Multi-Functional Genetic Engineering of Pluripotent Cell Lines for Universal Off-the-Shelf Natural Killer Cell Cancer Immunotherapy

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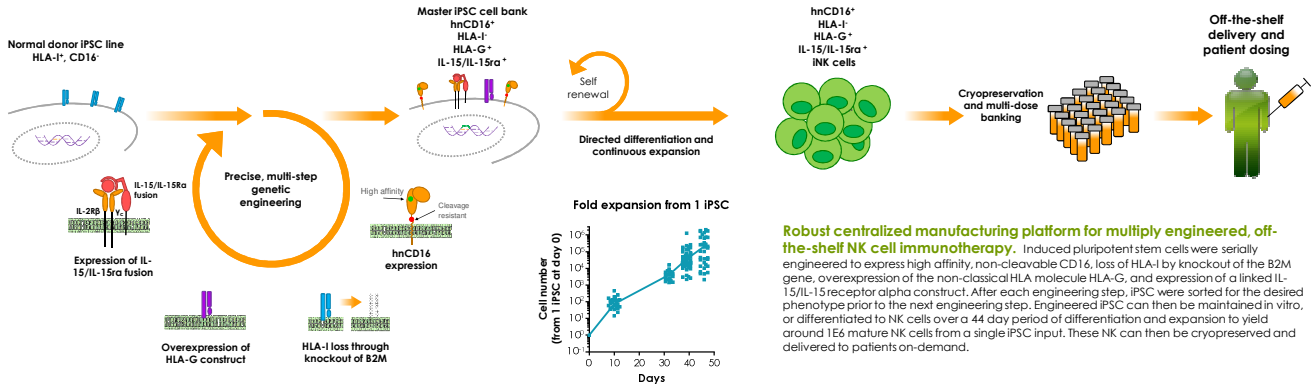
EXECUTIVE SUMMARY

- Induced pluripotent stem cells (iPSC) provide **renewable cell source for consistent and repeated manufacture of homogeneous cell products** and an ideal platform for the scalable production of precise, multi-step genetic engineered immunotherapies.
- Fate's unique iPSC technology allows for high throughput, **clonal selection of single input human iPSC** to create clonal iPSC master cell lines.
- Genetic engineering to produce both overexpression and knockout of desired genes are targeted toward enhancing the function and persistence of iPSC derived natural killer (NK) cells after allogeneic transfer
- Enforced expression of **high affinity, non-cleavable CD16 (hnCD16)** promotes enhanced NK cell responsiveness to CD16 stimulation and antibody dependent cellular cytotoxicity (ADCC).
- Genetic engineering of HLA molecules produces **reduced allogeneic rejection** in vitro of iPSC-derived NK cells across traditional histocompatibility barriers.
  - Deletion of the B2M gene removes cell surface HLA-I and prevents CD8+ T cell-mediated alloreactivity
  - Expression of the non-classical HLA gene HLA-G rescues B2M$^{-/-}$ iPSC from killing by allogeneic NK cells.
- Expression of IL-15 linked with the IL-15 receptor alpha chain (IL-15-IL-15ra) enhances NK cell differentiation and **survival in the absence of exogenous growth factors**
- Engineered, iPSC-derived NK cells retain effector function, and are highly effective in an ovarian cancer xenograft model in vivo

GRAPHICAL ABSTRACT



Robust centralized manufacturing platform for multiply engineered, off-the-shelf NK cell immunotherapy. Induced pluripotent stem cells were serially engineered to express high affinity, non-cleavable CD16, loss of HLA-I by knockout of the B2M gene, overexpression of the non-classical HLA molecule HLA-G, and expression of a linked IL-15/IL-15 receptor alpha construct. After each engineering step, iPSC were sorted for the desired phenotype prior to the next engineering step. Engineered iPSC can then be maintained in vitro, or differentiated to NK cells over a 44 day period of differentiation and expansion to yield around 1E6 mature NK cells from a single iPSC input. These NK can then be cryopreserved and delivered to patients on-demand.

RESULTS

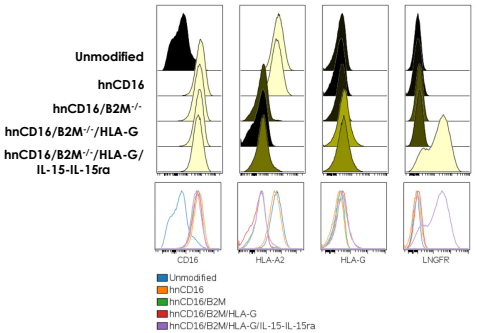


Figure 1. Validation of engineering steps in mature iNK cells. A. Flow cytometry of mature iPSC-derived NK cells demonstrates stepwise engineering of hnCD16 expression, B2M knockout (loss of HLA-A2 expression), HLA-G expression, and IL-15/IL-15ra (LNGFR) construct expression.

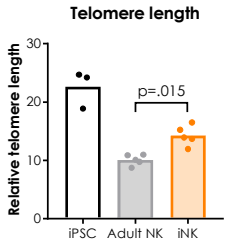


Figure 2. Mature iNK cells maintain longer telomeres compared to adult peripheral blood NK cells. Telomere length was determined by flow cytometry for iPSC, adult peripheral blood NK cells, and iPSC-derived NK cells using the 1301 T cell leukemia line as a control (100%) with correction for the DNA index of $G_{0/1}$ cells. iPSC-derived NK cells maintain significantly longer telomere length when compared to adult peripheral blood NK cells (p=.105, ANOVA).

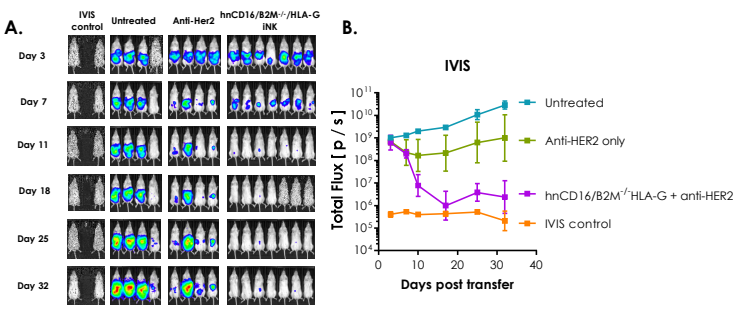


Figure 3. A single dose of hnCD16/B2M$^{-/-}$HLA-G iNK induce tumor regression in an in vivo xenograft model of ovarian cancer. NSG mice were transplanted with SKOV-3-Luciferase ovarian tumor cells IP prior to treatment with a single dose of anti-HER2 antibody on day 4, either alone or in combination with 8E6 hnCD16/B2M$^{-/-}$/HLA-G iNK cells. Tumor progression was measured by IVIS imaging to monitor tumor progression. Data are presented as **A.** IVIS images of each mouse or **B.** time course of tumor progression by IVIS imaging.

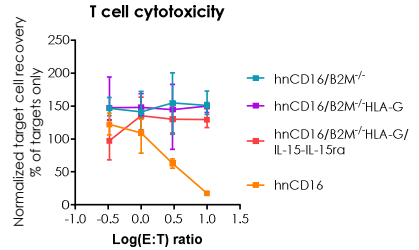


Figure 4. Knockout of B2M eliminates in vitro recognition of engineered cells by allogeneic CD8+ T cells. Mature, engineered iPSC-derived NK cells were incubated at the indicated effector:target (E:T) ratios with allogeneic CD8+ T cells primed against the same iNK donor. 48 hours later, remaining iNK target cells were counted by flow cytometry. Absence of B2M/HLA-I resulted in a loss of cytotoxicity by T cells. Results are the average of two donors and are normalized to % of target cells only for each target iNK cell genotype.

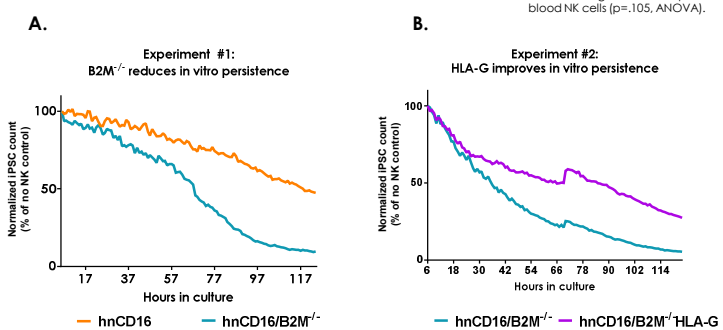


Figure5. Expression of HLA-G rescues B2M$^{-/-}$ iPSC from killing by allogeneic PBMC. A. Engineered iPSC were incubated with allogeneic PBMC, and loss of iPSC was measured over time using the Incucyte Zoom imaging system. Loss of cell surface HLA-I results in increased cytotoxicity **(A.)**(experiment 1), which can be at least partially reversed by expression of HLA-G on B2M$^{-/-}$ iPSC as shown in experiment 2 **(B.)** Data are normalized to the number of iPSC in wells without effectors, setting time = 0 to 100% for each condition.

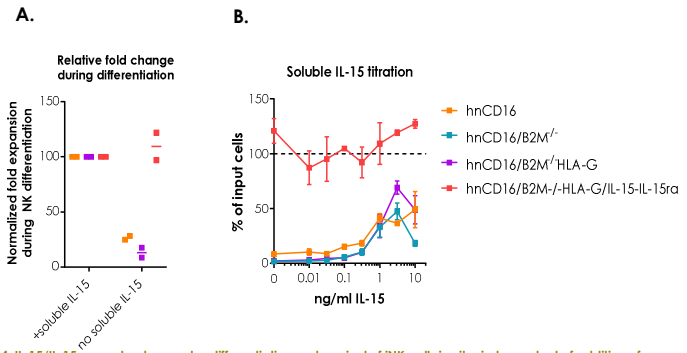


Figure 4. IL-15/IL-15ra construct promotes differentiation and survival of iNK cells in vitro independent of addition of soluble, exogenous IL-15. A. iNK cells of the indicated genotypes were differentiated with or without the addition of of soluble IL-15. IL-15/IL-15ra expressing cells differentiated equally well in both conditions. **B.** iNK cells were extensively washed and place back into culture in concentrations of soluble IL-15 ranging from 10ng/ml to 0 ng/ml for 7 days. Expression of the IL-15/IL-15ra construct rendered the cells independent of soluble IL-15.

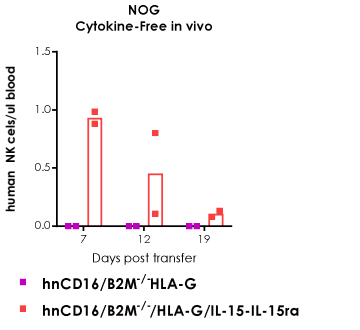


Figure 7. Expression of IL-15/IL-15ra construct enhances iNK persistence in vivo in the absence of soluble IL-15. Eight million hnCD16/B2M$^{-/-}$/HLA-G or hnCD16/B2M$^{-/-}$/HLA-G /IL-15-IL-15ra iNK cells were adoptively transferred to immunocompromised NOG mice. Only cells expressing the IL-15/IL-15ra construct persisted in the absence of soluble IL-15.