AsCas12a gene-edited iPSC-derived NK cells constitutively expressing CD16 and membrane-bound IL-15 demonstrate prolonged persistence and robust anti-tumor activities in a solid tumor mouse model

POSTER 562

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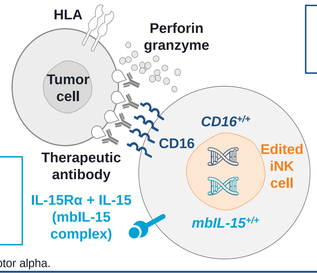
OBJECTIVE

To understand the functional enhancements of knocking-in CD16 and membrane-bound interleukin-15 (mbIL-15) into induced pluripotent stem cells (iPSCs) using AsCas12a and differentiating those iPSCs into induced natural killer (iNK) cells

INTRODUCTION

- Natural killer (NK) cell therapies are an attractive alternative therapy option to CAR-T cells, given their intrinsic tumor-killing capacity, few treatment-related toxicities, and ability to be administered to patients "off-the-shelf". Although most NK cell therapies are produced from healthy donor cells, deriving NK cells from induced pluripotent stem cells (iPSCs) allows generation of clones with any desired edits, conferring a unique advantage to this method.
- NK cells express CD16, which can recognize antibodies that bind tumor antigens, thus promoting antibody-dependent cellular cytotoxicity (ADCC).[1] Higher-affinity CD16 variants in the human population correlate with better clinical outcomes and antitumor response.[2] Therefore, increasing CD16 expression on iPSC-derived NK (iNK) cells is expected to increase binding of tumor antigens and result in more robust ADCC.
- Interleukin-15 (IL-15) is important for NK cell survival. The addition of a membrane-bound IL-15 (mbIL-15) is expected to prolong survival of iNK cells, without dependence on exogenous IL-15 supplementation.[3]
- By exploiting our SeLection by Essential-gene Exon Knock-in (SLEEK)[4] platform, which is powered by our proprietary AsCas12a, CD16 and mbIL-15 could be knocked into iNK cells with high efficiency and drive better ADCC and persistence of iNK cells.

Enhanced functions of CRISPR-AsCas12a-edited iNK cells



Enhanced ADCC
Constitutive overexpression of **CD16** through **CD16** knock-in

Enhanced survival and/or expansion of iNK cells
Increased **IL-15** signaling through knock-in of **mbIL-15 complex**

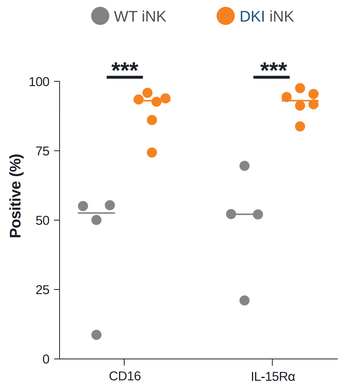
HLA, human leukocyte antigen; IL-15Rα, IL-15 receptor alpha.

METHODS

- Our proprietary engineered AsCas12a was used to generate double knocked-in (DKI) iPSC clones in which CD16 and mbIL-15 were knocked into an essential gene locus to increase the effector function and persistence of iNK cells. Constitutive surface expression of CD16 and mbIL-15 by the DKI iNK cells was demonstrated by flow cytometry.
- A 3D tumor spheroid killing assay using Incucyte® imaging of NucLight Red-tagged SKOV-3 cells was used to assess iNK cell cytotoxicity. In vitro persistence was measured by culturing wild type (WT) and DKI iNK cells in basal media without supporting cytokines for 21 days, as part of a 2D killing assay.
- The antitumor efficacy of the DKI iNK cells in vivo was evaluated using an SKOV3 ovarian cancer model. Tumor-bearing mice were treated with WT or DKI iNK cells intraperitoneally (IP) in combination with trastuzumab or treated with trastuzumab alone. No exogenous cytokines were administered.

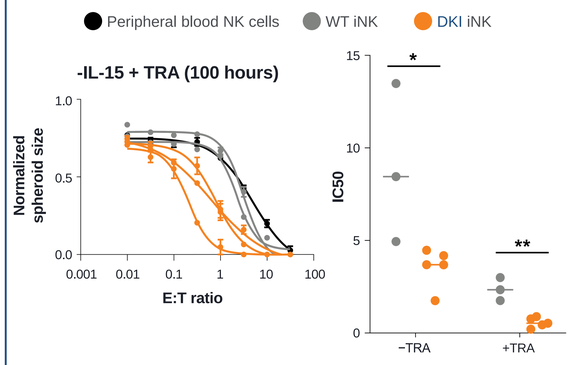
RESULTS

Figure 1. DKI iNK cells had increased CD16 and IL-15Rα expression compared with WT iNK cells



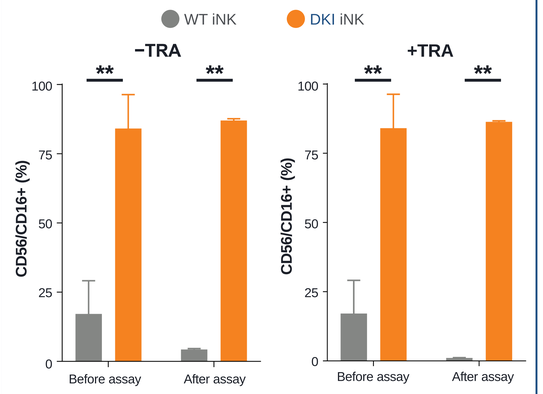
Number of independent experiments=4; ***ps0.001 (two-way ANOVA).

Figure 2. DKI iNK cells showed enhanced cytotoxicity compared with WT iNK cells in a 3D tumor spheroid killing assay



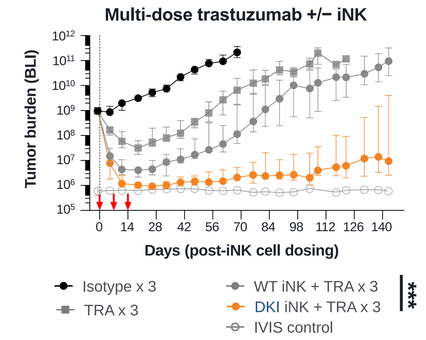
Number of independent experiments=2; *ps0.05, **ps0.01 (unpaired t-test). E, effector; IC50, half-maximal inhibitory concentration; T, target; TRA, trastuzumab.

Figure 3. CD16 surface expression was maintained in DKI iNK cells but decreased in WT iNK cells



Number of independent experiments=2; **ps0.01 (two-way ANOVA). TRA, trastuzumab.

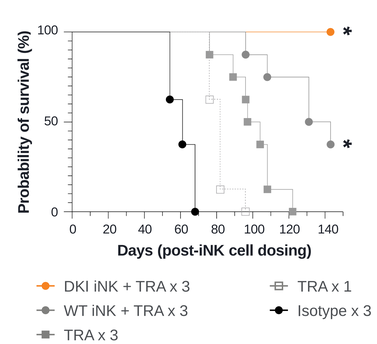
Figure 4. DKI iNK cells administered in combination with three doses of TRA significantly reduced tumor burden in a solid tumor (SKOV3) mouse model



- During the course of the 144-day experiment, 6/8 DKI iNK-treated mice had no detectable tumor

Number of mice per group=8; ***ps0.001; $5E^6$ iNK cells were injected IP; 2.5 mg/kg TRA or isotype were injected IP at indicated time points; BLI was monitored using an IVIS imager. BLI, bioluminescent imaging; IP, intraperitoneal; IVIS, in vivo imaging system; TRA, trastuzumab.

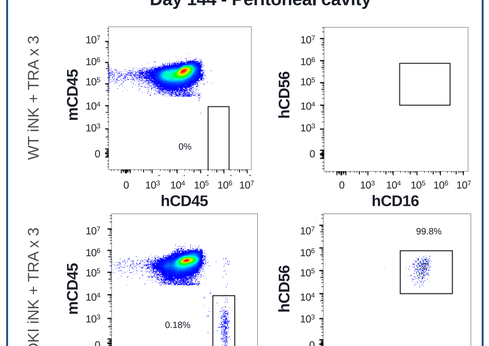
Figure 5. DKI iNK cells administered in combination with three doses of TRA significantly increased survival over WT iNK cells + TRA in a solid tumor (SKOV3) mouse model



- At Day 144, 100% of DKI iNK-treated mice were alive, compared with <50% of WT iNK-treated mice

Number of mice per group=8; *ps0.05; $5E^6$ iNK cells were injected IP; 2.5 mg/kg TRA or isotype were injected IP; multi-dose TRA or isotype were administered at Days 0, 7 and 14; single-dose TRA was administered at Day 0. TRA, trastuzumab.

Figure 6. DKI iNK cells persisted up to Day 144 in the peritoneal cavity when combined with TRA



Number of mice per group=8; representative flow plots are shown; mice were dosed with $5E^6$ WT or DKI iNK cells and 3 doses of TRA 2.5 mg/kg; staining represents human CD45 as the first gate, then human CD56 by CD16. TRA, trastuzumab.

CONCLUSIONS

- By exploiting our SLEEK platform, which is powered by our proprietary AsCas12a, CD-16 and mbIL-15 could be knocked into iNK cells with high efficiency, leading to dramatically increased persistence and antitumor activity compared with WT iNK cells (as shown both in vitro and in vivo).
- These data demonstrate that our gene editing platform enables the development of off-the-shelf iNK cell medicines that may be highly effective for treating solid tumors.

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DISCLOSURES

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