

GAPDH KNOCK-IN OF HIGH AFFINITY CD16 IN IPSC DERIVED NK CELLS DRIVES HIGH-LEVEL EXPRESSION AND INCREASED ANTI-TUMOR FUNCTION

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Background Natural killer (NK) cells have emerged as an alternative cell type for clinical utility given the low propensity for graft-versus-host disease, thereby making NK cells a potential off-the-shelf cell therapy. One critical pathway NK cells use to target tumor cells is through expression of Fc gamma receptor III alpha (CD16). Antibodies that bind tumor antigens are recognized by CD16 on NK cells, promoting NK-mediated tumor cell killing. High-affinity CD16 variants in the human population correlate with better clinical outcome and anti-tumor response. One mechanism tumors use to evade NK cell recognition is through down-regulation of CD16 expression on the NK cell surface. After being activated, CD16 is cleaved by A Disintegrin and Metalloprotease-17 (ADAM-17). By using a highly-active engineered AsCas12a to knock-in high-affinity CD16 (hCD16KI) at the GAPDH locus, hCD16 is constitutively expressed, continuously replacing hCD16, thereby allowing for repeated ADCC mediated killing.

Methods iPSCs were edited at the GAPDH locus with an engineered AsCas12a along with the CD16 donor construct. The bulk edited population was then plated at clonal density and single clones were selected and screened. iPSC clones were then differentiated into NK cells. A 3D tumor spheroid killing assay was used to demonstrate NK cell cytotoxicity against an ovarian cancer cell line (SKOV-3). In addition, a serial killing assay was used to better model NK cell serial killing.

Results Bi-allelic CD16KI iPSC clones were successfully generated. These iPSCs exhibited normal morphology and were able to differentiate into iNK cells. hCD16KI iNK cells showed normal differentiation and surface marker expression, such as CD45/CD56, compared to unedited iNK cells. CD16KI iNK cells demonstrated significantly increased cytotoxicity in the presence of antibody against tumor cells when compared with unedited iNK cells, as measured by reduction in tumor spheroid size in a 3D tumor spheroid killing assay. Importantly, enhanced surface expression of hCD16 on iNK cells after tumor exposure was detected, demonstrating the replenishment of cleaved hCD16. Notably, hCD16KI iNK cells demonstrated prolonged and enhanced tumor cell killing after being subjected to repeated tumor cell exposure in a serial killing assay.

Conclusions This work demonstrates a powerful new method to drive high-level constitutive hCD16 expression on the surface of iNK cells through transgene knock-in at the GAPDH locus using an engineered AsCas12a. The high level constitutive hCD16 expression enhances ADCC of iNK cells and enables enhanced serial tumor killing and is expected to exert enhanced anti-tumor activity in the clinic.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.191>