

# CS1 is expressed in Nasal type NK/T cell lymphomas: Implications for targeted therapy with Elotuzumab (HuLuc63)



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## ABSTRACT

CS1 (CRACC, SLAMF7, CD319) is a member of the signaling lymphocyte activating molecule-related receptor family. It is highly and uniformly expressed on the cell surface of benign and malignant plasma cells. We have recently reported on elotuzumab (formerly known as HuLuc63), a humanized monoclonal antibody targeting CS1, that is currently in phase 1 trials in relapsed multiple myeloma. Lower levels of the CS1 protein have also been reported on NK cells and NK-like T-cells (NK/T). CS1 expression in NK and T-cell lymphomas - aggressive lymphomas for which no effective therapy exists - is unknown. Here, we examined the expression of CS1 in normal NK/T cells and in a series of NK and peripheral T-cell lymphomas (PTCL).

Gene expression profiling showed CS1 expression in purified NK and NK/T cells. We confirmed cell surface expression of CS1 protein on normal blood NK and NK/T cells (n=12 samples) by flow cytometry with FITC (Fluorescein isothiocyanate)-elotuzumab. The majority of normal NK and NK/T cells expressed CS1 (mean% positive and standard deviation of 97% ± 4% and 67% ± 29%, respectively). We then evaluated tumor samples from patients with nasal type NK/T cell lymphoma as well as other peripheral T-cell lymphomas by immunohistochemistry using a paraffin-reactive CS1 antibody (clone 1G9). Biopsies from 13 patients (5 from the United States, 8 from Korea) with nasal type NK/T cell lymphomas were evaluated. 12 of 13 (92%) patient samples expressed CS1 with 1 being 1+, 3 being 2+, 5 being 3+, and 3 being 4+ in expression level. 37 PTCLs were also evaluated (including 8 AITL). Overall, 7/27 patients (29 samples) with PTCL NOS and 1/8 cases of AITL expressed CS1.

CS1 is expressed on nearly all nasal type NK/T cell lymphomas. These results support the rationale for exploring elotuzumab in the targeted treatment of NK/T-cell malignancies.

## INTRODUCTION

CS1 (CD319, CRACC, SLAMF7) is a member of the signaling lymphocyte activating-molecule-related receptor family. It has recently been identified as a cell surface antibody target selectively expressed in plasma cells.<sup>1</sup> Other members of the signaling lymphocyte activating-molecule (SLAM)-related receptor family include SLAM(CD150), 2B4 (CD244), CD84, NTB-A (Ly-108), and Ly-9 (CD229). These molecules are characterized by two or four extracellular immunoglobulin (Ig)-like domains and an intracellular signaling domain with immune receptor tyrosine-based switch motifs with the consensus amino acid sequence TxYxxV/I.

A humanized antibody to CS1 (elotuzumab, formerly known as HuLuc63) is currently under investigation in relapsed multiple myeloma.<sup>2</sup> Although selectively expressed in benign and malignant plasma cells, CS1 is also expressed at lower levels in normal NK cells and a subset of T-cells.<sup>3</sup> In this study, we characterize expression of CS1 in a series of T-cell lymphomas and nasal type NK cell lymphoma, an aggressive lymphoma without satisfactory treatment options.

## METHODS

**Cases and Immunohistochemistry:** Study cases were derived from the archives of the Cleveland Clinic and Samsung Hospital. Diagnoses were established according to established criteria of the WHO classification for hematolymphoid tumors. For the mature T-cell lymphomas a tissue microarray was constructed using duplicate 1mm diameter cores (Beecher Instruments, Sun Prairie, WI). Immunohistochemistry was performed as described using monoclonal antibodies to CD4 and CD8 (Ventana Medical Systems, Tucson AZ) or with the paraffin reactive anti-CS1 monoclonal antibody 1G9.1. Cases were considered positive if greater than 10% of tumor cells expressed CS1. Cases were scored as negative (<10%), 1+ (10-24%), 2+ (25-49%), 3+ (50-74%), or 4+ (75-100%). HANK1 cells are an IL-2 dependent cell line derived from a nasal type NK/T lymphoma as previously described.<sup>3</sup>

**Flow cytometry:** CS1 expression in normal NK and T-cells was assessed by gene expression profiling. Flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA) was performed on normal blood samples using a directly conjugated FITC-elotuzumab. Anti-CS1-PE mouse monoclonal antibodies (Clone 235614) for flow cytometry were obtained from R&D Systems (Minneapolis, MN).

## RESULTS

### Peripheral blood lymphocyte (PBL) flow cytometry (Table 1):

Prior gene profiling studies have demonstrated CS1 expression in normal NK cells.<sup>4,5</sup> Murine monoclonal antibodies to CS1 demonstrated high expression of CS1 in NK and NK/T cells.<sup>1</sup> Using directly conjugated elotuzumab, flow cytometry was performed in order to confirm expression patterns of CS1 in normal lymphocytes. 12 specimens were analyzed. B and CD4+ T-cells lacked appreciable amounts of CS1. However, NK cells and, to a lesser extent, CD16/56 positive-T-cells expressed CS1 (mean% positive and standard deviation of 97% ± 4% and 67% ± 29%, respectively) (Figure 1). Because of this expression pattern in normal lymphocytes, we then evaluated expression of CS1 in peripheral T-cell lymphoma, not otherwise specified (PTCL NOS), angioimmunoblastic T-cell lymphoma (AITL), and nasal type NK/T cell lymphoma.

### CS1 expression in T-cell Lymphoma and Nasal type NK/T cell lymphoma:

A paraffin reactive CS1 antibody (clone 1G9) was developed and used for immunohistochemistry in archival fixed tissues. Figure 2 shows the pattern in normal tonsil in which scattered T-cells and plasma cells (enriched in subepithelial areas) are positive. 37 peripheral T-cell lymphomas consisting of 29 PTCL NOS from 27 patients, and 8 AITLs were studied. 7/29 (24%) of PTCL NOS cases were positive. Two patients each had two biopsies. In one, both specimens were negative while in the other, one relapse specimen was negative while the initial specimen was positive. In the 6 CS1+ PTCL NOS specimens for which CD4 or CD8 expression could be assigned, 4 were CD8+.

Only 1 of 8 (15%) AITLs were positive and this case was CD4+ (Figure 3). Because NK and NK-like T-cells expressed CS1, we also examined a series of nasal type NK/T cell lymphomas. 12 of 13 cases (92%) expressed CS1. The scores were 1+ (1), 2+ (3), 3+ (5), and 4+ (3) (Figure 3). Thus, as a group this lymphoma type more frequently expressed CS1 compared to the most common types of mature T-cell lymphomas (P<.0001, Fisher exact). Examination of the nasal NK/T cell lymphoma cell line HANK1 by flow cytometry confirmed CS1 surface expression (Figure 4).

Table 1: CS1 expression in lymphocyte subsets from normal blood

| cell type        | phenotype                                  | n  | mean | SD |
|------------------|--|----|------|----|
| NK cells         | % Elotuzumab+ of True NK (CD3-/CD(16+56)+) | 12 | 97   | 4  |
| NKT cells        | % HuLuc63+ of CD3+/CD(16+56)+              | 12 | 67   | 29 |
| CD3+CD8+         | % Elotuzumab + of CD3+CD8+                 | 12 | 51   | 20 |
| CD3+CD8+(16+56)± | % Elotuzumab + (CD16+56)+ of CD3+CD8+      | 12 | 17   | 13 |
| CD3+CD8+(16+56)± | % Elotuzumab + (CD16+56)- of CD3+CD8+      | 12 | 34   | 18 |
| CD4 cells        | % Elotuzumab + of CD3+CD8-                 | 12 | 10   | 7  |
| B cells          | % Elotuzumab + of CD20+HLA-DR+             | 12 | 4    | 7  |

Figure 1: Flow cytometry of peripheral blood NK and NK/T cells

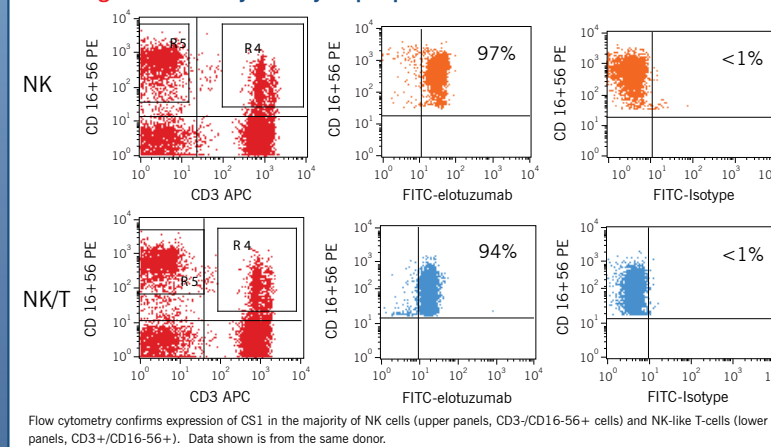


Figure 4: CS1 expression in T-cell and NK lymphomas

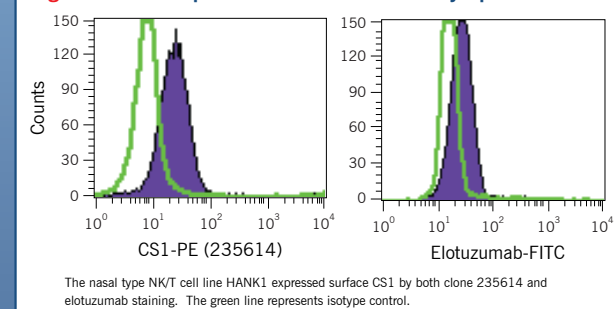
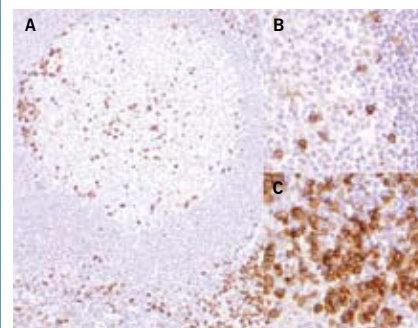
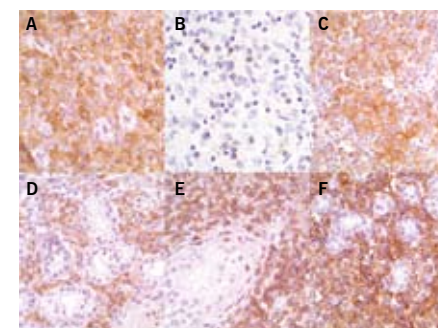


Figure 2: CS1(1G9) expression in tonsil



CS1 expression in tonsil. **A**) A germinal center is negative in B-cell areas with only scattered individual lymphocytes and plasma cells. **B**) High magnification at the border of the germinal center and mantle zone. **C**) Sub-epithelial area as in the lower right corner of A. Numerous plasma cells express CS1.

Figure 3: CS1 expression in T-cell and NK lymphomas



Upper panels: CS1 is expressed in a PTCL NOS (A) and AITL (C). Panel B illustrates and PTCL NOS that was negative for CS1. Lower panels: Examples of 3 different nasal type NK/T cell lymphomas expressing CS1.

## CONCLUSIONS

1. The great majority of nasal type NK/T-cell lymphomas express CS1, as might be expected from expression in normal NK and NK/T-cells.
2. CS1 is expressed in a minority of PTCL NOS and AITL
3. These data support rationale for further examination of elotuzumab as a potential targeted therapeutic for the treatment of nasal type NK/T cell lymphoma.

## REFERENCES

1. Hsi ED, Steinle R, Balasa B et al. CS1, a Potential New Therapeutic Antibody Target for the Treatment of Multiple Myeloma. Clin Cancer Res. 2008;14:2775-2784.
2. Tai YT, 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. 2007.
3. Kagami Y, Nakamura S, Suzuki R et al. Establishment of an IL-2-dependent cell line derived from 'nasal-type' NK/T-cell lymphoma of CD2+, sCD3-, CD3epsilon+, CD56+ phenotype and associated with the Epstein-Barr virus. Br J Haematol. 1998;103:669-677.
4. Boles KS, Stepp SE, Bennett M, Kumar V, Mathew PA. 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;234-249.
5. Boles KS, Mathew PA. Molecular cloning of CS1, a novel human natural killer cell receptor belonging to the CD2 subset of the immunoglobulin superfamily. Immunogenetics. 2001;52:302-307.