Knock-out of CISH and TGFβR2 or knock-in of CD16 and mblL-15 in iPSC-derived NK cells promotes high cytotoxicity and enhances in vivo tumor killing


Editsa Medicine, Inc., Cambridge, MA, USA

OBJECTIVE

To evaluate the in vitro and in vivo anti-tumor efficacy of induced pluripotent stem cell (iPSC)-derived natural killer (INK) cell therapies modified using Editsa’s engineered highly active and specific Cas12a to double knock-out (DKO) CISH and TGFβR2 or double knock-in (DKI) CD16 and mblL-15.

INTRODUCTION

• Natural killer (NK) cells are strong effectors of the innate immune system that can directly recognize and kill tumor cells, hence making them attractive for use as cellular therapies in cancer.

• NK cell effectiveness is diminished by the lack of persistence due to low levels of critical cytokines required for NK survival, such as interleukin-15 (IL-15), as well as tumour-intrinsic immunosuppressive mechanisms, such as high levels of transforming growth factor beta (TGFβ) within the tumor microenvironment.

• Furthermore, NK cells ability to exert antibody-dependent cellular cytotoxicity (ADCC) is impaired when CISH is cleaved off after cell activation.

• Editsa's proprietary AsCas12a-based gene editing method was used to modify INK cells to overcome these biologic limitations and enhance NK cell function.

• We hypothesized that DKO of the cytokine-inhibitory SH2-containing protein (CISH) gene, a negative regulator of IL-21L-15 signaling, and of the TGFβ-receptor 2 (TGFβR2) gene would improve NK cell effector function and make them resistant to TGFβ-mediated suppression. Additionally, we hypothesized that DKI of membrane-bound IL-15 (mblL) and CD16 into INK cells would prolong NK persistence and increase cytotoxicity when combined with ADCC, inducing tumor-targeting antibodies, such as trastuzumab.

RESULTS

Enhanced functions of AsCas12a-edited INK cells

• IPSCs were edited at the glycoaldehyde-3-phosphate dehydrogenase (GAPDH) locus with an engineered AsCas12a to knock-in CISH and mblL-15 using the SelectSeq by Editorial-genome exon insertion (SelectSeq) method. Separate IPSCs were edited with AsCas12a to knock-out CISH and TGFβR2. IPSC clones were then differentiated into IPSC-derived NK (INK) cells.

• A 3D tumor spherical assay using Incucyte® imaging of NuLight Red (NLR)-tagged SKOV-3 tumor cells was used to determine the NK cell cytotoxicity.

• Non-obese diabetic (NOD) severe combined immunodeficient (scid) gamma null (NSG) mice were inoculated with 2.5x10⁶ tumor cells expressing SKOV-3 cell line into SCID-hu women mice and treated with trastuzumab.

METHODS

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CONCLUSIONS

• DKO and DKI INK cells induced enhanced anti-tumor activity against 3D SKOV-3 tumor spheroids compared with WT INK cells (Figure 1 and 2). DKO INK cells had enhanced resistance to TGFβ and, when stimulated, produced elevated levels of cytotoxic inflammatory cytokines, including IL-15, GM-CSF, and TNF alpha (Figure 1).

• DKO INK cells, administered IP (20M cells) as monotherapy, induced significant reduction in tumor burden compared with WT INK cells in SKOV-3-luc tumor-bearing mice (Figure 3).

• DKI INK cells, administered IP (5M cells) in combination with single or multiple doses of trastuzumab, induced significant to complete tumor clearance in multiple mice (Figure 4).

• DKI INK cells were detected in vivo for more than 3 months, indicating that mblL-15 is expressed at a level sufficient to maintain INK survival for a prolonged time without exogenous cytokine support (Figure 5).

REFERENCES

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DISCLOSURES

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