POTENTIATING THE LARGE-SCALE EXPANSION AND ENGINEERING OF PERIPHERAL BLOOD-DERIVED CAR NK CELLS FOR OFF-THE-SHELF APPLICATION

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Background Peripheral blood natural killer (NK) cells are mature cytotoxic innate lymphocytes possessing an inherent capacity for tumor cell killing, thus making them attractive candidates for adoptive cell therapy. These NK cells are also amenable to CRISPR and chimeric antigen receptor (CAR) genomic engineering for enhanced functions. Moreover, NK cells possess an inherent capacity for off-the-shelf therapy since they are not known to cause graft-versus-host disease, unlike T cells. Presently, approved CAR cell therapy is custom-made from each patient's own T cells, a process that can limit patient pool, narrow therapeutic window, and contribute to product variability. In this study, we investigate whether peripheral blood NK cells from a selected donor can be edited, engineered, and expanded sufficiently for off-the-shelf use in a wide patient population.

Methods Using the CRISPR/Cas9 system, we knocked out CISH expression in isolated peripheral blood NK cells from 3 healthy donors. Subsequently, we expanded edited NK cells by using IL-2 and sequential stimulations using NKSTIM, a modified K562 stimulatory cell line expressing membrane-bound form of IL-15 (mbIL-15) and 4-1BBL. IL-12 and IL-18 were added twice during expansion to drive memory-like NK cell differentiation. We transduced the expanded NK cells to express engineered CD19-targeted CAR and mbIL-15 during an interval between the first and second NKSTIM pulses. We assessed NK cell cytotoxicity against Nalm6 target cells by IncuCyte.

Results Isolated peripheral blood NK cells from 3 healthy donors were successfully edited using CRISPR/Cas9, engineered to express high levels of CAR, extensively expanded using a series of NKSTIM pulses in the presence of IL-2, and differentiated into memory-like NK cells using IL-12 and IL-18. Interestingly, NK cells from the 3 donors exhibited distinct outcomes. NK cells from one donor reached a peak expansion limit of approximately 7-million-fold before undergoing contraction whereas NK cells from two donors continued to expand over the length of the study surpassing 100-million-fold expansion, without appearing to have reached a terminal expansion limit. At the end of the study, NK cells from one donor exceeded 1-billion-fold expansion and maintained 88% cytolytic activity compared to Nkarta's standard process control in a 72-hour IncuCyte assay.

Conclusions In this study, we demonstrate that healthy donor-derived peripheral blood NK cells are capable of expanding over billion-fold while maintaining potency. These results provide a rationale for the development of off-the-shelf CAR NK cell therapies using NK cells from donors selected to provide optimal product characteristics.

Ethics Approval Human samples were collected with written informed consent by an approved vendor.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.151>