Elotuzumab Promotes Self-Engagement of SLAMF7 Between Natural Killer and Multiple Myeloma Cells to Enhance Cytotoxicity

Tatiana Pazina,¹ Ashley M. James,² Amy Jhatakia,³ Robert F. Graziano,³ Natalie A. Bezman,³ Michael D. Robbins,³ Adam D. Cohen,⁴ Kerry S. Campbell¹

¹Fox Chase Cancer Center, Philadelphia, PA; ²American Association for Cancer Research, Philadelphia, PA; ³Bristol-Myers Squibb, Princeton, NJ; ⁴University of Pennsylvania, Philadelphia, PA

Background

- Elotuzumab (elo) is a humanized immunoglobulin G1 monoclonal antibody (mAb) that targets human signaling lymphocytic activation molecule family member 7 (SLAMF7).^{1,2}
- SLAMF7, a self-ligand, is highly expressed on multiple myeloma (MM) cells, natural killer (NK) cells, and other immune cells.^{1,2} On NK cells, SLAMF7 exists as two isoforms—SLAMF7-L and SLAMF7-S—which can influence signaling³
- SLAMF7 self-adhesion between macrophages and tumor cells has a critical role in mediating tumor cell phagocytosis⁴
- Elo binds SLAMF7 on MM cells and promotes NK-mediated antibody-dependent cellular cytotoxicity (ADCC) via Fc interaction with FcγRIIIA (CD16),^{2,5,6} which is further enhanced when combined with immunomodulatory drugs (IMiDs).^{5,6}
- Elo also mediates antibody-dependent cellular phagocytosis of SLAMF7⁺ MM cells⁷
- Elo, in combination with an IMiD (lenalidomide) and dexamethasone (dex), has been shown to improve progression-free survival (PFS) in relapsed/refractory MM (RRMM)⁸
- A recent trial has also shown a significant PFS benefit for elo plus another IMiD, pomalidomide, and dex in RRMM⁹
- Previous preclinical studies also suggest that elo enhances NK cell activity via a costimulation mechanism independent of CD16 by binding directly to SLAMF7 expressed on the NK cell surface^{1,10}

Objective

- To characterize the mechanism through which elo affects interactions between NK and MM cells to enhance NK cell-mediated cytotoxicity

Methods

Cell lines

- RPMI8226 MM (RPMI8226+SLAMF7) and SKOV3 ovarian adenocarcinoma (SKOV3+SLAMF7) cell lines were engineered to express high constitutive levels of SLAMF7
- CRISPR/Cas9 technology was used to generate SLAMF7-deficient human NK92 cells (SLAMF7⁻, CD16⁻)
- SLAMF7⁻ and SLAMF7⁺ NK92 cells were retrovirally transduced with human CD16 cDNA to generate SLAMF7⁻, CD16⁺ and SLAMF7⁺, CD16⁺ cells
- SLAMF7⁻ NK92 cells were retrovirally transduced with full-length SLAMF7 (SLAMF7-L) or truncated SLAMF7 missing the cytoplasmic intracellular immunoreceptor tyrosine-based switch motif (ITSM) (SLAMF7-S)

Antibodies

- An elo variant containing a mutation in the Fc portion of the antibody that prevents binding to CD16 (elo Fc mut), as well as two mAbs binding different SLAMF7 epitopes (PDL241, 162.1), were provided by Bristol-Myers Squibb (Princeton, NJ)
- The anti-SLAMF7 mAb ChLuc90 was provided by AbbVie Inc. (North Chicago, IL)

Cell assays

- NK cell-mediated cytotoxicity was measured using the CytoTox 96[®] non-radioactive assay (Promega, Madison, WI) and the xCELLigence[®] real-time cell analysis platform (ACEA Biosciences, San Diego, CA)
- To measure the effect of elo on SLAMF7-SLAMF7 interaction, a human (h) SLAMF7 extracellular/T-cell receptor (TCR)-ζ intracellular fusion construct was transfected into a 3A9 mouse T-cell line and used as a reporter of SLAMF7 binding by measuring interleukin (IL)-2 production with an enzyme-linked immunosorbent assay
- For statistical analysis, 3–6 repeats were performed under each experimental condition

Figure 1. Elo can enhance natural cytotoxicity by NK cells in a CD16-independent manner

(A) Effect of elo (0.001–300 µg/mL) on natural cytotoxicity of NK92 (SLAMF7⁺, CD16⁻) cells toward RPMI8226 cells expressing low or high (+SLAMF7) levels of SLAMF7. Comparison between NK92+RPMI8226 vs NK92+RPMI8226+SLAMF7 using unpaired Welch's t test, *p<0.05, **p<0.01; comparison between indicated groups (overhead bars) using paired Student t test, *p<0.05, **p<0.01. (B) Schematic of elo effect on cytotoxicity of NK cells against SLAMF7⁺ target cells. (C) Kinetics of natural cytotoxicity of NK92 (SLAMF7⁺, CD16⁻) cells toward adherent SKOV3 (+SLAMF7) target cells in the presence of elo as measured using the xCELLigence assay. Her2+ SKOV3 cells and Herceptin (Her) were used as a control. (D) Mean (SD) target cell death at 10-h timepoint by xCELLigence assay. Comparison between indicated groups using paired Student t test, *p<0.05, **p<0.01

Results

- Consistent with previous reports,^{1,10} elo enhanced the natural cytotoxicity of SLAMF7⁺ NK92 cells against SLAMF7-expressing target cells
 - No cell death occurred when target cells lacked SLAMF7 (Figure 1A–B)
- Elo-mediated enhanced NK92 cell natural cytotoxicity was independent of CD16
 - Elo significantly enhanced killing of RPMI8226 (+SLAMF7) and SKOV3 cells (+SLAMF7) by CD16-deficient NK92 effector cells (Figure 1A, C, D)
- Elo, however, did not enhance the cytotoxicity of NK cells lacking expression of both SLAMF7 and CD16 (Figure 2)

Figure 2. Elo cannot enhance natural cytotoxicity by NK cells lacking SLAMF7 expression

(A) Mean (SD) target cell death at 10-h timepoint from xCELLigence assays for elo and NK92 (SLAMF7⁺, CD16⁻) effector cells toward SKOV3 (+SLAMF7) target cells. Comparison between indicated groups was performed using the paired Student t test, **p<0.01. (B) Schematic showing requirement for SLAMF7 expression on NK cells for CD16-independent NK cell-mediated cytotoxicity. NS, not significant

- Engagement with SLAMF7 on NK cells by elo costimulates ADCC (Figure 3)
 - Both elo and elo Fc mutant (Fc mut, which cannot bind to CD16) significantly enhanced killing of SKOV3 (+SLAMF7) cells by NK92 (SLAMF7⁺, CD16⁻) cells, although elo was more potent
 - By contrast, elo Fc mut had no effect on the cytotoxicity of SLAMF7-deficient NK92 (SLAMF7⁻, CD16⁻) cells on SKOV3 (+SLAMF7) targets

Figure 3. Effect of elo and elo Fc mutant on cytotoxicity of NK92 (SLAMF7⁺, CD16⁻) cells against SKOV3 (+SLAMF7) target cells

Mean (SD) target cell death at 10-h timepoint from xCELLigence assays for elo and elo Fc mut and NK92 (SLAMF7⁺, CD16⁻) cells and NK92 (SLAMF7⁻, CD16⁻) cells toward SKOV3 (+SLAMF7) cells. Comparison between indicated groups using paired Student t test, **p<0.01

- Among different anti-SLAMF7 antibodies, elo uniquely promoted SLAMF7-SLAMF7 interactions
 - Elo induced notable IL-2 secretion when added to the 3A9 reporter cell line in plates coated with SLAMF7, whereas other antibodies to different SLAMF7 epitopes induced only low levels of IL-2 secretion (Figure 4)
- Only elo enhanced killing of SLAMF7-expressing target cells by SLAMF7⁺ CD16⁻ NK92 cells; other SLAMF7 mAbs modestly suppressed natural cytotoxicity under the same conditions (Figure 5)
- Elo enhanced the natural cytotoxicity of NK cells only if SLAMF7 contained the full cytoplasmic domain (Figure 6)

Figure 4. Elo is unique among several mAbs in promoting SLAMF7-SLAMF7 interactions in a reporter model

(A) Elo (0.1–3 µg/mL) induced high-level IL-2 secretion when added to 3A9 reporter cells in plates coated with recombinant hSLAMF7; other SLAMF7 mAbs induced low levels of IL-2 at higher doses (1–3 µg/mL). Plotted as mean (SEM). (B) Reporter assay system schematic. Elo promoted IL-2 secretion by the reporter cell line when plates were coated with recombinant hSLAMF7, but not when coated with control protein mesothelin. Isotype control antibody had no effect. ECD, extracellular domain; ICD, intracellular domain; mAb #1, ChLuc90; mAb #2, 162.1; mAb #3, PDL241

Figure 5. Elo uniquely promotes cytotoxicity of CD16-deficient NK92 cells toward SLAMF7-expressing target cells

Mean (SD) target cell death at 10-h timepoint by xCELLigence assay with different SLAMF7 mAbs. Comparison between indicated groups using paired Student t test, **p<0.01. mAb #1, ChLuc90; mAb #2, 162.1; mAb #3, PDL241

Figure 6. Elo promotes natural cytotoxicity of CD16-deficient NK92 cells expressing full SLAMF7 (+SLAMF7-L), but not a truncated (+SLAMF7-S) isoform

(A) Mean (SD) target cell death at the 10-h timepoint from xCELLigence assays using elo on NK92 (+SLAMF7-L, CD16⁻) or NK92 (+SLAMF7-S, CD16⁻) cells toward SKOV3 (+SLAMF7) cells. Comparison between indicated groups using paired Student t test, *p<0.05. (B) Schematic showing requirement of cytoplasmic ITSM motif of SLAMF7 on NK cells for elo effect on SLAMF7-SLAMF7 interaction

Conclusions

- Elotuzumab's additional function as an NK cell-activating mAb required SLAMF7 expression on both NK and target cells and the presence of the ITSM motif in the long version of SLAMF7 on NK cells
- Elotuzumab uniquely facilitates or enhances SLAMF7-SLAMF7 interactions between SLAMF7⁺ NK cells and SLAMF7⁺ MM targets in a CD16-independent manner (Figure 7)

Figure 7. Elotuzumab mechanism of action

ADCC, antibody-dependent cellular phagocytosis

Future investigations

- Additional studies are needed to understand the impact of combination treatment with IMiDs on elotuzumab-mediated SLAMF7-SLAMF7 interactions, and to investigate SLAMF7 isoform expression on NK cells as a biomarker for elotuzumab efficacy
- Further studies are required to address the ability of elotuzumab to enhance SLAMF7 self-adhesion interactions to augment macrophage-mediated killing of SLAMF7⁺ MM cells

References

- Collins SM et al. *Cancer Immunol Immunother* 2013;62:1841–9
- Hsi ED et al. *Clin Cancer Res* 2008;14:2775–84
- Lee JK et al. *Eur J Immunol* 2004;34:2791–9
- Chen J et al. *Nature* 2017;544:493–7
- Tai YT et al. *Blood* 2008;112:1329–37
- Balasa B et al. *Cancer Immunol Immunother* 2015;64:61–73
- Kurdi AT et al. *Mol Cancer Ther* 2018;17:1454–63
- Lonial S et al. *N Engl J Med* 2015;373:621–31
- Dimopoulos MA et al. *EHA* 2018 [Oral LB2606]
- Pazina T et al. *Oncol Immunology* 2017;6:e1339853

Acknowledgments

Support for this work from Bristol-Myers Squibb. Professional medical writing and editorial assistance was provided by Janice Zhou of Caudex, and was funded by Bristol-Myers Squibb.

Disclosures

AJ, RFG, NAB: employment: Bristol-Myers Squibb. MDR: employment, stock ownership: Bristol-Myers Squibb. ADC and KSC received grant funding from and served on scientific advisory boards for Bristol-Myers Squibb. TP, AMJ: nothing to disclose.

Copies of this poster obtained through Quick Response (QR) code are for personal use only and may not be reproduced without permission from the authors