KIR HAPLOTYPE CAN INFORM DONOR SELECTION PRODUCTION OF ALLOGENEIC MEMORY-LIKE CAR NK CELLS FOR CLINICAL APPLICATION

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Background NK cells expanded on membrane-bound (mb) IL-15 and 41BBL expressing K562 stimulatory cells (NKSTIM) for clinical use can be genetically modified to express activating chimeric receptors.^{1 2 3} NK cells activated in the presence of IL-12, IL-15 and IL-18 develop cytokine induced memory-like (CIML) phenotype and function; these cells have shown clinical promise.⁴ Additionally, HSCT AML transplants using NK KIR Haplotype Group B donors with better and best Group B profiles (≥ 2 activating genes) show better survival.⁵ ⁶ Here we investigate whether KIR profiles impact healthy allogeneic donor NK cell function and phenotype when these cells are expanded on NKSTIM in the presence of IL-12 and IL-18 (12–18).

Methods Healthy donor PBMC NK were genotyped for HLA and KIR and expanded on K562-mbIL15-41BBL stimulatory cells with IL-2 alone or with IL-2 plus IL-12 and IL-18 (12–18). Expanded NK were transduced with CAR constructs including CD19, and then evaluated for NK cell expansion, cytokine secretion, RNA profiles, cytotoxicity against tumor lines, and cell surface phenotypes. Expanded CD19 NK donors with varying numbers of activating KIR vs inhibitory KIR were tested for effector function, and these donors were then tested for in vivo efficacy and pharmacokinetics. A KIR ranking score was developed by considering both the number of activating and inhibitory KIR genes expressed by each donor. This score was correlated with functional properties of CAR NK cells.

Results Addition of 12–18 to the K562-mbIL15-41BBL stimulatory cells improves CD19-CAR NK potency 2-fold relative to the stimulatory cell line alone ($P=.02$) while NK cell expansion is unchanged. 12–18 also drove an increase in effector cytokine accumulation on exposure of CAR-NK to CD19 tumor. CIML CAR NK cells from donors with higher KIR scoring also had higher cytotoxicity (Pearson's $R=0.74$, $P=0.006$); this correlation was not observed following expansion in the absence of 12–18. 12–18 also drove more potent in vivo activity against tumor with an increased presence of circulating NK cells over 4 weeks in the mice.

Conclusions CIML CAR NK cells derived from donors with favorable KIR scoring have greater cytotoxic activity, effector cytokine production, and *in vivo* pharmacokinetics and efficacy. These findings may provide an important criterion for donor selection in the development of more robust and potent engineered NK cells for clinical use.

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Ethics Approval Animal studies were conducted with IACUC approval.

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