Generation of Natural Killer Cells with Enhanced Function from a CRISPR/Cas12a-Edited Induced Pluripotent Stem Cell Line


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Disclosures


• Previous employment with bluebird bio: R.A.M.
Editing of *CISH* and *TGFBR2* is hypothesized to enhance effector function and anti-tumor activity of iPSC-derived NK cells

**Advantages of NK cells**

- Recognize broad array of tumor ligands
- Recognize malignant cells that lack MHC I
- Rapid degranulation leads to robust tumor cytotoxicity
- Recruit and engage cells of the adaptive immune system to enhance tumor cytotoxicity

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KO target 1: *CISH*  
KO target 2: *TGFBR2*

CISH KO →  
enhanced IL-15 signaling →  
↑ NK cell effector function

TGFBR2 KO →  
Θ TGF-β signaling →  
↑ NK cell effector function

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CISH: cytokine-inducible SH2-containing protein gene; IL: interleukin; iPSC: induced pluripotent stem cell; KO: knock out; MHC: major histocompatibility complex; NK: natural killer; TGF-β: tumor growth factor beta; TGFBR2: TGF-β receptor II gene
Development of a CRISPR-Cas12a-edited iPSC platform for the generation of enhanced CD56+ iNK cells

CRISPR-Cas12a RNPs targeting CISH + TGFBR2

CISH-/-TGFBR2-/- iPSCs
- Editing efficacy (next-generation sequencing)
- Pluripotency & differentiation potential (IHC)

CRISPR-Cas12a RNPs targeting CISH + TGFBR2

Master iPSC bank

Differentiate 32–46 days

CISH-/-TGFBR2-/- CD56+ iNK cells

Differentiation assays
- Flow cytometry
- Embryoid bodies

Molecular and functional analyses
- pFLOW
- Cytokine production
- Spheroid killing assay (SK-OV-3)
- Serial killing assay

Objective:
To evaluate the enhanced effector function of CRISPR-Cas12a CISH-/-TGFBR2-/- human iPSC-derived NK cells in vitro

CRISPR-Cas12a: clustered regularly interspaced short palindromic repeats-Caspase 12a; IHC: immunohistochemistry; iNK: natural killer cell derived from induced pluripotent stem cells; pFLOW: phosphorylating flow cytometry; RNP: ribonucleic protein
Successful generation of $CISH^{-/-}/TGFBR2^{-/-}$ iPSCs that retain normal stemness marker expression

Robust multiplex editing

<table>
<thead>
<tr>
<th></th>
<th>CISH</th>
<th>TGFB2</th>
<th>CISH</th>
<th>TGFB2</th>
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<tr>
<td>Edited iPSCs</td>
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<tr>
<td>EP control</td>
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Normal stemness

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<th>SSEA4</th>
<th>Nanog</th>
<th>Tra-1-60</th>
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</thead>
<tbody>
<tr>
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<td>250 μM</td>
<td>250 μM</td>
<td>250 μM</td>
<td>250 μM</td>
</tr>
<tr>
<td>$CISH^{-/-}/TGFBR2^{-/-}$</td>
<td>250 μM</td>
<td>250 μM</td>
<td>250 μM</td>
<td>250 μM</td>
</tr>
</tbody>
</table>

EP: electroporation
CISH<sup>−/−</sup>/TGFBR2<sup>−/−</sup> iPSCs retain their potential for trilineage differentiation and have normal karyotypes.
CISH⁻/⁻/TGFBR2⁻/⁻ iPSCs successfully differentiated into CD56⁺ iNK cells with canonical NK cell markers

**Identification of distinct iNK cell populations by flow cytometry**

Unedited iNK cells

CISH⁻/⁻/TGFBR2⁻/⁻ iNK cells

**Edited iNK cells expressed NK cell markers, similar to unedited controls**

Unedited iNK cells  CISH⁻/⁻/TGFBR2⁻/⁻ iNK cells

Average values from >5 differentiations

Representative plots from a single experiment
**CISH^-/-/TGFBR2^-/-** iNK cells demonstrated enhanced anti-tumor activity against SK-OV-3 ovarian tumor spheroids

SK-OV-3 ovarian cells

3D spheroid of SK-OV-3 ovarian cells

**CISH^-/-/TGFBR2^-/-** iNK cells + IL-15 + TGF-β

**CISH^-/-/TGFBR2^-/-** iNK cells kill SK-OV-3 tumor spheroids

Greater reduction of SK-OV-3 spheroid size

*<p<0.05; **p<0.01; ***p<0.001 vs unedited iNK cells (two-way ANOVA, Sidak’s multiple comparisons test)
**CISH−/−/TGFBR2−/− iNK cells demonstrated enhanced sustained serial killing of a B cell leukemia cell line (Nalm6)**

Serial killing assay challenges NK cells with new Nalm6 cells every 48 hours for up to 12 days

<Diagram>

- **Normalized total red object area (µm²/well)**
  - Start assay
  - Time (hours): 0, 48, 96, 144, 192, 240
  - + IL-15 (10 ng/mL)
  - + TGF-β (10 ng/mL)

Enhanced sustained serial killing of Nalm6 cells

<Diagram>

- **Normalized total red object area (µm²/well)**
  - Start assay
  - Time (hours): 0, 50, 100, 150, 200, 250
  - + IL-15 (10 ng/mL)
  - + TGF-β (10 ng/mL)

- **Continued killing**

- **Unedited iNK cells (n=3)**
- **CISH−/−/TGFBR2−/− iNK cells (n=3)**

= Tumor cell addition (5×10⁶ cells)
Conclusions

Established a robust, consistent, and highly effective CRISPR-Cas12a editing platform in iPSCs that may be generalized to other targets.

Unedited and $CISH^{-/-}/TGFBR2^{-/-}$ iPSCs differentiated into iNK cells with canonical NK cell markers.

$CISH^{-/-}/TGFBR2^{-/-}$ iNK cells demonstrated enhanced anti-tumor activity against tumor cell lines derived from both solid and hematological malignancies.

This demonstrates the utility of this iPSC platform to create multiple, off-the-shelf edited cell therapy medicines for future application to a broad range of oncology indications.
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