170 NOVEL GENE EDITING APPROACH TO ENHANCE CD38-DIRECTED ANTITUMOR ACTIVITY OF PRIMARY HUMAN NATURAL KILLER CELLS

Joseph Clara*, Emily Levy, Robert Reger, Mala Chakraborty, David Allan, Richard Childs.
National Institutes of Health, Bethesda, MD, USA

Background Natural killer (NK) cells play an important role in the antitumor responses of therapeutic monoclonal antibodies (mAbs) by mediating antibody-dependent cellular cytotoxicity (ADCC). ADCC occurs when tumor-ligated mAbs trigger NK cell killing by engaging CD16 on NK cells. Further, infusions of ex vivo expanded NK cells to potentiate the antitumor effects of mAbs is a promising immunotherapeutic approach. Daratumumab (DARA) is an anti-CD38 mAb used in the treatment of multiple myeloma (MM) that efficiently induces NK-mediated ADCC. However, CD38 is also expressed on NK cells, which leads to DARA-mediated NK cell destruction and impaired ADCC against MM. Harnessing NK cells to fully exploit the ADCC mechanism of DARA has the potential to improve DARA's efficacy.

Methods We developed a novel approach to maximize DARA-mediated ADCC against MM by protecting NK cells from DARA targeting while simultaneously boosting their ADCC capacity. We designed a CRISPR/Cas9-based gene editing platform to couple the disruption of CD38 expression with site-specific insertion of a gene encoding a high-affinity variant of CD16 at the CD38 locus in ex vivo expanded NK cells. To achieve this, we delivered a ribonucleoprotein composed of a single guide RNA and Cas9 nuclease to induce a double strand break within CD38, followed by infection with engineered AAV particles to deliver a homology directed repair template encoding either a truncated CD34 reporter or a FLAG-tagged high-affinity CD16 to be integrated at the site of CD38 gene disruption. Gene editing and insertion efficiency were assessed by flow cytometry for CD38, CD34, and/or FLAG. Transgene integration was confirmed by junction PCR.

Results High CD38 knockout efficiency was achieved (90.1 ± 4.1%). Using an EF-1 alpha promoter-containing CD34 donor template, stable CD34 expression was seen in 90.2 ± 5.1% NK cells. Insertion of high-affinity CD16, as assessed by FLAG expression, was observed in 47.7 ± 6.1% of edited NK cells. NK cells modified to express high-affinity CD16 also exhibited a higher number of CD16-positive NK cells compared to unedited control NK cells (10.94 ± 1.8%). Functionally, CD38 knockout NK cells expressing high-affinity CD16 demonstrated augmented degranulation in ADCC assays and killing of daratumumab-treated MM cells compared to unedited control and CD38 knockout NK cells.

Conclusions Novel gene editing techniques can be successfully applied to generate NK cells with enhanced antitumor capabilities. We established an efficient gene editing platform that can be utilized to produce NK cells optimized for adoptive combination with daratumumab.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.170