Background Healthy adult peripheral blood natural killer (PBNK) cells are mature cytotoxic innate lymphocytes possessing an inherent capacity for tumor cell killing, making them attractive candidates for adoptive cell therapy. These NK cells are also amenable to 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. Approved CAR cell therapies are custom-made from each patient’s own T cells, a process that can limit patient eligibility and contribute to product variability. In this study, we compare PBNK cells to umbilical cord blood NK (CBNK) cells to evaluate both as candidate starting materials for clinical and commercial supply of CAR NK cells.

Methods PBNK and CBNK cells were expanded using either a 14-day protocol and a single stimulation with Nkarta’s NKSTIM cell line plus IL-2, or with 5 stimulations over 70 days. IL-12 and IL-18 were added at the beginning and end of the 70-day expansion to drive memory-like NK cell differentiation. We transduced NK cells to express CD19-targeted CAR and membrane-bound IL-15 following the first NKSTIM pulse. We measured cytotoxicity against 3 tumor cell lines by IncuCyte, and phenotyped cells for NK markers including differentiation markers CD57 and NKG2A, and NKG2A and KIR.

Results Purified NK cells from 1 PBNK donor and 4 CBNK donors were successfully expanded and engineered to express high levels of CAR. The 70-day final product (FP) CBNK cells were CD57⁻KIR⁻ and NKG2A+, consistent with an immature phenotype, whereas the FP PBNK cells were educated, at more than 80% NKG2A KIR⁺. CBNK cells expanded to approximately 11-million-fold, whereas PBNK cells surpassed 250-billion-fold expansion, without appearing to have reached a terminal expansion limit. At the end of the study, Nkarta’s standard 14-day process (SP) cells [1], and FP PBNK cells were as potent or trended towards greater potency than CBNK cells against 3 different tumor targets in a 72-hour IncuCyte assay. Furthermore, FP PBNK cells were as or more potent than SP PBNK cells, depending on the tumor target.

Conclusions We demonstrate healthy donor-derived PBNK cells can expand over 250 billion-fold while maintaining potency. These results show robust expansion capability of educated, potent NK cells and 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.

REFERENCE