GAPDH knock-in of high-affinity CD16 and mbIL-15 in iPSC-derived NK cells drives high-level expression and increased anti-tumor function

POSTER
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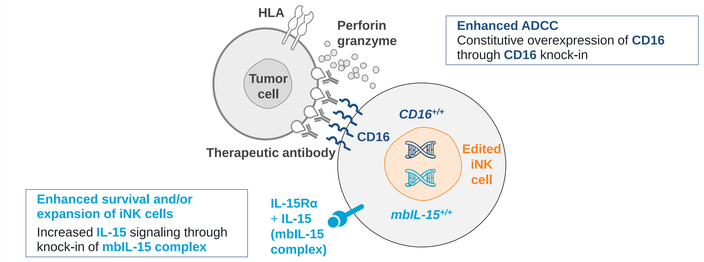
OBJECTIVE

To evaluate the level of cytotoxicity and persistence against tumor cells using CRISPR-AsCas12a-mediated knock-in of CD16 and membrane-bound interleukin-15 (mbIL-15) in natural killer (NK) cells derived from induced pluripotent stem cells (iPSCs).

INTRODUCTION

- NK cells are good candidates for off-the-shelf immunotherapy given their high tumor killing capacity and low propensity for graft-versus-host disease.
- NK cells express CD16, which can recognize antibodies that bind tumor antigens, thus promoting antibody-dependent cellular cytotoxicity (ADCC).[1] High-affinity CD16 variants in the human population correlate with better clinical outcomes and anti-tumor response[2]. Increasing CD16 expression on NK cells is expected to increase binding of tumor antigens and result in stronger ADCC.
- Interleukin-15 (IL-15) is important for NK cell survival. The addition of mbIL-15 would allow for prolonged survival of NK cells, without dependence upon exogenous supplementation of IL-15.[3]
- Exploiting the ability of CRISPR-AsCas12a-mediated SeLection by Essential-gene Exon Knock-in (SLEEK) of CD16 and mbIL-15 in NK cells is expected to enhance cytotoxicity and prolong survival, resulting in greater tumor killing capacity.

Enhanced functions of CRISPR-AsCas12a-edited iNK cells

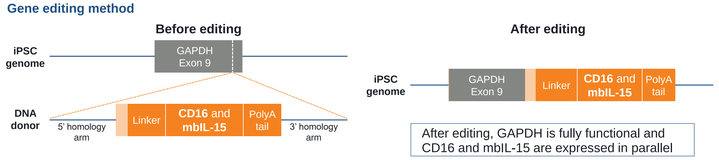


HLA, human leukocyte antigen; IL-15Rα, IL-15 receptor alpha; iNK cell, iPSC-derived NK cell.

METHODS

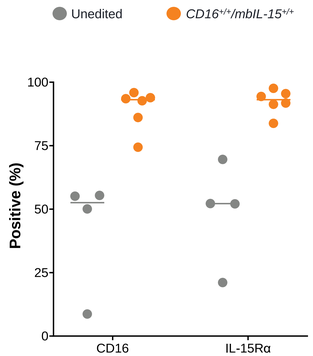
- iPSCs were edited at the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) locus with an engineered CRISPR-AsCas12a to knock-in CD16 and mbIL-15 using the SLEEK method;[4] iPSC clones were then differentiated into iPSC-derived NK (iNK) cells.
- Flow cytometry was used to detect CD16 and the IL-15 receptor alpha (IL-15Rα) chain.
- A fluorescent lactate dehydrogenase release assay using the ovarian cancer cell line SKOV-3 was used to assess 2D cell killing; CD16 shedding was analyzed by extracting iNK cells from the 2D killing assay after 48 hours and running flow cytometry.
- A 3D tumor spheroid killing assay using Incucyte® imaging of NucLight Red-tagged SKOV-3 cells was used to assess NK cell cytotoxicity.
- *In vitro* persistence was measured by culturing unedited and edited iNK cells in basal media without supporting cytokines for 21 days.

Gene editing method



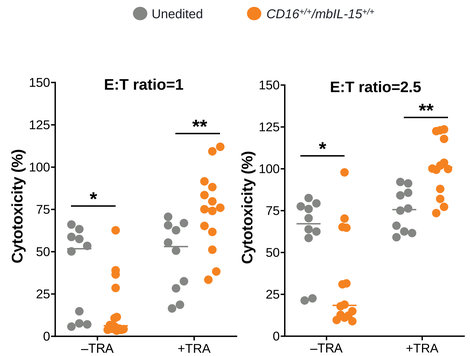
RESULTS

Figure 1. *CD16$^{+/+}$/mbIL-15$^{+/+}$* iNK cells had increased CD16 and IL-15Rα expression compared with unedited iNK cells



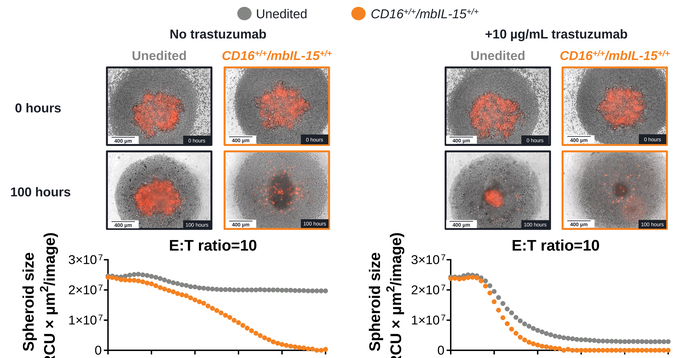
n=4 independent experiments.

Figure 2. *CD16$^{+/+}$/mbIL-15$^{+/+}$* iNK cells demonstrated increased ADCC compared with unedited iNK cells in a 2D cell killing assay



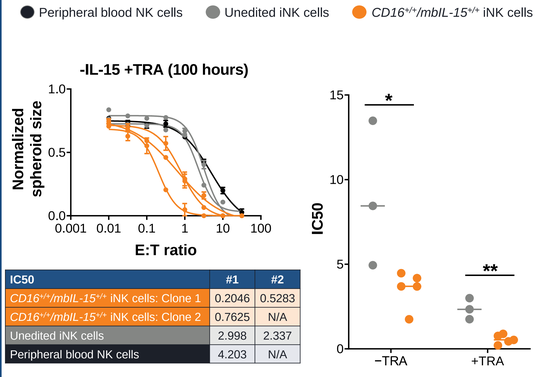
n=10–14 independent experiments. 5–7 biological replicates with technical duplicates. *p<0.05, **p<0.01 (two-way ANOVA).
ANOVA, analysis of variance; E, effector; T, target; TRA, trastuzumab.

Figure 3. *CD16$^{+/+}$/mbIL-15$^{+/+}$* iNK cells elicited greater reductions in tumor spheroid size compared with unedited iNK cells in a 3D tumor spheroid killing assay



E, effector; RCU, red calibrated unit; T, target.

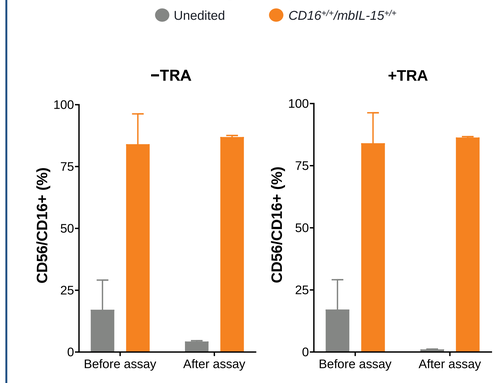
Figure 4. *CD16$^{+/+}$/mbIL-15$^{+/+}$* iNK cells showed enhanced cytotoxicity compared with unedited iNK cells in a 3D tumor spheroid killing assay



IC50	#1	#2
CD16$^{+/+}$/mbIL-15$^{+/+}$ iNK cells: Clone 1	0.2046	0.5283
CD16$^{+/+}$/mbIL-15$^{+/+}$ iNK cells: Clone 2	0.7625	N/A
Unedited iNK cells	2.998	2.337
Peripheral blood NK cells	4.203	N/A

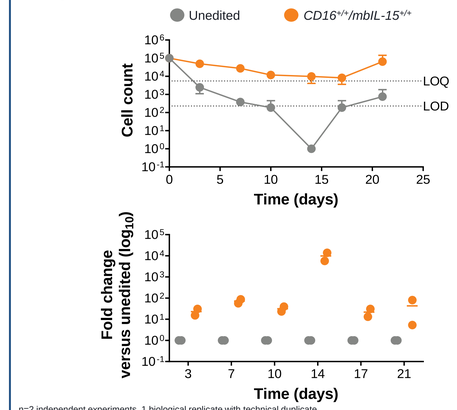
n=2 independent experiments. *p<0.05, **p<0.01 (unpaired t-test).
E, effector; IC50, half-maximal inhibitory concentration; T, target; TRA, trastuzumab.

Figure 5. CD16 surface expression was maintained in *CD16$^{+/+}$/mbIL-15$^{+/+}$* iNK cells but decreased in unedited iNK cells in a 2D killing assay



n=2 independent experiments.
TRA, trastuzumab.

Figure 6. mbIL-15 knock-in resulted in increased *in vitro* persistence in *CD16$^{+/+}$/mbIL-15$^{+/+}$* iNK cells compared with unedited iNK cells



n=2 independent experiments. 1 biological replicate with technical duplicate.
LOD, limit of detection; LOQ, limit of quantification.

CONCLUSIONS

- CRISPR-AsCas12a-mediated SLEEK knock-in of CD16 and mbIL-15 at the GAPDH locus in iNK cells increased expression of CD16 and mbIL-15 on the surface of iNK cells.
- *CD16$^{+/+}$/mbIL-15$^{+/+}$* iNK cells demonstrated enhanced cytotoxicity (due to increased and maintained CD16 expression) and increased persistence (due to mbIL-15 expression) versus unedited iNK cells.

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