iPSC-derived CD38-null NK Cells in Combination with CD38-targeted Antibody: a Dual Therapeutic Strategy to Enable ADCC and Eliminate Host Immune Cells in Multiple Myeloma

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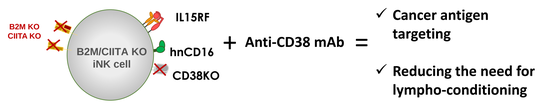
INTRODUCTION

Anti-CD38 therapeutic monoclonal antibodies (mAbs) such as daratumumab (dara) have shown wide application and therapeutic benefit for CD38-expressing Multiple Myeloma (MM) patients. However, the ability of natural killer (NK) cells to mediate antibody-dependent cell-mediated cytotoxicity (ADCC), a critical mechanism of action associated with many mAbs, is limited by fratricide due to their own expression of CD38. To develop a therapeutic strategy that captures the full breadth of the anti-tumor capacity of dara and other anti-CD38 mAbs, we utilized our induced pluripotent stem cell (iPSC) platform, where the generation of precisely engineered master iPSC lines serve as the starting material for the manufacture of uniformly engineered immune cells for off-the-shelf cell therapies. To generate an iPSC-derived NK (iNK) cell capable of maximizing ADCC and reducing the need for lympho-conditioning in combination with dara, a master iPSC line was created containing five unique edits; a high-affinity, non-cleavable CD16 (hnCD16) to potentiate ADCC in combination with a mAb, a recombinant IL-15 receptor fusion (IL-15RF) for cytokine-autonomous persistence, elimination of B2M and CIITA to reduce T cell detection, and deletion of CD38 expression to prevent anti-CD38 mAb mediated fratricide and to enhance NK cell fitness.

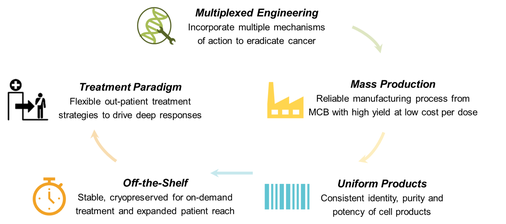
Novel Dual-Purpose Strategy

iPSC derived iNK cells engineered to enable ADCC and to avoid host immune rejection when delivered with anti-CD38 mAbs



WT iNK cell – CD38KO, hnCD16, IL15RF (HLA I & II sufficient)
B2M/CIITA KO iNK cell – CD38KO, hnCD16, IL15RF, HLA-I &HLA-II KO

Off-the-Shelf Platform



CONCLUSIONS

- We utilized our novel induced pluripotent stem cell (iPSC) platform to create multiplexed-engineered clonal iPSC lines and facilitate the generation of five-point edited NK cells uniformly expressing all edits, including lack of CD38 surface expression to evade fratricide and promote ADCC when delivered in combination with anti-CD38 antibodies, including daratumumab.
- Preclinical data show that in combination with daratumumab, we can i) selectively eliminate multiple myeloma cells and ii) selectively deplete alloreactive immune cells, thus maximizing anti-tumor efficacy and reducing the need for lympho-conditioning with CD38KO alone or with additional edits such as B2M/CIITA KO.
- Clinical data show the addition of daratumumab to lympho-depleting chemotherapy (LDC) delays CD38+ host immune reconstitution in multiple myeloma patients receiving FT538
- Taken together, these data support a therapeutic strategy combining iPSC-derived CD38-null NK cells with a CD38-targeted antibody to eliminate alloreactive CD38+ host immune cells and to enable ADCC in multiple myeloma.

RESULTS

iNK cells generated from clonally-derived multiplexed-engineered iPSCs uniformly express various genetic edits

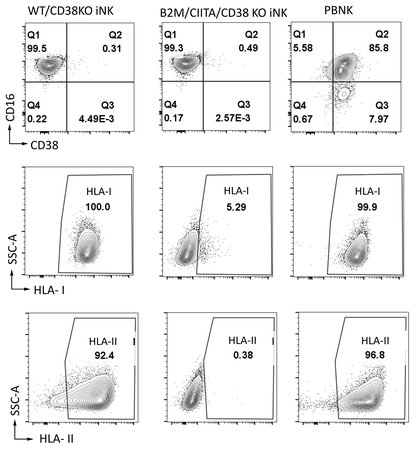


Figure 1. Fully differentiated and expanded iNK cells engineered on Fate's CD38 knockout (KO) platform were evaluated for expression of all engineered elements by flow cytometry compared to expanded peripheral blood NK cells (PBNKs) from a healthy donor. WT and B2M/CIITA KO iNK cell populations lacked CD38 expression compared to PBNKs. All cell populations expressed CD16. Compared to WT iNK cells and PBNKs, the B2M/CIITA KO iNK cells did not express HLA-I and HLA II.

CD38 KO iNK cells are uniquely protected from Daratumumab mediated fratricide and cytotoxicity

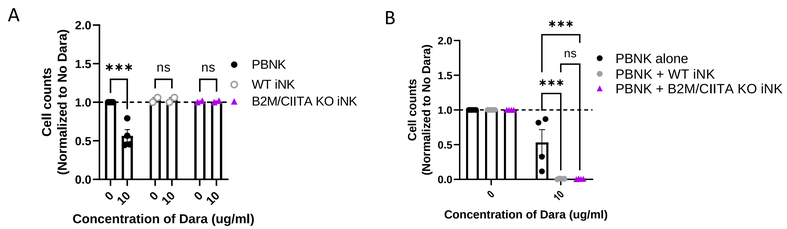


Figure 2. CD38 KO iNK cells are protected from daratumumab mediated fratricide and cytotoxicity. PBNKs, WT and B2M/CIITA KO iNK cells were cultured in the presence of daratumumab for 48hrs. PBNKs, which expressed CD38, were depleted in the presence or daratumumab while WT and B2M/CIITA KO iNK cells which lack CD38 were protected from fratricide (A). PBNKs were cocultured with either WT or B2M/CIITA KO iNK cells in the presence or absence of daratumumab. PBNK counts in the daratumumab coculture wells were completely depleted compared to the PBNK alone wells with daratumumab indicating not only depletion by fratricide but also via iNK cell mediated ADCC (B). N= 4 donors, *** p<0.0005

Daratumumab protects CD38 KO iNK cells from allogeneic T- and NK-cell elimination

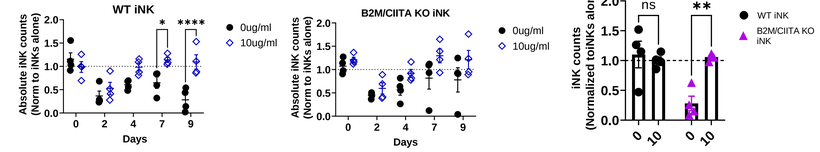


Figure 3. Daratumumab protects CD38 KO iNK cells from allogeneic T- and NK-cell elimination. In the absence of daratumumab WT iNK cells were depleted by allogeneic T cells. However, with daratumumab, WT iNK cells were protected from rejection (A). B2M/CIITA iNK cells persisted in the presence of allogeneic T cells due to lack of HLA-I and HLA-II expression and slightly enhanced in the presence of daratumumab (B). Furthermore, in the presence of activated PBNK, B2M/CIITA iNK cells were depleted due to missing self response. This depletion was avoided in the presence of daratumumab (C). N= 4 donors, *p<0.05, **p<0.005, ****p<0.00005

Daratumumab in combination with hnCD16 prevents the expansion of allogeneic T cells

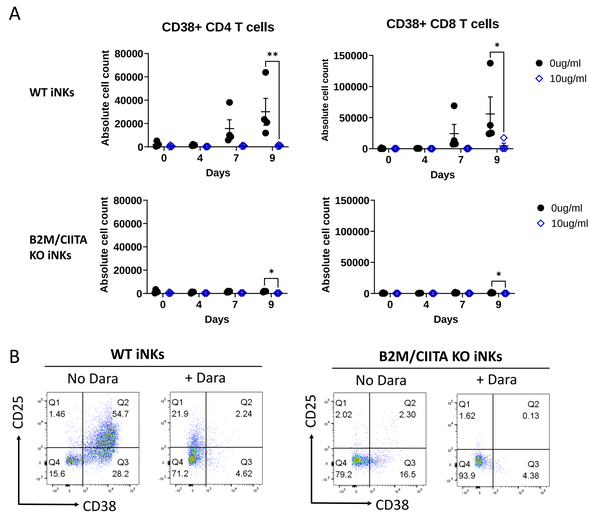


Figure 4. Daratumumab in combination with hnCD16 prevents expansion of allogeneic T cells. PBMCs, WT and B2M/CIITA KO iNK cells were co-cultured in the presence or absence of daratumumab. Over time, the WT iNK cells stimulated an expansion in allogeneic T cells which was significantly decreased in the B2M/CIITA KO co-culture wells. This expansion was prevented in the presence of daratumumab as evident by a decrease in the CD38+ T cells and CD25+ T cells in the both WT iNK and B2M/CIITA KO iNK co-culture groups (A & B). N=4 PBMC donors (*p<0.05, ** p<0.001).

Daratumumab in combination with hnCD16 protects allogeneic iNK cells from PBNK- and T cell-mediated allorejection in vivo

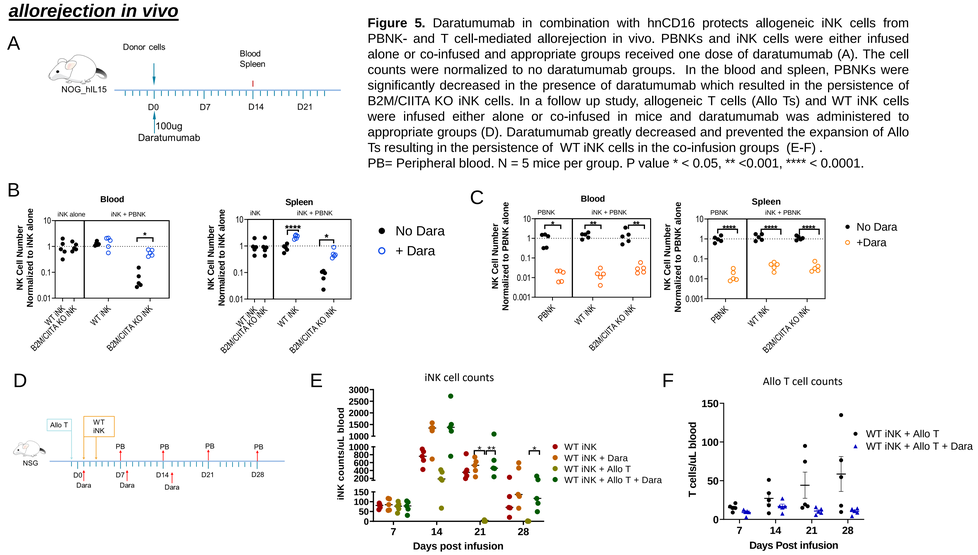


Figure 5. Daratumumab in combination with hnCD16 protects allogeneic iNK cells from PBNK- and T cell-mediated allorejection in vivo. PBNKs and iNK cells were either infused alone or co-infused and appropriate groups received one dose of daratumumab (A). The cell counts were normalized to no daratumumab groups. In the blood and spleen, PBNKs were significantly decreased in the presence of daratumumab which resulted in the persistence of B2M/CIITA KO iNK cells. In a follow up study, allogeneic T cells (Allo Ts) and WT iNK cells were infused either alone or co-infused in mice and daratumumab was administered to appropriate groups (D). Daratumumab greatly decreased and prevented the expansion of Allo Ts resulting in the persistence of WT iNK cells in the co-infusion groups (E-F) .
PB= Peripheral blood. N = 5 mice per group. P value * < 0.05, ** <0.001, **** < 0.0001.

Use of Daratumumab with lympho-depleting chemotherapy suppresses host CD38+ immune cell reconstitution

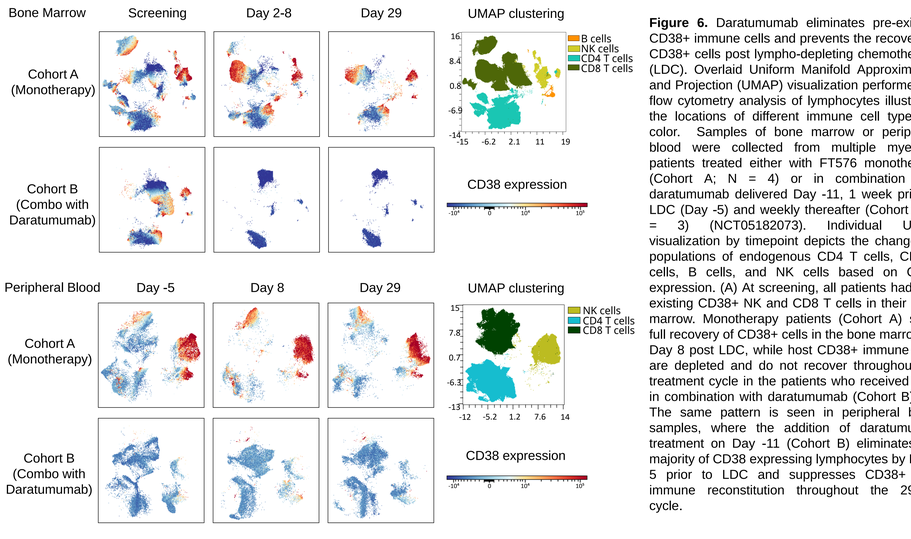


Figure 6. Daratumumab eliminates pre-existing CD38+ immune cells and prevents the recovery of CD38+ cells post lympho-depleting chemotherapy (LDC). Overlaid Uniform Manifold Approximation and Projection (UMAP) visualization performed on flow cytometry analysis of lymphocytes illustrates the locations of different immune cell types by color. Samples of bone marrow or peripheral blood were collected from multiple myeloma patients treated either with FT576 monotherapy (Cohort A; N = 4) or in combination with daratumumab delivered Day -11, 1 week prior to LDC (Day -5) and weekly thereafter (Cohort B; N = 3) (NCT05182073). Individual UMAP visualization by timepoint depicts the changes in populations of endogenous CD4 T cells, CD8 T cells, B cells, and NK cells based on CD38 expression. (A) At screening, all patients had pre-existing CD38+ NK and CD8 T cells in their bone marrow. Monotherapy patients (Cohort A) show full recovery of CD38+ cells in the bone marrow by Day 8 post LDC, while host CD38+ immune cells are depleted and do not recover throughout the treatment cycle in the patients who received LDC in combination with daratumumab (Cohort B). (B) The same pattern is seen in peripheral blood samples, where the addition of daratumumab treatment on Day -11 (Cohort B) eliminates the majority of CD38 expressing lymphocytes by Day -5 prior to LDC and suppresses CD38+ host immune reconstitution throughout the 29-day cycle.