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CXCR4 transgene improves in vivo migration and efficacy of engineered iPSC-derived natural killer cells

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Introduction

Background Chimeric antigen receptor (CAR)-engineered induced pluripotent stem cell (iPSC)-derived natural killer (iNK) cells have shown a favorable safety profile and early evidence of clinical efficacy in the treatment of patients with certain hematologic malignancies. CAR-iNK cells retain phenotypic and functional similarities to primary natural killer cells, with additional capabilities for recognition of tumors. It is critical that iNK cells can migrate efficiently to relevant sites of disease, which includes bone marrow for hematologic malignancies. CXCR4 (C-X-C chemokine receptor type 4) is a chemokine receptor specific for stromal-derived-factor-1 (SDF-1) and mediates the migration of hematopoietic cells to the bone marrow upon binding SDF-1. There is 91% homology between mouse and human CXCR4, and 99% homology between mouse and human SDF-1. Here we describe novel engineering of iPSCs resulting in CAR-specific killing and enhanced iNK migration and subsequent tumor killing in the bone marrow. The addition of the CXCR4 transgene in iPSC-derived effector cells may allow for a higher therapeutic response in axial-skeletal located tumors.

Methods

- iPSCs were sequentially engineered at defined loci with Crispr/MAD7/HDR to incorporate Allo-Evasion™ edits, a CAR targeting antigens expressed on different hematological malignancies, constitutive interleukin-15 (IL-15) cytokine support, with and without wild-type CXCR4.
- iPSCs were differentiated into natural killer cells using a proprietary differentiation process.
- CXCR4+ and CXCR4- iNK underwent in vitro and in vivo functional analysis.
- iNK migration and tumor cell killing were tested in vitro and in vivo.

Results

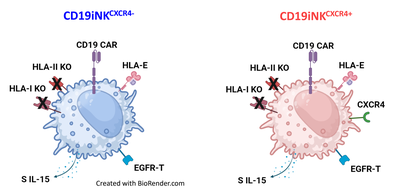
- iPSCs were successfully engineered with Allo-Evasion™ edits, CAR, constitutive IL15 cytokine, and wild-type CXCR4.
- CXCR4 engineering yielded a bulk population of iNK with heterogenous CXCR4 expression, and CAR-specific potency was not affected.
- CXCR4 transgene resulted in significant anti-tumor efficacy in heme malignancy xenograft models.
- CXCR4(+) iNK have greater migration potential in vitro and in vivo.

Summary and Conclusions

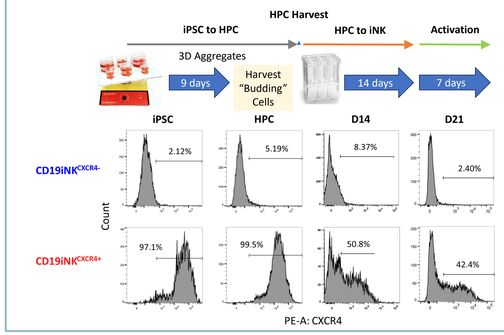
- iPSCs engineered with Allo-Evasion, cytokine, and CAR edits, were engineered with CXCR4 and differentiated into iNK cells.
- CXCR4 engineering did not affect functionality of CAR transgenes, and iNKs maintained high potency against multiple hematologic malignancies.
- CXCR4(+) iNK cells have enhanced migration to the bone marrow and various other tissues involved in hematological malignancies.
- Bone marrow migration of iNK cells targeting various antigens results in superior tumor cell killing in the marrow in aggressive disseminated heme xenograft models.
- Century's iPSC-derived iNK cell platform provides a scalable and customizable platform for development of cellular immunotherapies for axial-skeletal located malignancies.

Precision Engineering of iPSC

Engineering of CD19-iNK without (blue) and with (red) CXCR4 transgene.



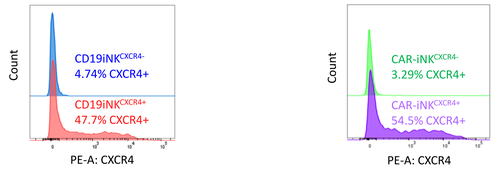
Bulk iNK Differentiation and CXCR4 Expression



iPSCs were precision engineered with desired transgenes at specific loci. iPSCs are differentiated into Hematopoietic Progenitor Cells (HPCs), then into iNK cells, followed by iNK activation. This yields a **bulk** population of iNK with heterogenous surface expression of CXCR4.

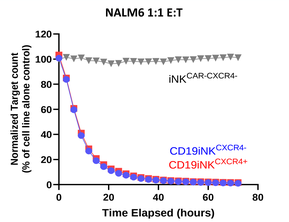
Addition of the CXCR4 Transgene Does Not Affect iNK Potency

CXCR4 Expression of Different CAR-iNK Cells Targeting Heme Malignancies

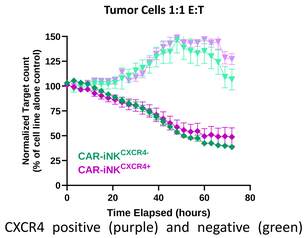


Engineering with wild-type CXCR4 yields bulk populations of iNK with ~50% CXCR4 expression. Endogenous CXCR4 expression of iNK is low at <5%.

72-hour Cytolysis of Tumor Cells by Different CAR-iNK Lines Engineered with CXCR4



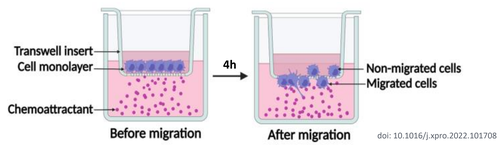
CD19iNK negative for CXCR4 (blue), CD19iNK with CXCR4 transgene (red), and CAR/CXCR4-negative iNK (gray), were tested for in vitro potency against NALM6 leukemia. At 1:1 E:T, both CD19iNK cells were equally potent.



CXCR4 positive (purple) and negative (green) iNK engineered with a CAR specific to tumor antigen (Ag), were tested for in vitro potency against tumor cell lines positive (♦) and negative (▾) for Ag. Both iNK were equally potent in Ag⁺ tumor cells.

iNK Engineered with CXCR4 Transgene Have Enhanced Migration In Vitro

Transwell Migration Assay



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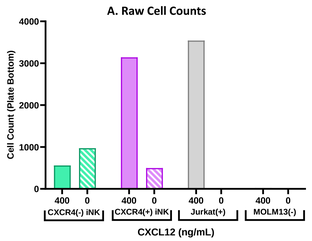
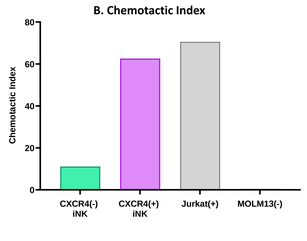
$$\text{ChI} = \left(\frac{\textit{Number of cells in experimental wells}}{\textit{Average of cells in baseline wells}}\right)$$

400 ng CXCL12 (SDF-1) is loaded into the bottom of a 24-well plate with transwell insert. 2x10^5 iNK (or control tumor cells) in media are loaded into each transwell insert. After 4 hours, iNK in the plate bottom are counted and Chemotactic Index (ChI) is calculated.



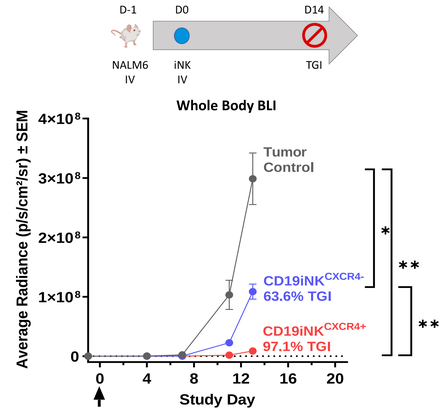


CXCR4(+) cells (iNK^{CXCR4+} and Jurkat) and CXCR4(-) cells (iNK^{CXCR4-} and MOLM13) were tested for migration in a transwell assay format.

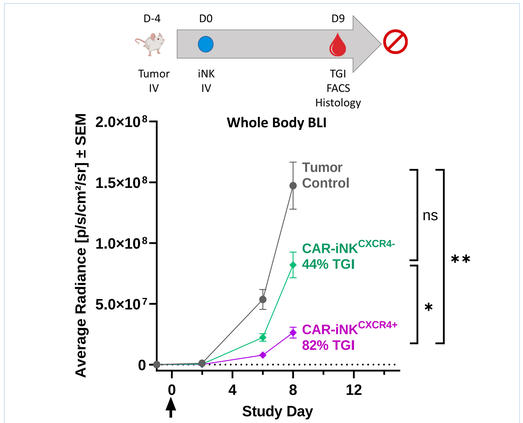
Raw iNK counts at migration assay completion (A) and calculated Chemotactic Index (ChI) (B) indicate pronounced migration of CXCR4(+) cells in the presence of CXCL12.

iNK Engineered with CXCR4 Transgene Have Enhanced Migration and Efficacy In Vivo

In Vivo Efficacy in Aggressive Disseminated Heme Xenograft Models

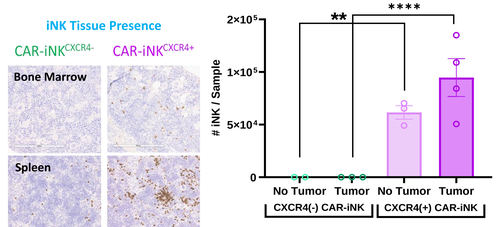


NALM6 cells were intravenously implanted into mice. The next day, mice were intravenously injected with CD19iNK negative for CXCR4 (blue) or CD19iNK with CXCR4 transgene (red). On Day 14, CXCR4-positive CD19iNK elicit superior TGI (97%) over CXCR4-negative CD19iNK (64%). Tumor growth is clearly diminished in long bones, vertebrae and sternum.



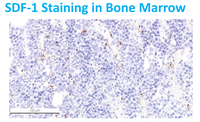
CXCR4(-) CAR-iNK (green) or CXCR4(+) CAR-iNK (purple) were intravenously injected into an aggressive established disseminated xenograft model in NSG mice. On Day 9, CAR-iNK^{CXCR4+} elicit superior TGI (82%) over CAR-iNK^{CXCR4-} (44%).

Bone Marrow Presence of iNK

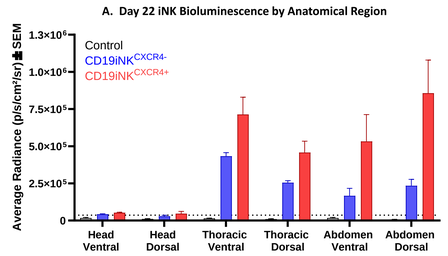


FACS analysis of bone marrow from tumor- and non-tumor-bearing mice treated with CXCR4(-) and CXCR4(+) CAR-iNK. Only CAR-iNK^{CXCR4+} cells were found in the bone marrow.

SDF-1 Staining in Bone Marrow



In Vivo Tracking of iNK Utilizing Bioluminescent Imaging of a NanoLuc Reporter



CD19iNK positive (red) and negative (blue) for CXCR4 transgene were engineered with a bioluminescent NanoLuc reporter, Antares, and were intravenously injected into naïve NSG mice on Day 0. Mice were injected with fluorofurimazine substrate and imaged to evaluate iNK biodistribution. CXCR4+ iNK had greater in vivo biodistribution quantified by bioluminescent signal on Day 22 (A) and visually evident in images below (B).