinophil; Baso, basophil; Mo, monocyte; MP, macrophage; HP, multipotent hematopoietic progenitor; HSP, hematopoietic stem cell; PLT, platelet; Grn, granulocyte; DC, dendritic cell; PC, plasma cell, C2 and V, C2- and V-type immunoglobulin domain. Adapted and reproduced with permission from Bhat, R., Eissmann, P., Endt, J., Hoffmann, S., Watzl, C. *Fine-tuning of immune responses by SLAM-related receptors*. J Leukoc Biol 2006, 79(3): 417-24. Copyright 2006 by Journal of Leukocyte Biology.

Interestingly, the consequences of CS1 ligation, whether via effector/target contact, effector cell/effector cell interaction or *cis* engagement, may depend on whether EAT-2 is present or not. In NK cells (where EAT-2 is present) activation of cytotoxic activity is seen. However, in $CS1^+$-activated $CD4^+$ T cells (which lack EAT-2) exposed to appropriate CS1-expressing antigen-presenting cells (APCs), an inhibitory effect may be seen (decreased proliferation and cytokine production) (13).

IMMUNOHISTOCHEMISTRY USING CS1 mAbs

As part of a research program to identify cell-surface markers specific to plasma cells, a cDNA library was prepared using subtractive hybridization. A naïve B-cell cDNA library ($CD19^+/IgD^+/CD38^{-/lo}/CD27^-$) was subtracted from a memory B-cell/plasma cell library ($CD19^{+/lo}/IgD^-/CD38^{int/-}/CD27^+$). CS1 cDNA was found to be differentially expressed and passed bioinformatic screening for molecules with potential cell-surface expression. CS1 expression in plasma cells was confirmed by custom Affymetrix GeneChip analysis of $CD138^+$ purified normal plasma cells and plasma cells from patients with plasma cell dyscrasias. In fact, CS1 expression was observed in multiple myeloma from all patient populations, irrespective of cytogenetic abnormalities and molecular profiles that determine the risk of disease recurrence (4). Specificity was also confirmed via analysis of a variety of normal adult tissue extracts (4, 5).

A series of mAbs were generated that were used for Western blotting and flow cytometry (MuLuc63 and MuLuc90) and immunohistochemistry in paraffin sections (1G9) (4). MuLuc63 and MuLuc90 antibodies recognize extracellular epitopes, while 1G9 recognizes a membrane proximal intracellular epitope. These antibodies were used to further characterize the expression of CS1 and demonstrated expression in peripheral blood lymphocytes, with the highest expression in NK cells, NK-like T cells and a subset of $CD8^+$ T cells (Fig. 2). Little or no expression was detected on $CD4^+$ T cells, resting B cells, monocytes and granulocytes. Immunostaining showed expression of CS1 in lymphoid tissue in a small subset of lymphocytes and in most plasma cells (Fig. 3). Plasma cells from bone marrow of multiple myeloma patients and from plasmacytoma also showed high expression of CS1, whereas CS1 expression in B-cell lymphomas was limited to those with plasmacytic differentiation, such as lymphoplasmacytic lymphoma (4). Malignant plasma cells expressed CS1 regardless of the stage of disease. Low levels of soluble CS1 protein (clipped or shed) were also detected in the sera of myeloma patients, with higher levels observed in patients with stage

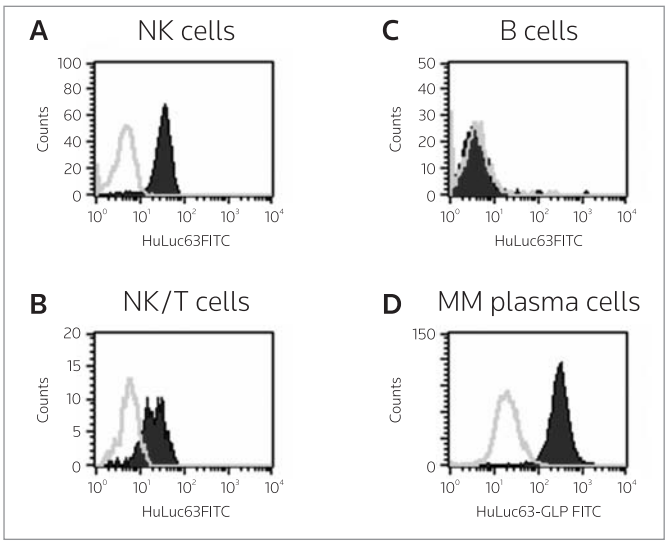

Figure 2. Flow cytometry of blood lymphocytes and myeloma cells. Normal human blood lymphocytes were stained with antibodies to CD3, CD45, CD16/56, CD19 and elotuzumab-fluorescein isothiocyanate (FITC) or isotype control. **A:** $CD3^-/CD19^-/CD16^-56^+$ NK cells; **B:** $CD3^+/CD16^-56^+$ NK-like T cells express CS1; **C:** $CD19^+$ B cells are negative for CS1 staining; **D:** bone marrow plasma cells ($CD45^-/CD138^+$) from a multiple myeloma (MM) patient stain brightly positive for CS1. Solid purple = elotuzumab-FITC histogram, while the green line represents FITC-labeled isotype control.

II/III disease compared to patients with stage I disease (5). A subset of peripheral T-cell lymphomas also expressed CS1, and more recently, analysis of NK cell lymphomas showed that the majority expressed CS1 (Fig. 4) (3).

ELOTUZUMAB – DEVELOPMENT OF A POTENTIAL THERAPEUTIC ANTIBODY

Because of the high levels of CS1 expression in virtually all cases of multiple myeloma and the significant unmet medical need in this disease, further development of anti-CS1 antibodies was undertaken, leading to the production of a humanized version of MuLuc63 (HuLuc63), named elotuzumab (Facet Biotech, Bristol-Myers Squibb), which has entered phase I clinical trials.

Biological activity of elotuzumab

Preclinical studies showed that elotuzumab specifically bound to malignant plasma cells from myeloma patients, but did not bind to $CD34^+$ hematopoietic stem cells and most other normal tissues. Although elotuzumab did bind resting NK cells and a subset of $CD8^+$ T cells, in vitro incubation of whole blood with the antibody did not result in significant changes in the absolute counts of lymphocyte subsets. In contrast, using similar methods, the murine/human chimeric anti-CD20 mAb rituximab and the humanized anti-CD52 mAb alemtuzumab depleted B cells and total lymphocytes, respectively (4).

Functionally, elotuzumab appears to be capable of mediating NK cell-dependent antibody-dependent cellular cytotoxicity (ADCC) of primary myeloma cells and myeloma cell lines (4). Indeed, recent studies show that CS1 antibodies activate human NK cells and monocytes and cause release of the chemokines MCP-1 and IP-10

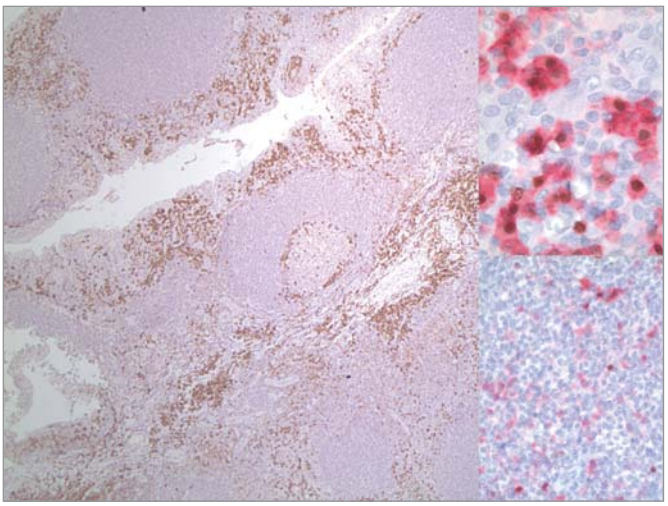

Figure 3. CS1 immunostaining in the tonsil shows expression in plasma cells located in subepithelial areas and in reactive germinal centers. The upper inset shows dual color staining with anti-CS1 (red) and anti-MUM1 (brown) antibodies. Anti-MUM1 antibody labels nuclei of plasma cells that also stain positive with anti-CS1 antibodies. The lower inset is a similar stain in the T-cell zone of the tonsil. Scattered, small MUM1-negative lymphocytes corresponding to T cells are $CS1^+$.

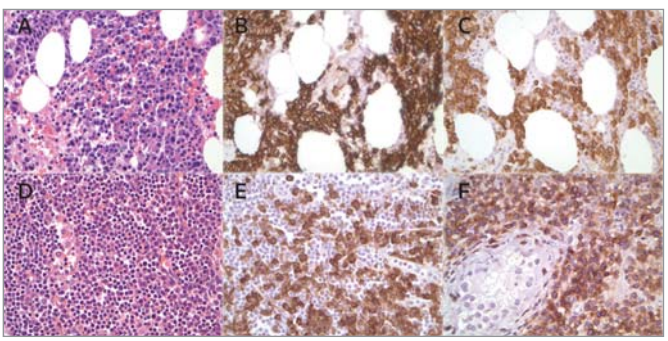

Figure 4. A-C: Multiple myeloma bone marrow, serial sections. Hematoxylin and eosin stain (**A**) shows sheets of plasma cells that strongly express CD138 (**B**) and CS1 (**C**); **D, E:** hematoxylin and eosin stain of a lymphoplasmacytic lymphoma in a lymph node shows small lymphocytes and numerous plasmacytic cells (**D**). The plasmacytic cells express CS1 (**E**); F: a nasal-type NK/T-cell lymphoma in testis strongly expresses CS1.

(15). Furthermore, elotuzumab was active in inhibiting the growth of several murine myeloma xenograft models in a dose-dependent fashion (5). This activity was largely dependent on the presence of functional NK cells, indicating that in vivo antitumor activity is also mediated by ADCC (4). The limited normal tissue expression profile of CS1, as well as the binding specificity and preclinical activity of elotuzumab, support its development as a potential therapeutic mAb for multiple myeloma.

The biological role for CS1 in normal and malignant plasma cells has yet to be elucidated. Studies exploring the function of CS1 in myeloma cells have suggested that CS1 is involved in adhesion to bone marrow stromal cells (BMSCs). Detailed subcellular localization studies showed that CS1 localized to uropod membrane domains in polarized myeloma cell lines and primary myeloma cells (5).

Experimental evidence for an adhesion role for CS1 was also demonstrated. Using a short interfering (si)RNA strategy, myeloma cells with downregulated expression of CS1 were generated, showing decreased adhesion to BMSC-coated culture plates compared with nontransduced cells. In addition, elotuzumab blocked adhesion of myeloma cells to BMSCs (5). This suggests that elotuzumab treatment may affect myeloma cell survival not only by inducing ADCC, but also possibly by inhibiting the survival signals that arise from adhesion to BMSCs.

Most recently, CS1-depleted cells generated using short hairpin (sh)RNA have been shown to have altered signaling and survival properties. Specifically, OPM2 myeloma cells with decreased CS1 demonstrated decreased phosphorylation of ERK1/2, STAT3 and AKT –pathways known to be activated by CS1 in NK cells (11, 16). CS1-depleted cells also had decreased survival via enhanced apoptosis compared with control cells when exposed to serum deprivation. Thus, CS1 appears to mediate prosurvival signals in plasma cells. Finally, the CS1 knockdown OPM2 cells had an impaired ability to form tumors in mouse models, supporting an important role for CS1 in the biology of myeloma and also providing a further rationale for targeting CS1 in this disease (16).

Clinical studies with elotuzumab

Following promising preclinical results, phase I trials of elotuzumab as both monotherapy in patients with relapsed/refractory myeloma and in combination with standard-of-care agents in relapsed myeloma were initiated. Interim data for the monotherapy trial indicated that at doses up to 20 mg/kg of elotuzumab administered intravenously every other week, the maximum tolerated dose (MTD) had not been reached. Five of six drug-related serious adverse events were associated with a first-dose infusion reaction, which resolved the same day (17). Interim data, reported at the 2008 American Society of Hematology annual meeting, indicated that during the dose-escalation phase, 6 of 18 patients with relapsed and refractory multiple myeloma experienced stable disease as assessed by EBMT (European Group for Blood and Marrow Transplantation) criteria, suggesting that elotuzumab has biological activity (17).

In another phase I study in relapsed myeloma, doses of up to 20 mg/kg elotuzumab administered every 10 days were well tolerated when combined with the standard dose of the multiple myeloma therapy bortezomib (18). In this study, an objective response (partial response or better) was observed in 55% (6 of 11) of patients during the dose-escalation phase of the trial. Similarly, when elotuzumab was combined with lenalidomide and dexamethasone to treat patients with relapsed myeloma, no unexpected safety issues were observed. Four of six patients treated experienced an objective response (19). Although these studies are still ongoing and the activity of elotuzumab remains to be determined, this novel therapeutic candidate may represent a significant advance in the treatment of myeloma. Depending on the final results of these safety and future efficacy studies, elotuzumab may represent a new member of a class of drugs aimed at utilizing target specificity and the immune system to treat cancer.

DISCLOSURE

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REFERENCES

1. Boles, K.S., Mathew, P.A. *Molecular cloning of CS1, a novel human natural killer cell receptor belonging to the CD2 subset of the immunoglobulin superfamily*. Immunogenetics 2001, 52(3-4): 302-7.
2. Bouchon, A., Cella, M., Grierson, H.L., Cohen, J.I., Colonna, M. *Activation of NK cell-mediated cytotoxicity by a SAP-independent receptor of the CD2 family*. J Immunol 2001, 167(10): 5517-21.
3. Hsi, E.D., Steinle, R., Balasa, B., Rice, A., Ko, Y.-H., Afar, D.E.H. *CS-1 is expressed in nasal type NK/T cell lymphomas and angioimmunoblastic T-cell lymphomas: Implications for targeted therapy with elotuzumab (HuLuc63).* Blood [50th Annu Meet Am Soc Hematol (Dec 6-9, San Francisco) 2008] 2008, 112(11): Abst 1779.
4. Hsi, E.D., Steinle, R., Balasa, B. et al. *CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma*. Clin Cancer Res 2008, 14(9): 2775-84.
5. Tai, Y.T., Dillon, M., Song, W. et al. *Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu*. Blood 2008, 112(4): 1329-37.
6. Boles, K.S., Stepp, S.E., Bennett, M., Kumar, V., Mathew, P.A. *2B4 (CD244) and CS1: Novel members of the CD2 subset of the immunoglobulin superfamily molecules expressed on natural killer cells and other leukocytes*. Immunol Rev 2001, 181: 234-49.
7. Sayos, J., Wu, C., Morra, M. et al. *The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM.* Nature 1998, 395(6701): 462-9.
8. Bhat, R., Eissmann, P., Endt, J., Hoffmann, S., Watzl, C. *Fine-tuning of immune responses by SLAM-related receptors*. J Leukoc Biol 2006, 79(3): 417-24.
9. Kumaresan, P.R., Lai, W.C., Chuang, S.S., Bennett, M., Mathew, P.A. *CS1, a novel member of the CD2 family, is homophilic and regulates NK cell function*. Mol Immunol 2002, 39(1-2): 1-8.
10. Lee, J.K., Mathew, S.O., Vaidya, S.V., Kumaresan, P.R., Mathew, P.A. *CS1 (CRACC, CD319) induces proliferation and autocrine cytokine expression on human B lymphocytes.* J Immunol 2007, 179(7): 4672-8.
11. Tassi, I., Colonna, M. *The cytotoxicity receptor CRACC (CS-1) recruits EAT-2 and activates the PI3K and phospholipase Cgamma signaling pathways in human NK cells*. J Immunol 2005, 175(12): 7996-8002.
12. Morra, M., Lu, J., Poy, F. et al. *Structural basis for the interaction of the free SH2 domain EAT-2 with SLAM receptors in hematopoietic cells.* EMBO J 2001, 20(21): 5840-52.
13. Cruz-Munoz, M.E., Dong, Z., Shi, X., Zhang, S., Veillette, A. *Influence of CRACC, a SLAM family receptor coupled to the adaptor EAT-2, on natural killer cell function*. Nat Immunol 2009, 10(3): 297-305.
14. Lee, J.K., Boles, K.S., Mathew, P.A. *Molecular and functional characterization of a CS1 (CRACC) splice variant expressed in human NK cells that does not contain immunoreceptor tyrosine-based switch motifs.* Eur J Immunol 2004, 34(10): 2791-9.
15. Balasa, B., Huseni, M., Cherukuri, J. et al. *Elotuzumab (HuLuc63) activates CD56^{dim} natural killer cells and monocytes resulting in the release of IP-10 and MCP-1.* Blood [50th Annu Meet Am Soc Hematol (Dec 6-9, San Francisco) 2008] 2008, 112(11): Abst 108.
16. Tai, Y.T., Soydan, E., Song, W. et al. *CS1 promotes multiple myeloma cell adhesion, clonogenic growth, and tumorigenicity via c-maf-mediated interactions with bone marrow stromal cells*. Blood 2009, 113(18): 4309-18.
17. Zonder, J.A., Singhal, S., Bensinger, W. et al. *Phase I study of elotuzumab (HuLuc63) in phase I study of elotuzumab (HuLuc63) in relapsed/refractory multiple myeloma.* Blood [50th Annu Meet Am Soc Hematol (Dec 6-9, San Francisco) 2008] 2008, 112(11): Abst 2773.

18. Jakubowiak, A.J., Bensinger, W., Zimmerman, T., Channan-Khan, A., Singhal, A., Siegel, D., Anderson, K. *Phase I study of elotuzumab in combination with bortezomib ± dexamethasone in MM patients with 1-3 prior therapies: Interim results*. Clin Lymphoma Myeloma (Int Myeloma Workshop) 2009, Abst 264.

19. Lonial, S., Vij, R., Westland, C., Singhal, A., Jagannath, S. *Phase 1b study of elotuzumab (HuLuc63) in combination with lenalidomide/dexamethasone in relapsed multiple myeloma: Interim results*. Clin Lymphoma Myeloma (Int Myeloma Workshop) 2009, Abst 129.