Potentiating the Large-Scale Expansion and Engineering of Peripheral Blood-Derived CAR NK Cells for Off-the-Shelf Application

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nkarta THERAPEUTICS

Introduction

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. NK cell cytotoxicity was assessed against multiple tumor target cells by Incucyte.

NK cell expansion schema

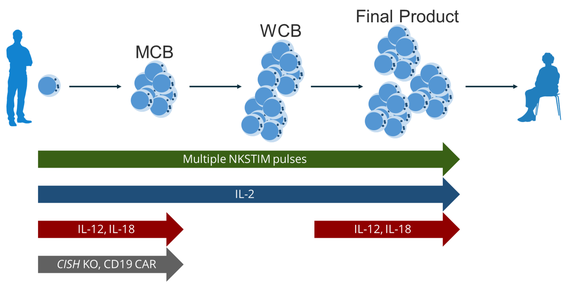

Figure 1. Schematic diagram of healthy donor-derived NK cell expansion for off-the-shelf use.

MCB: master cell bank; WCB: working cell bank; FP: final product

Results

Isolated peripheral blood NK cells from 3 healthy donors were successfully edited using CRISPR/Cas9, engineered to express high levels of CAR and mbIL-15, 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 Donor 1 reached a peak expansion limit of approximately 7-million-fold before undergoing contraction whereas NK cells from Donor 2 and Donor 3 continued to expand over the length of the study surpassing 2-billion and 200-million-fold expansion, respectively, without appearing to have reached a terminal expansion limit and maintained potency.

Substantial expansion of engineered peripheral blood NK cells can be achieved using multiple NKSTIM pulses

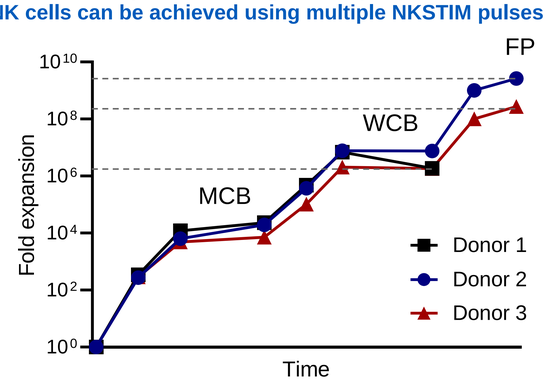

Figure 2. Up to 2-billion-fold expansion was achieved for Donor 2 NK cells.

Cell surface markers associated with exhaustion are upregulated on Donor 1 NK cells

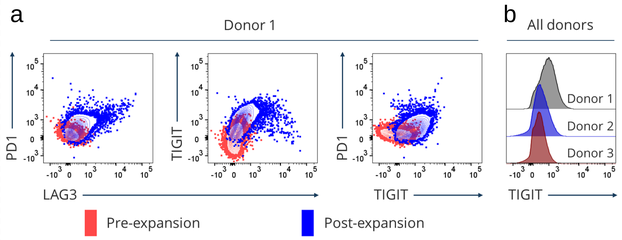

Figure 3. (a) Multi-color flow cytometry analysis of Donor 1 NK cells show increased expression of markers associated with exhaustion. **(b)** Highest expression of TIGIT was observed in Donor 1 NK cells at WCB stage.

Cell surface expression of many activating receptors are upregulated on NK cells during expansion

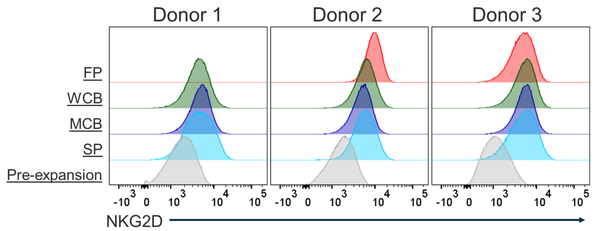

Figure 4. Cell surface expression of NKG2D increases during expansion. Similar trends were observed for NKp30, NKp44, NKG2C, DNAM-1, KIR2DS4, and KIR3DS1 in most donor NK cells, data not shown. Standard process (SP) NK cells are used as a benchmark reference. SP NK cell expansion protocol closely follows Nkarta's clinical trial drug manufacturing process.

CAR/mbIL-15$^+$ NK cells are preferentially enriched during expansion

	CAR$^+$ NK cells (%)			
	SP	MCB	WCB	FP
Donor 1	85	89	93	N/A
Donor 2	73	93	91	99
Donor 3	65	80	84	90

Table 1. Bi-cistronic expression of CD19-targeted CAR and mbIL-15 enforces IL-15 signaling in CAR$^+$ NK cells. The combination of mbIL-15 expression and *CISH* gene editing further enriches CAR$^+$ cells during expansion.

Chromosomal integrity is well-maintained in NK cells after >200-million-fold expansion

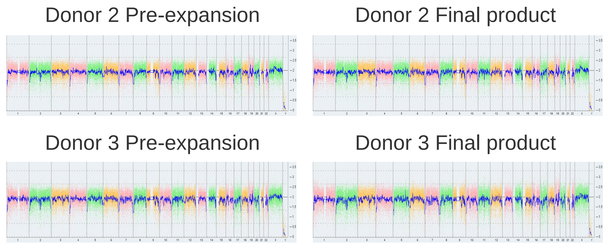

Figure 5. KaryoStat^{TM} analysis shows no chromosomal aberrations in Donor 2 and Donor 3 NK cells. 150k SNPs spread across the genome were analyzed and compared between donor matched pre- and post-expansion NK cells. X-axis indicates chromosome number and Y-axis indicates copy number.

Potency of peripheral blood NK cells can be maintained after >200-million-fold expansion

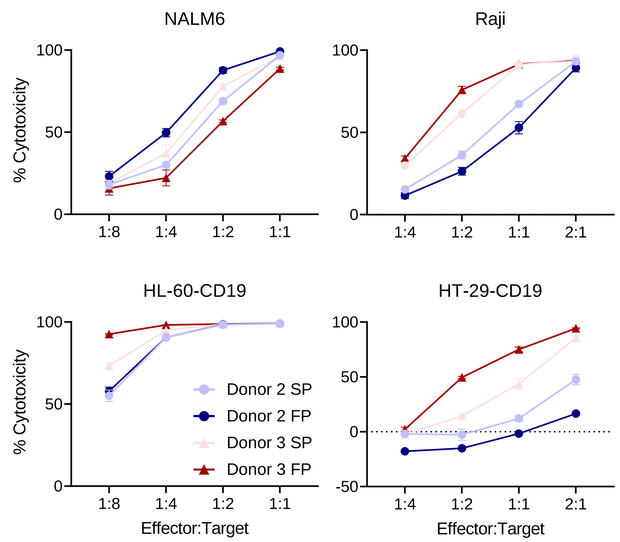

Figure 6. Extensively expanded healthy donor-derived peripheral blood NK cells are capable of killing multiple tumor cell types. Standard process and final product NK cells from Donor 2 and Donor 3 were used in a cytotoxicity assay against B cell tumor cell lines that naturally express CD19 (NALM6 and Raji) and non-B cell tumor cell lines that ectopically express CD19 (HL-60-CD19 and HT-29-CD19). Percent cytotoxicity was calculated based on Incucyte images collected at 72-hours after co-culture. % cytotoxicity = [(control – experiment) / control] x 100.

Conclusion

In this study, we describe the development of manufacturing methods for NK cells derived from a limited pool of donors selected to provide optimal product characteristics. We show that NKG2D expression is increased on the cell surface during expansion, *CISH* edited CAR/mbIL-15$^+$ NK cells are enriched in a self-selecting manner, and chromosomal integrity is well-maintained despite >2-billion-fold expansion. Most importantly, final product NK cells are highly cytotoxic against multiple tumor cell lines. These results support large-scale expansion and engineering of peripheral blood-derived CAR NK cells for off-the-shelf application.

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