

Progress to Date:

Aim 1: To engineer NK cells with increased CD16a expression for enhanced ADCC function. NK cells express high levels of the IgG Fc receptor FcγRIIIa (CD16a), which is their sole means of recognizing anti-tumor antibodies for the induction of antibody-dependent cell-mediated cytotoxicity (ADCC). This receptor, however, undergoes a very rapid and efficient downregulation in expression by a proteolytic process (i.e., ectodomain shedding) mediated by the metalloprotease ADAM17 upon NK cell activation, which decreases their ADCC efficiency. Induced pluripotent stem cells (iPSC) are being used to generate a renewable source of natural killer (NK) cells to enhance current immunotherapies and the patient's immune system in killing cancer cells. However, this process results in NK cell activation and CD16a downregulation. The goal of this project is to engineer NK cells so they can be grown to significant numbers in culture without diminishing their ability to mediate ADCC. Our objective is to genetically modify iPSCs to produce NK cells with increased CD16a expression and ADCC function. This will be accomplished by three approaches, over-expression of wild-type CD16a; expression of non-cleavable CD16a; and *ADAM17* gene targeting to prevent the cleavage of endogenous CD16a.

Aim 2: To evaluate the anti-cancer activity of iPSC-generated NK cells with increased CD16a expression. From these studies we will compare the ADCC efficiency of iPSC-NK cells expressing endogenous, wildtype or noncleavable CD16a, as well as iPSC-NK cells expressing or lacking ADAM17.

Dr. Dan Kaufman was originally a co-PI on this grant, but in 2016 he relocated to the University of California, San Diego. This caused an initial setback in generating iPSC-NK cells (iNK cells). We have developed and improved this methodology are now able to produce engineered iNK cells in our lab. iPSCs (UCBiPS7 cells) were transduced using a *Sleeping Beauty* transposon system to stably express either a non-cleavable version of CD16a or wild-type human CD16a. The engineered iPSCs were then differentiated into a hemogenic endothelium population defined by CD34 expression. These cells were further differentiated into iNK cells in two stages. They were first differentiated into early iNK cells expressing CD56 and early markers of NK cells, and then mature iNK cells and at the same time expanded using K562/mblL21/41BBL cells. The engineered iNK cells expressed high levels of transduced CD16a (non-cleavable or wildtype; **Fig. 1A and B**), whereas control differentiated NK cells expressed lower levels of endogenous CD16a (**Fig. 1C**). Overt activation of iNK cells caused a downregulation in expression of endogenous and wildtype CD16a, but not non-cleavable CD16a (**Fig. 1D**). Of interest is that the expression of either wildtype or non-cleavable CD16a in iPSCs ablated endogenous CD16a expression in the differentiated NK cells. iNK cells expressing non-cleavable CD16a also demonstrated higher levels of ADCC than iPSC-NK cells expressing wildtype CD16a (**Fig. 1E**). Target cells in this assay consisted of the ovarian cancer cell line Skov3 expressing HER2 treated with the anti-HER2 therapeutic mAb Herceptin.

In addition to the expression of wildtype or non-cleavable CD16a, we have also targeted the *ADAM17* gene in iPSCs by CRISPR/Cas9 to prevent the cleavage of endogenous CD16a. UCBiPS7 cells were transfected with a vector that expresses a gRNA sequence targeting exon-1 of the *ADAM17* gene. This vector also expresses GFP, which was used to initially sort transfected cells. Following their sorting, cells were passaged by limiting dilution to isolate iPSCs clones. The individual clones were then stained with an ADAM17 mAb to assess its surface expression by flow cytometry. Several clones were identified that expressed or lacked ADAM17 cell surface expression (**Fig. 2**). ADAM17 gene targeting was confirmed by PCR amplification and sequencing of ADAM17 exon 1. We are currently generating iNK cells that lack ADAM17 expression. Their ADCC potency will be compared to iNK cells expressing ADAM17, as well as to iNK cells expressing wildtype and non-cleavable CD16a.

List any of the following that have resulted from the Minnesota Regenerative Medicine grant funding:

Publications and/or manuscripts submitted for publication:

In progress.

Disclosures/patents:

Intellectual property pertaining to the expression of noncleavable CD16a in genetically engineered cells that exhibit increased anti-tumor activity is encompassed in our patent application: WO2015/148926 *Polypeptides*,

Award #: MRM 2015 4925
 cells, and methods involving
 engineered cd16, now
 US15/300,024 (unpublished)
 and counterpart foreign
 applications (Europe, Australia,
 Canada, China and Japan).

PI: Walcheck, Bruce

Fate Therapeutics has signed an exclusive license and is working in collaboration with the UMN for the clinical development of a first-of-kind, off the shelf iNK product expressing noncleavable CD16a in combination with therapeutic antibodies as a cancer immunotherapy. Fate's in-house research has shown that iNK cells expressing noncleavable CD16a exhibit superior ADCC of ovarian cancer and lymphoma cell lines when combined with the therapeutic mAbs Eributux and Rituxan, respectively, by *in vitro* assays, as compared to conventional NK cells sourced from peripheral blood and cord blood. iNK cells expressing noncleavable CD16a also demonstrated augmented anti-tumor activity of an ovarian cancer cell line in combination with Herceptin *in vivo*, as compared to mice treated with Herceptin alone.

Grant applications and/or awards:

1R01CA203348-01 [PI: Walcheck] 01/01/2016-12/31/2021; NIH/NCI; \$2,235,171 total; Title: Blocking NK cell shedding of CD16a to increase cancer cell killing.

The goal of this grant is to block the shedding of CD16a through engineered mutations in the receptor and with unique ADAM17 inhibitors to increase NK cell killing of primary cancer cells *in vivo*. Data from the RMM grant was instrumental in obtaining this grant.

Budget Update:
 NA

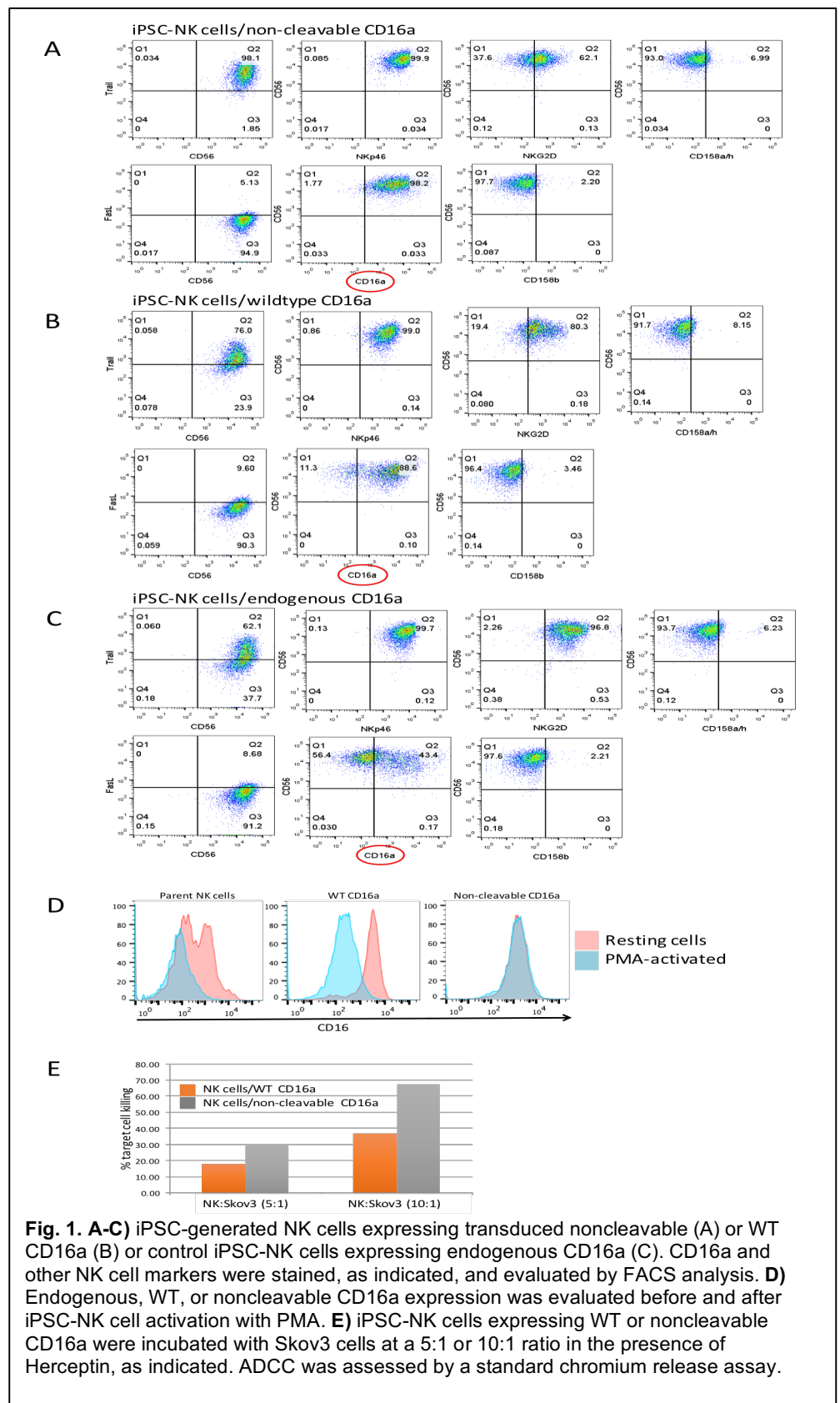


Fig. 1. A-C iPSC-generated NK cells expressing transduced noncleavable (A) or WT CD16a (B) or control iPSC-NK cells expressing endogenous CD16a (C). CD16a and other NK cell markers were stained, as indicated, and evaluated by FACS analysis. **D**) Endogenous, WT, or noncleavable CD16a expression was evaluated before and after iPSC-NK cell activation with PMA. **E**) iPSC-NK cells expressing WT or noncleavable CD16a were incubated with Skov3 cells at a 5:1 or 10:1 ratio in the presence of Herceptin, as indicated. ADCC was assessed by a standard chromium release assay.

Overview:

Induced pluripotent stem cells (iPSCs) are derived from adult cells obtained from healthy donors or patients and they provide a platform for producing an unlimited source of immune cells for cell-based therapies to treat cancer. Our goal was to engineer iPSCs to generate a renewable source of enhanced natural killer (NK) cells to improve current therapies and the patient's immune system in killing cancer cells. A key advantage of NK cells is that they can be targeted to multiple types of cancer by their recognition of anti-tumor antibodies. However, NK cells can downregulate the expression of key receptors important for

their anti-cancer function during their expansion for transfer into patients as well as in the tumor environment. The objective of this project was to engineer iPSCs to generate NK cells that maintain their ability to kill cancer cells. We have achieved this objective of engineering enhanced versions of NK cells. This has resulted in a US and international patent application; the licensing of our technology by a pharmaceutical company that is working in collaboration with the University of Minnesota in the development of a first-of-kind, off the shelf NK cell product as a cancer immunotherapy; and external funding from the National Institutes of Health.

