Stimulatory cells plus IL-12 and IL-18 augments NK cell expansion, transduction, memory phenotype, and *in vitro* and *in vivo* CAR NK cytotoxicity & persistence

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Introduction

NK cells have been expanded on K562 stimulatory cells expressing membrane-bound (mb) IL-15 and 41BBL (NKSTIM) for clinical use and can be genetically modified to express activating chimeric receptors [1,2,3]. Engineered NK cells targeting CD19 show *in vitro* and *in vivo* cytotoxicity against relevant tumor targets that can overcome endogenous resistance to NK cells. NK cells activated in the presence of IL-12, IL-15 and IL-18 develop cytokine induced memory-like phenotype and function; these cells have shown clinical promise [4]. Here we describe NK cell function and phenotype achieved by combining robust expansion driven by K562-mbIL15-41BBL with the induction of a cytokine-induced memory phenotype achieved after exposure to IL-12 and IL-18.

Methods

NK cell expansion, cytokine secretion, cytotoxicity against tumor lines at various time points, and persistence in culture over 5 weeks was compared with or without exposure to IL-12 and IL-18 (12-18). The expanded NK were transduced with a CD19 CAR construct, and the resulting cells were evaluated for CAR expression, cytotoxicity and *in vivo* efficacy against relevant cell lines.

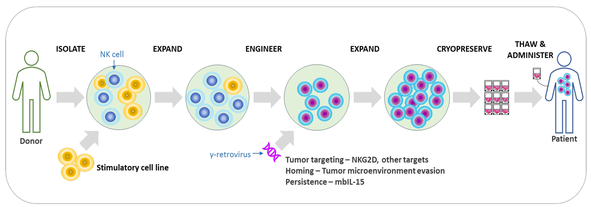
NK Expansion with IL-12 and IL-18

Healthy donor PBMC NK were expanded on K562-mbIL15-41BBL stimulatory cells (NKSTIM) with IL-2 alone or with IL-2 plus IL-12 and IL-18. Expanded NK were then measured for expansion, cytotoxicity and memory-like phenotype.

Transduction with CAR constructs & in vivo experiments

Expanded NK were genetically modified with a retroviral construct consisting of the FMC63 scFv CD19-OX40-CD3zeta CAR and mbIL-15 to drive persistence. Modified NK were then assayed *in vitro* and *in vivo*.

Figure 1: Nkarta Natural Killer (NK) cell platform showing ex vivo (NK) cell expansion, activation and genetic modification procedures



Results

Figure 2: Expansion 6 healthy PBMC donors were expanded 7 days in a matrix of 4 concentrations each ranging from 20-0.08 ng/ml IL-12 and IL-18 inoculated at day 0. Day7 fold expansion is shown for IL18 @ 4ng/ml (A) and 20ng/ml (B)

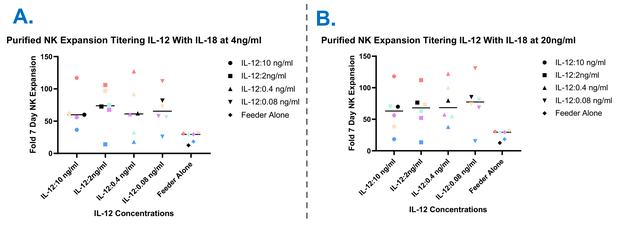
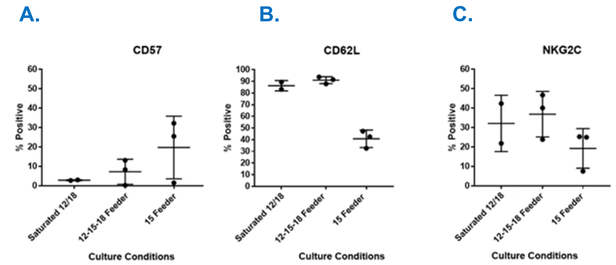


Figure 3: Flow Phenotype 3 donors were expanded on NKSTIM with or without soluble 12-18 (10 & 20ng/ml respectively) spiked at day 0 or with NKSTIM feeder engineered to express membrane bound IL-12 and IL-18. Flow cytometry was used to characterize the cells over 7-35 days of expansion. 12-18 drives a decrease in CD57 (A) and increase in CD62L (B) and NKG2C (C) over 21 days. Weekly tracking of memory markers over 5 weeks (D) shows an increase, and then constant NKG2C D21-35 expression and an initial spike and subsequent drop in CD62L. Both NKG2C NK percentages and surface level expression (E) increase in an example donor from D14 to D21 with a concurrent loss of CD57 expression by day 21.



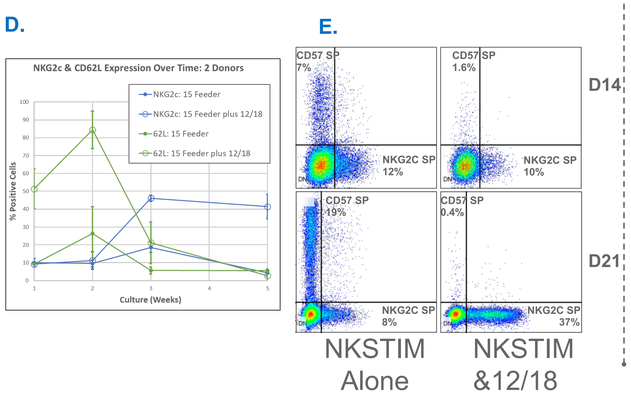


Figure 4: Cytokine Production IL-12 & 18 titrations on NKSTIM expanded NK drive potent IFNγ accumulation over 3 days. Peripheral blood NK were expanded 7 days and plated with varying concentrations of IL-12 or 18 alone or in combination. Activation with NKSTIM plus 12-18 synergizes to drive high levels of IFNγ accumulation.

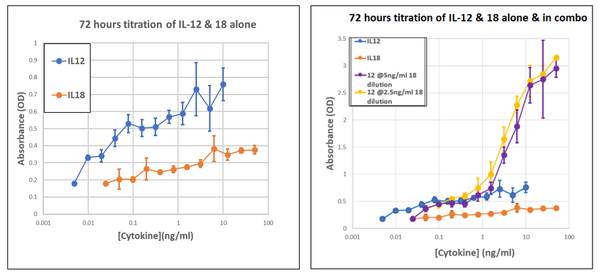


Figure 5. Nkarta CD19 based chimera (NKG2D.aCR)

Figure 6: Genetic Modification & Cytotoxicity 12-18 plus NKSTIM expansion drives comparable CD19 CAR expression (A) over 3 weeks and significantly more potent CAR-CD19 driven cytotoxicity against NALM-6 (B & C). 3 donors were expanded with or without 12-18 plus NKSTIM and genetically modified with a retroviral CD19-CAR-mbIL-15 construct. Cells were assayed for cytotoxicity 7 days post transduction for 4 days against NALM-6 and both unmodified and CAR expressing cells showed significantly greater in vitro cytotoxicity over 4 days (B) and at the final time point at day4 (C).

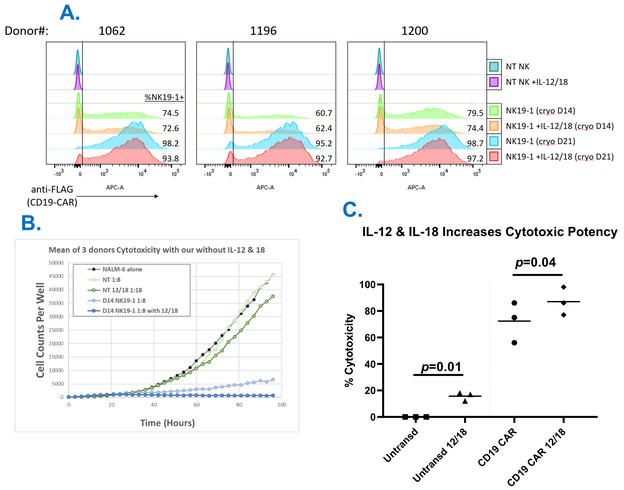
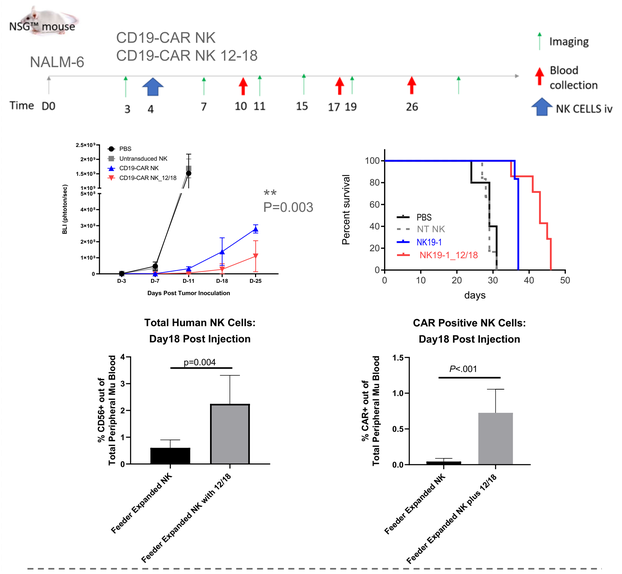


Figure 7: Preclinical Model Feeder stimulated NK with IL-12 & IL-18 drives significantly greater activity and persistence in a CD19 CAR-NK NALM-6 xenograft tumor model. 1x10^5 NALM-6 were injected at Day 0 and 2x10^7 CD19-CAR NK with or without 12-18 were inoculated 4 days later,



Conclusions

IL-12 and IL-18 in combination with Nkarta's NKSTIM feeder NK expansion platform significantly augments NK phenotype and function.

- **Addition of IL-12 & IL-18 drives significantly greater in vitro expansion.**
- **NK innate and CD19-CAR driven cytotoxicity significantly improves with addition of IL-12 & 18 to Nkarta's expansion platform**
- **Exposure to IL-12 and IL-18 in Nkarta's platform drives greater persistence and improved potency in a NALM-6 preclinical model**

References

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2. Chihaya I., et al. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. Blood. (2005)
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4. Romee R., et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. Sci Trans Med. (2016)

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