NEO-201-recognized truncated Core 1 O-glycans represent a new target for CAR-NK therapy in AML

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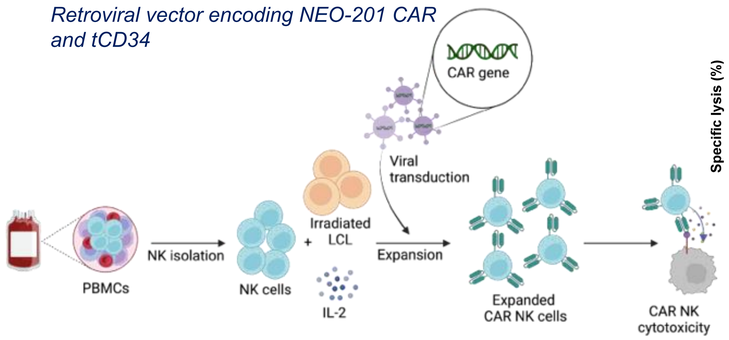
INTRODUCTION

- Acute myeloid leukemia (AML) continues to have poor long-term outcomes, particularly in high-risk and relapsed/refractory disease.
- Progress in AML immunotherapy has been limited by the scarcity of antigens selectively expressed on leukemic cells while sparing hematopoietic stem/progenitor cells (HSPCs).
- Aberrant surface glycans represent an underexplored class of tumor-associated targets.
- NEO-201 is a humanized IgG1 monoclonal antibody that binds truncated Core 1 O-glycans with limited reactivity to most normal tissues and an acceptable safety profile in a phase I solid tumor trial (NCT03476681).
- NEO-201-associated neutrophil depletion in clinical studies suggests expression of this antigen within the myeloid lineage.
- We hypothesized that NEO-201–recognized truncated O-glycans represent a novel AML-associated antigen and evaluated their expression across AML subtypes while developing a CAR NK cell therapy targeting this antigen.

METHODS

- AML cell lines representing diverse molecular subtypes were analyzed for NEO-201 binding by flow cytometry.
- NK cells were isolated from healthy donor peripheral blood buffy coats and expanded using a clinically established ex vivo expansion method.

Retroviral vector encoding NEO-201 CAR and tCD34



Schema of NK cell ex vivo expansion and retroviral transduction workflow

- CAR NK cells were generated via retroviral transduction using a second-generation CAR incorporating a NEO-201–binding domain, CD8α transmembrane region, 4-1BB co-stimulatory domain, CD3ζ signaling domain, and a truncated CD34 (tCD34) marker.
- Cytotoxicity was assessed in co-culture assays using calcein-AM–labeled AML targets at varying effector-to-target ratios.
- Antigen expression across neutrophil maturation stages was evaluated by flow cytometry in G-CSF–mobilized peripheral blood to assess potential on-target/off-tumor toxicity.

RESULTS

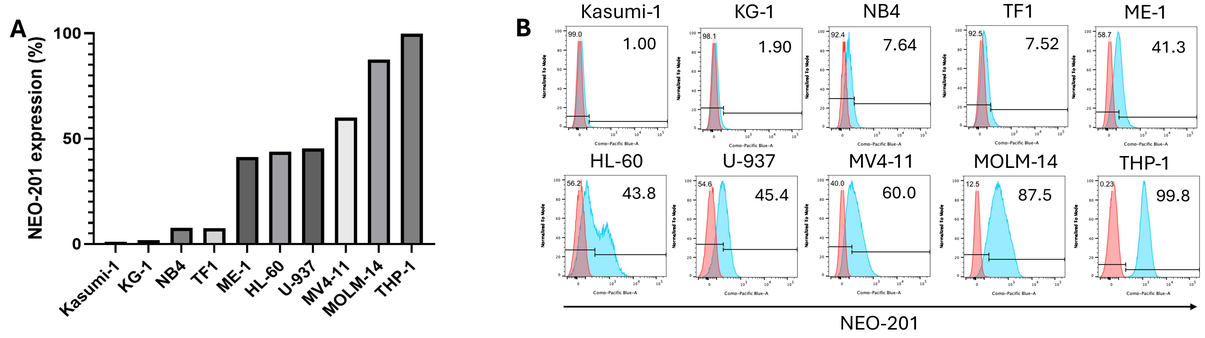


Figure 1. NEO-201 expression in AML cell lines. (A) Surface expression of NEO-201 across AML cell lines, quantified as percent positive cells by flow cytometry. **(B)** Representative histograms demonstrating variable NEO-201 expression, shown as overlay of NEO-201 staining and FMO control. Percent positive cells are indicated.

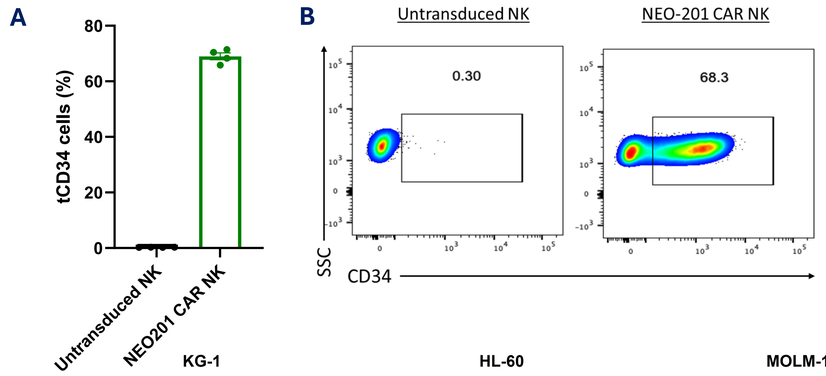


Figure 2. Generation and characterization of NEO-201 CAR NK cells. (A) NEO-201 CAR expression in NK cells following retroviral transduction, quantified as percentage of tCD34⁺ cells by flow cytometry. Data represent mean ± SD from 4 donor-derived NK cell experiments (mean transduction efficiency: 69% ± 2.5%). **(B)** Representative flow cytometry plots demonstrating efficient CAR expression in NEO-201 CAR NK cells compared with untransduced controls.

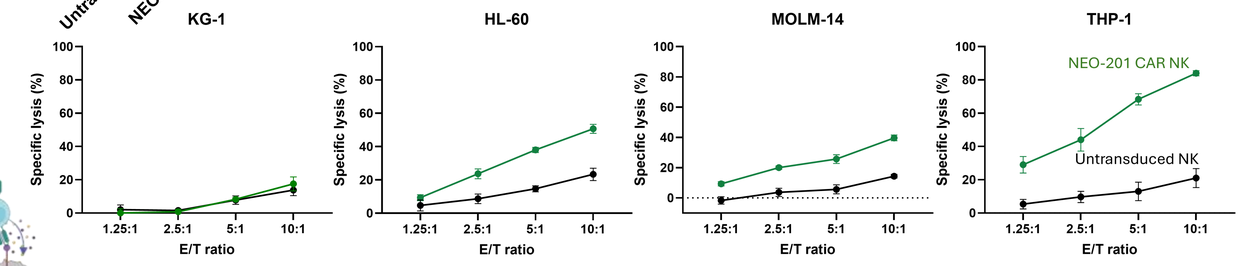


Figure 3. Cytotoxic activity of NEO-201 CAR NK cells against AML cell lines. NEO-201 CAR NK cells (green) exhibit dose-dependent cytotoxicity against AML cell lines across increasing effector-to-target (E:T) ratios. Cytotoxicity was assessed after 4 h co-culture and is greatest in NEO-201-expressing cell lines, with minimal activity observed in the NEO-201-negative cell line KG-1, consistent with antigen-dependent activity. Data represent mean ± SD from 3 donor-derived NK cell experiments.

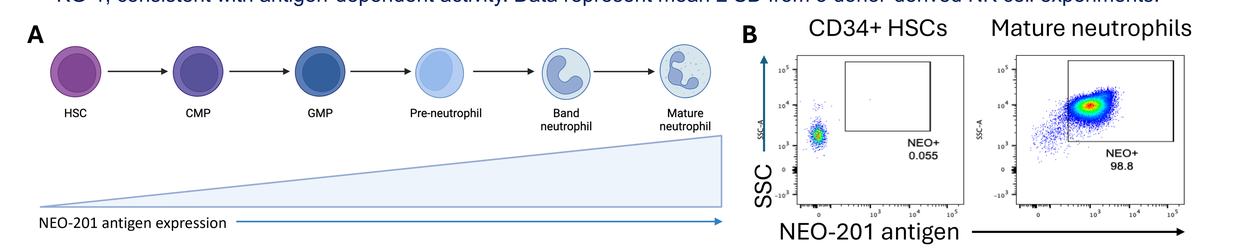


Figure 4. NEO-201 antigen expression across neutrophil maturation. (A) Schematic illustrates progressive myeloid differentiation from hematopoietic stem cells (HSCs) to mature neutrophils, with increasing NEO-201 antigen expression. **(B)** Representative flow cytometry plots demonstrate minimal expression in CD34⁺ HSCs and high expression in mature neutrophils, supporting lineage-restricted and maturation-dependent expression. Data derived from G-CSF–mobilized peripheral blood from healthy donors.

RESULTS (cont.)

- NEO-201 binding revealed variable surface expression of truncated Core 1 O-glycan antigens across AML cell lines representing diverse molecular subtypes, with absent expression in select models, demonstrating target heterogeneity.
- NEO-201 CAR NK cells were efficiently generated using retroviral transduction, achieving consistent CAR expression across donor-derived NK cells.
- NEO-201 CAR NK cells exhibited dose-dependent cytotoxicity against AML targets, with the greatest activity observed in NEO-201-expressing cell lines.
- Minimal cytotoxicity was observed in the NEO-201-negative cell line KG-1, consistent with antigen-dependent activity.
- Evaluation of NEO-201 expression across neutrophil maturation demonstrated minimal expression in CD34⁺ hematopoietic stem cells and increased expression in mature neutrophils, indicating maturation-dependent and lineage-restricted expression.

CONCLUSIONS

- NEO-201 recognizes truncated Core 1 O-glycans expressed on AML cells across multiple subtypes
- NEO-201 CAR NK cells can be reliably generated using clinically relevant expansion and transduction methods
- NEO-201 CAR NK cells demonstrate antigen-dependent cytotoxicity against AML targets in vitro
- Minimal binding to CD34+ hematopoietic progenitors suggests a potentially favorable therapeutic window
- These findings support truncated O-glycans as a novel and targetable antigen class in AML and provide a rationale for further development of NEO-201–directed cellular therapy

NEXT STEPS

- Validate antigen expression and cytotoxicity in primary AML patient samples
- Perform colony-forming unit and extended hematopoietic toxicity assays to further assess HSPC safety
- Evaluate in vivo efficacy and persistence in AML xenograft models
- Optimize CAR design and NK cell engineering to enhance persistence and activity
- Explore combination strategies to augment NK cell function in the AML microenvironment
- Advance toward early-phase clinical translation of NEO-201 CAR NK therapy

Selected References

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