# iPSC-derived CD38-null NK cells in Combination with CD38-targeted Antibody Represent a Novel Therapeutic Strategy to Reduce the Requirement of Conditioning Chemotherapy for Off-the-Shelf Cell-based Cancer Immunotherapy

**Rina M Mbofung[1], Alan M Williams[1], Mandana Karimi, Ken Hayama[1], Yijia Pan[1], Brian Groff[1], Thomas Daley[1], Miguel Meza[1], Sajid Mahmood[1], Kethan Mathavan[1], Greg Bonello[1], Tom Lee[1], Quirin Hammer[2], Karl-Johan Malmberg[2,3], Peter M Szabo[1], Lily Wong[1], Sarah Cooley[1], Ryan Bjordahl[1], Jode Goodridge[1], Bahram Valamehr[1]**

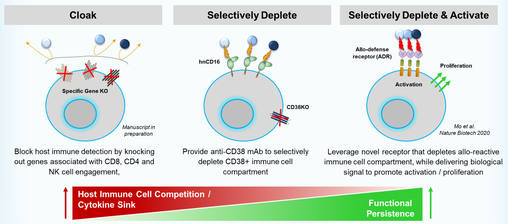
1. Fate Therapeutics, San Diego, CA; 2. Karolinska Institute, Stockholm, Sweden; 3. University of Oslo, Norway

## INTRODUCTION

Autologous and allogeneic chimeric antigen receptor (CAR) T-cell therapies are commonly combined with conditioning chemotherapies that suppress a patient's immune system by creating a suitable window of activity to elicit clinical response. However, protracted lympho-conditioning also affects immune reconstitution and can negatively impact the rate of infection. Alternative approaches to limit lympho-conditioning and prevent allorejection may therefore help to enhance the efficacy of the therapy while preserving the immune system of the patient. In this study, we provide details of a bona fide off-the-shelf strategy where iPSC derived NK-cell (iNK cell) therapy is engineered with a novel immune-evasion modality; CD38 KO to enable combination with anti-CD38 mAbs, which can be administered to deplete activated alloreactive lymphocytes, including NK cells.

## NOVEL STEALTH STRATEGIES

**Approaches to eliminate the need for conditioning chemotherapy**




**Mo F et al.** Engineered off-the-shelf therapeutic T cells resist host immune rejection. Nat. Biotechnol. 2020 doi: 10.1038/s41587-020-0601-5.
**Malmberg et al.** manuscript under preparation

## CONCLUSIONS

- Utilizing our unique ability to multiplex engineer clonal iPSC lines, NK cells for the first time were successfully derived to be uniformly deficient of CD38, as a means of evading host immune cell mediated allorejection in combination with anti-CD38 antibody, daratumumab.
- B2M null iNK cells were resistant to rejection by alloreactive T cells *in vitro*.
- Daratumumab was able to protect both WT, and B2M KO iNK cells from 'missing self' induced rejection, by selective depletion of CD38+ alloreactive NK cells both in vitro and in vivo.
- Addition of Daratumumab to lympho-depleting chemotherapy (LDC) delays CD38+ host immune reconstitution in the patient setting
- Taken together, this data provides evidence of CD38 conditioning as a viable strategy to reduce or even perhaps eliminate the need for conditioning chemotherapy.

## RESULTS

***Generated from a clonally-derived multiplexed-engineered iPSC, iNK cells are uniformly engineered for tailor-made applications, including reducing the need for cy/flu conditioning***

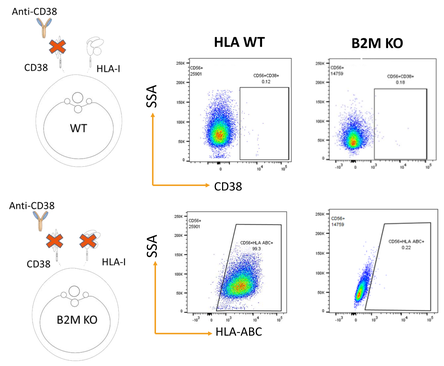



**Figure 1.** Fully differentiated and expanded iNK cells engineered on Fate's CD38 knockout (KO) platform were evaluated for expression of all engineered elements by flow cytometry. All cell populations lacked CD38 expression. Compared to HLA WT (WT), the B2M KO iNKs did not express HLA-I.

***Inhibitory ligand overexpression does not protect HLA null allogeneic cells from all subsets of NK cells but immune disengagement does***

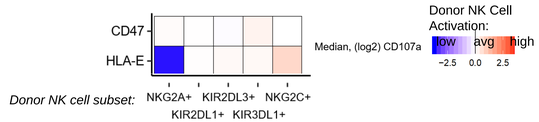



**Figure 2.** Overexpression of individual inhibitory ligands does not protect against all subsets of NK cells: Heat map indicating fold change in cytotoxicity of NK cell subsets expressing specific HLA receptors against K562-targets expressing indicated inhibitory ligands. The data indicate that expression of molecules such as HLA-E or CD47 does not prevent recognition by all NK cell subsets, thus additional strategies are needed. The data were collected using NK cells from 18 independent donors.

***CD38 KO iNK cells are protected from Daratumumab mediated fratricide***

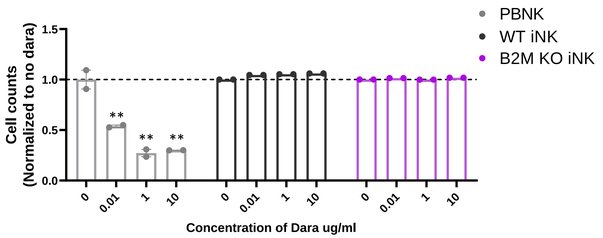



**Figure 3.** CD38 KO iNKs are protected from daratumumab mediated fratricide. Peripheral blood NK cells (PBNKs), WT and B2M KO iNKs were cultured in the presence of daratumumab for 48hrs, PBNKs, which expressed CD38, were depleted in the presence or daratumumab in a dose dependent manner while WT and B2M KO iNKs which lack CD38 were protected from fratricide ( P value ** <0.001).

***Allogeneic iNK cells deficient in CD38 are resistant to allogeneic T- and NK-cell elimination in vitro when co-cultured with daratumumab***

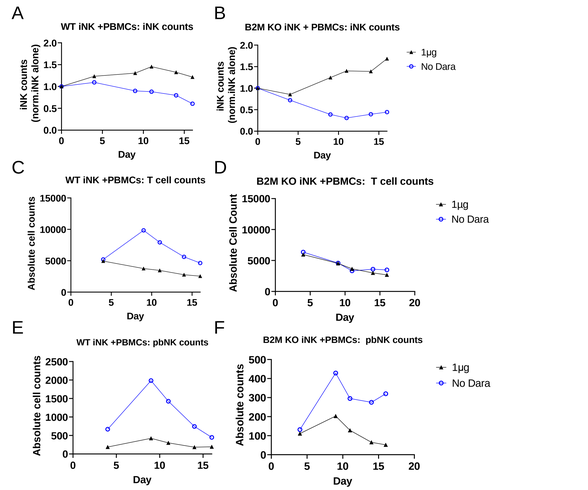



**Figure 4.** Allogeneic iNK cells deficient in CD38 are resistant to allogeneic T- and NK-cell attack in vitro. In the absence of daratumumab both WT and B2M KO iNK cells were depleted. However, with daratumumab, WT and B2M KO iNK cells were protected from rejection (A & B). Absolute T cell counts in WT & B2M KO iNK cell co-cultures (C) indicate that daratumumab prevented T cell expansion and that B2M KO on iNK cells (D) was sufficient to prevent T cell expansion. Absolute pbNK cell counts in both WT iNK cell co-cultures (E) and B2M KO iNK cell co-cultures (F) indicate that daratumumab prevented pbNK cell expansion.

***Daratumumab protects allogeneic iNK cells from PBNK-mediated allorejection in vivo***

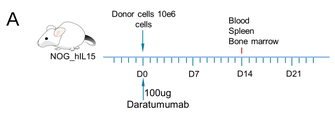



**Figure 5.** Daratumumab protects allogeneic iNK cells from PBNK-mediated allorejection in vivo. PBNKs and iNK cells were either infused alone or co-infused and appropriate groups received one dose of daratumumab. (B - D) The cell counts were normalized to no daratumumab groups. In the blood, spleen and bone marrow, PBNKs were significantly decreased in the presence of Daratumumab which resulted in the persistence of B2M KO iNK cells. In addition, daratumumab significantly decreased the number of CD38+ PBNK which contributed to protection of iNKs from PBNK allorejection (E-G) ( n = 5 mice per group. P value * < 0.05, ** <0.001, **** < 0.0001).

***Use of Daratumumab with lympho-depleting chemotherapy delays host immune reconstitution potentially prolonging the opportunity window for cell therapy***

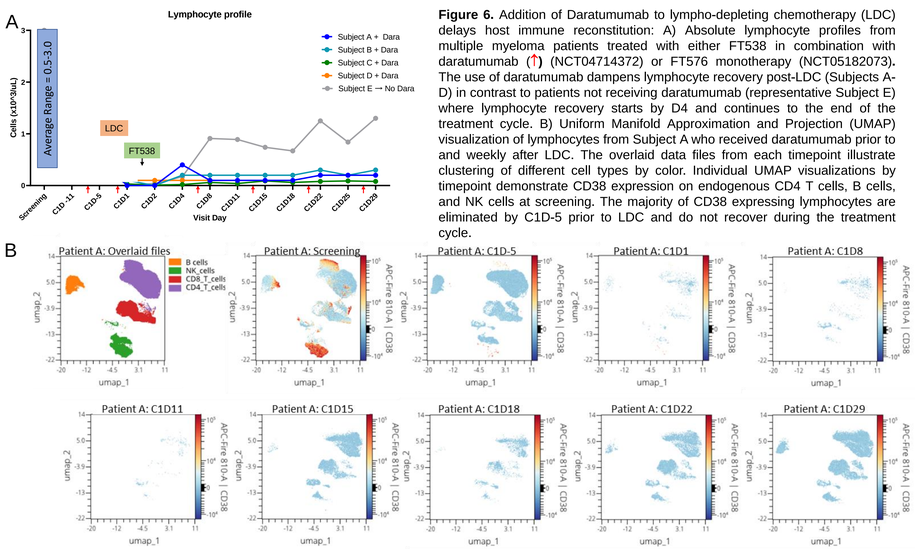



**Figure 6.** Addition of Daratumumab to lympho-depleting chemotherapy (LDC) delays host immune reconstitution: A) Absolute lymphocyte profiles from multiple myeloma patients treated with either FT538 in combination with daratumumab (↑) (NCT04714372) or FT576 monotherapy (NCT05182073). The use of daratumumab dampens lymphocyte recovery post-LDC (Subjects A-D) in contrast to patients not receiving daratumumab (representative Subject E) where lymphocyte recovery starts by D4 and continues to the end of the treatment cycle. B) Uniform Manifold Approximation and Projection (UMAP) visualization of lymphocytes from Subject A who received daratumumab prior to and weekly after LDC. The overlaid data files from each timepoint illustrate clustering of different cell types by color. Individual UMAP visualizations by timepoint demonstrate CD38 expression on endogenous CD4 T cells, B cells, and NK cells at screening. The majority of CD38 expressing lymphocytes are eliminated by C1D-5 prior to LDC and do not recover during the treatment cycle.