KIR haplotype can inform donor selection production of allogeneic memory-like CAR NK cells for clinical application

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Introduction

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]. 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 [2]. Additionally, HSCT AML transplants using NK KIR Haplotype Group B donors with better and best Group B profiles (≥2 activating genes) show better survival [3]. 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 engineered to express a CD19 CAR and mbIL-15, 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. 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.

Figure 1. Nkarta platform for expansion, activation, and engineering of allogeneic CAR NK cells for off-the-shelf use

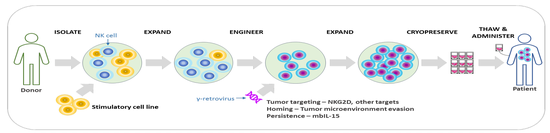


Figure 2. NK Donor Genotype DNA-based high-resolution genotypic analysis of HLA & KIR was performed on 12 NK donors. Furthermore, KIR B content group was determined using IPD-KIR database [4].

Donor HLA Genotype Contribution to Licensing

IUK Donor ID	KIR B-Content Calculator (Cooley Miller 2010)	HLA-A 3DL2	3DL1 Bw4	C1 HLA	C2 HLA	Total # HLA	2DL1	C2 HLA	2DL2	2DL3	C1 HLA	2DL4	2DL5	3DL1	3DL2	3DL3	3DS1	2DS1	2DS2	2DS3	2DS5	2DS4	2DP1	3DP1
		KIR Ligands:					C2				C1	HLA-G	PVR	Bw4	A3/A8	HHLA2	HLA-F/ Bw4 + HIVpep	C2	A11 pept		006=<2	A & C	NA	NA
451	B - Best	0	1	2	0	3	NEG	0	POS	NEG	2	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	NEG	POS
454	B	0	0	1	1	2	POS	1	NEG	POS	1	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	POS
512	B -Better	0	0	1	1	2	POS	1	POS	POS	1	POS	POS	NEG	POS	POS	POS	POS	NEG	NEG	POS	NEG	POS	POS
548	B	1	2	1	1	5	POS	1	NEG	POS	1	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	POS
558	B - Better	0	1	2	0	3	POS	0	POS	POS	2	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	POS
624	A	0	0	2	0	2	POS	0	NEG	POS	2	POS	NEG	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS	POS
709	B	0	1	0	2	3	POS	2	POS	POS	0	POS	NEG	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS	POS
744	A	0	0	1	1	2	POS	1	NEG	POS	1	POS	NEG	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
784	B	0	1	1	1	3	POS	1	POS	POS	1	POS	NEG	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS	POS
828	A	1	2	0	2	5	POS	2	NEG	POS	0	POS	NEG	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
845	B	1	1	0	2	4	POS	2	POS	POS	0	POS	POS	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS	POS
909	B	0	0	2	0	2	POS	0	NEG	POS	2	POS	POS	POS	POS	POS	NEG	NEG	NEG	POS	NEG	POS	POS	POS

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. Furthermore, NK memory associated cell surface markers and natural cytotoxicity receptors (NCR) were upregulated with 12-18. Significant increase in gene expression linked to NK activation in RNAseq data correlated with 12-18 treatment. 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. Cytotoxic activity of unmodifed NK Haplotype B donors were slightly higher than Haplotype A against 2 of the 4 tumor types tested.

Figure 3: Flow Phenotype 12 NK donors were expanded on NKSTIM with or without soluble IL-12/IL-18 cytokines and genetically modified with a retroviral CD19-CAR-mbIL-15 construct. Cells were characterized by flow cytometry on Day 0 & 14. Genetically modified NKs express increased levels of activation markers & activating NK receptors, namely TIGIT, Lag3, CD69, NKp30, NKp44, NKp46. Moreover, a notable increase in NK memory associated markers, NKG2C, NKG2A, CD69 and a decrease in terminal differentiation marker, CD57 are observed in expanded NKs (A). IL-12 & IL-18 drives an increase in CD62L, while downregulates inhibitory receptor ILT2 (B).

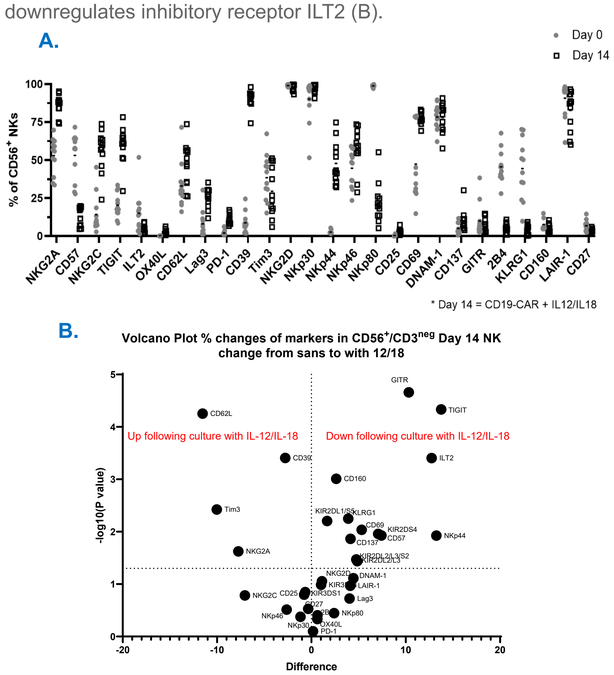


Figure 4: RNAseq Differential Expression Addition of IL-12/IL-18 during expansion drives upregulation of genes associated with activation of NK cells.

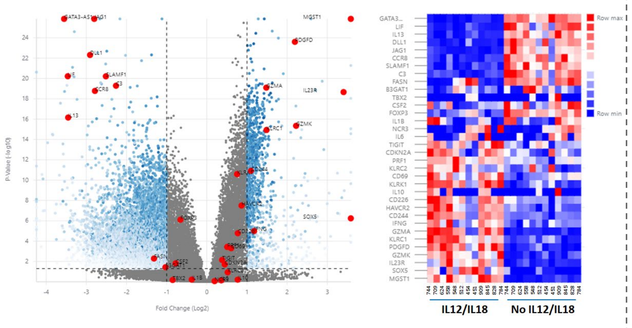


Figure 5: Expansion & Cytotoxicity Addition of IL-12/IL-18 during expansion significantly enhances CD19 CAR directed cytotoxicity against NALM-6 tumor line (B) but not fold-expansion of the NKs (A). 12 healthy purified NK donors were expanded on NKSTIM with or without soluble IL-12/IL-18 cytokines and genetically modified with a retroviral CD19-CAR-mbIL-15 construct. Eight days post transduction, cytotoxicity against the ALL cell line NALM-6 was measured using Incucyte at a 1:4 E:T ratio.

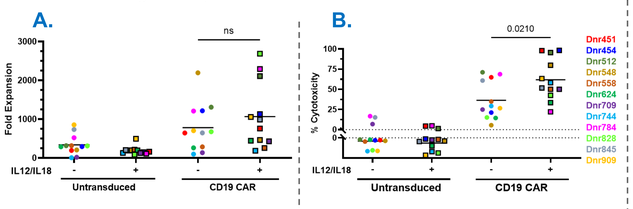


Figure 6: Luminex Profile Supernatants from NALM-6 tumor cytotoxicity assays (Figure 5) were evaluated on a multi-plex panel consisting of 11 analytes. Increased levels of IFN-g (A), GM-CSF (B), MIP-1a (C), and Perforin (D) are detected in IL12/IL18 expanded CD19-CAR transduced NKs, consistent with cytotoxic potency ranking of individual NK donors.

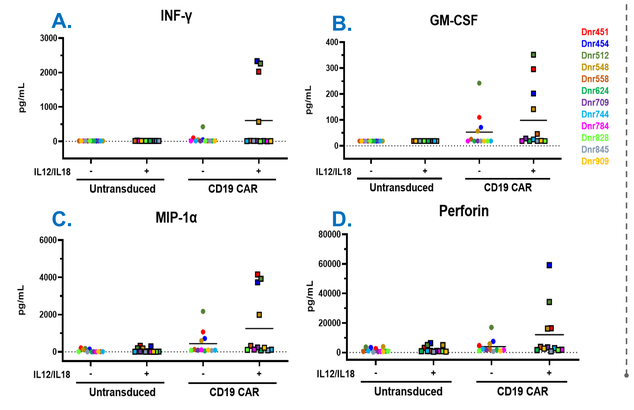


Figure 8: KIR Content Ranking A total KIR ranking score based on both activating KIR (aKIR) and inhibitory KIR (iKIR) correlates significantly with CAR NK cytotoxic potency when cells are expanded in the presence of IL-12 and -18. (A), but not in their absence (B). Donors with KIR B-content groups Best & Better rank amongst the highest in killing potency.

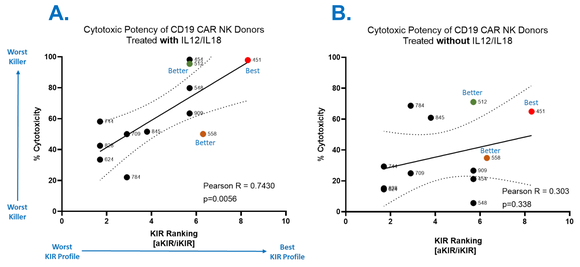
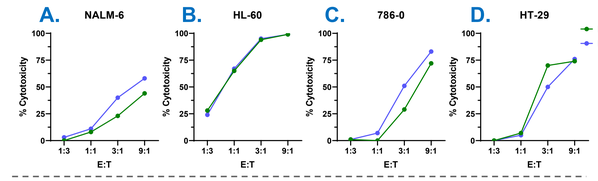


Figure 9: KIR Haplotype A & B Cytotoxic Potency Unmodified KIR haplotype B donor NKs treated with IL-12/IL-18 during expansion effectively kill tumor cells across different indications, namely NALM-6 – B cell leukemia (A), HL-60 – myeloid cell leukemia (B), 786-0 – renal cancer (C), and HT-29 – colorectal adenocarcinoma (D). 6 donors were tested: 2 haplotype A and 4 haplotype B, group averages are shown.



Conclusions

CIML CAR NK cells derived from donors with favorable KIR scoring have greater cytotoxic activity and effector cytokine production. Transcription and cell surface expression of several NCRs and markers of NK activation are increased in CAR NK cells treated with IL12/IL-18. The contribution of KIR scoring to NK cell potency may vary with target, as the cytotoxicity of unmodified NK cells measured across 4 tumor cell lines showed only a marginal dependence on KIR haplotype. These findings may provide an important criterion for donor selection in the development of more robust and potent engineered NK cells for clinical use.

References

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