336 A NOVEL METHOD FOR EFFICIENT CGMP PRODUCTION OF NATURAL KILLER CELLS FROM CLONAL MASTER INDUCED PLURIPOTENT STEM CELLS FOR NEXT GENERATION, OFF-THE-SHELF CANCER IMMUNOTHERAPY

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Background Natural killer (NK) cells are innate immune cells that play a key role in in tumor immune surveillance. Recent advances allow for the derivation of immune cells, including NK cells, from human induced pluripotent stem cells that can be utilized for cancer immunotherapy. High doses of NK cells and multiple doses are both safe and likely necessary for clinical efficacy. Manufacturing large number of NK cells from a clonal master iPSC line provides a promising strategy to enable next generation, off-the-shelf, cancer immunotherapies.

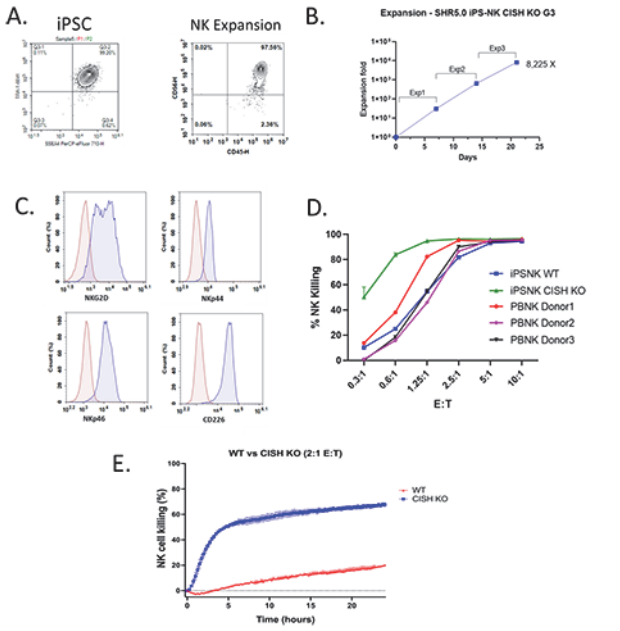
Methods We have developed a novel process to produce high purity, clinical scale NK cells efficiently and consistently. Briefly, hematopoietic progenitor cells are derived using an improved spin embryoid body (EB) method using defined cytokines including bone morphogenic protein-4 (BMP-4), stem cell factor (SCF), vascular endothelial growth factor (VEGF), fms-like tyrosine kinase 3 (FLT-3) etc. The hematopoietic progenitor cells are subsequently differentiated into mature NK cells using a second set of defined cytokines such as IL-3, IL-7, IL-15, SCF etc.

Results Using this novel method, fully functioning NK cells could be expanded more than 8,000-fold to enable us to produce 1 x 10^{12} NK cells starting from 1 x 10^{6} undifferentiated iPSCs.This cell production scale can supply >200 clinical doses from one cGMP manufacturing campaign. NK cells produced using this method are CD56-positive and display the typical phenotype of NK cell activating receptors including NKG2D, NKp44, NKp46, DNAM-1. Importantly, these NK cells demonstrate better anti-tumor activities and produce higher levels of IFNγ and TNFα compared to peripheral blood-derived NK cells isolated from healthy donors. Moreover, this protocol has been adapted and optimized for clinical scale manufacturing of NK cells from a genetically engineered iPSC cell line bearing a knock-out of *CISH*, a key intracellular checkpoint of NK cell activation.[1] *CISH* KO NK cells produced using this optimized method show significantly better anti-tumor activities against multiple liquid and solid tumor cell lines (figure 1), including K562, Raji (B cell lymphoma), Daudi (B cell lymphoma), SUP-B15 (B cell lymphoma), MOLT-4 (T cell lymphoma), SKOV-3 (ovarian cancer), OVCAR-4 (ovarian cancer), HCC-827 (lung cancer), ZR-75 (breast cancer), and BT-474 (breast cancer), in standard cytotoxicity assays compared with unmodified NK cells.

Conclusions Overall, this novel cell production strategy paves the way for clinical trials using higher doses of iPSC-derived NK cells with increased potency, thus enabling next-generation CAR-NK cell-based immunotherapies.

REFERENCE

1. Zhu H, Blum R, Bernareggi D, Ask E, Wu Z, Hoel H, Meng Z, Wu C, Guan K, Malmberg KJ, Kaufman D. Metabolic reprograming via deletion of CISH in human iPSC-derived NK cells promotes in vivo persistence and enhances anti-tumor activity. *Cell Stem Cell*. 2020; **27**: 224-237.



Abstract 336 Figure 1 CISH-KO iPSCs can be used to generate highly functional iNK
(A) Flow cytometric analysis of CISH KO iPSCs and iNK cells following differentiation and expansion. (B) Graph showing cumulative in vitro fold expansion of CISH-KO iNK cells over three expansion periods of 7d each. (C) Flow cytometric analysis of expression of indicated surfaces markers by CISH-KO iNK cells. (D) Comparison of cytotoxicity of WT iNK, CISH-KO iNK, and PBNK cells co-cultured with K562 cells using a flow cytometry killing assay. Error bars represent mean +/- SEM from three independent experiments. (E) xCELLigence killing assay comparing cytotoxicity of WT vs CISH-KO iNK cells co-cultured with BT474 target cells.

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