Co-expression of a CD19-OX40-CD3ζ CAR with membrane bound IL-15 enhances natural killer cell function

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Introduction

Chimeric antigen receptors (CARs) have been used successfully to retarget T cells in patients with hematologic malignancies. Natural killer (NK) cells offer an alternative to T cells for cellular immunotherapy and are suitable for allogeneic use as they are not HLA-restricted and patients that receive NK cell treatment do not develop graft-versus-host disease (GVHD). Therefore, NK cells can provide an attractive alternative for 'off-the-shelf' cellular therapy. Here, we investigated multiple approaches to engineer and enhance CD19 CAR activity in NK cells. It has been previously reported that co-stimulatory domains play an important role in proliferation, efficacy and persistence of CAR T cells both *in vitro* and *in vivo*. To understand how CAR structure effects the functional behavior of NK cells, we assessed the capability of various co-stimulatory signaling domains, including CD28, OX40, CD28-41BB and others, all coupled to CD3ζ, to enhance CD19 CAR signaling and cytotoxicity in NK cells. We demonstrate that NK activity and persistence can be elevated by simultaneous expression of chimeric constructs directing the expression of a CD19 CAR and a membrane-bound form of IL-15 (mbIL-15)[1].

Methods

NK cells were generated by co-culture of peripheral blood mononuclear cells (PBMC) with genetically modified irradiated K562 feeder cells[2]. NK cells were transduced at a MOI of 1-2 with a γ-retrovirus encoding a CD19 CAR and mbIL-15. NK expansion and CAR expression were evaluated by flow cytometry. *In vitro* cytotoxicity of transduced NK cells was measured using both flow cytometry and the IncuCyte S3 live cell analysis system. The *in vivo* activity of engineered NK cells was further assessed in a xenograft tumor model in NSG mice, using a Nalm6 leukemia cell line.

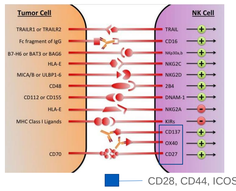

Figure 1. Natural Killer (NK) cells and Co-stimulatory signal on NK cell biology.

Chester et al, Front.Immunol., 2015[3]

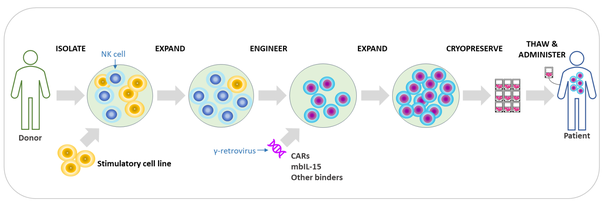

Figure 2. Nkarta Natural Killer((NK) cell platform showing ex vivo natural killer (NK) cell activation and expansion procedures

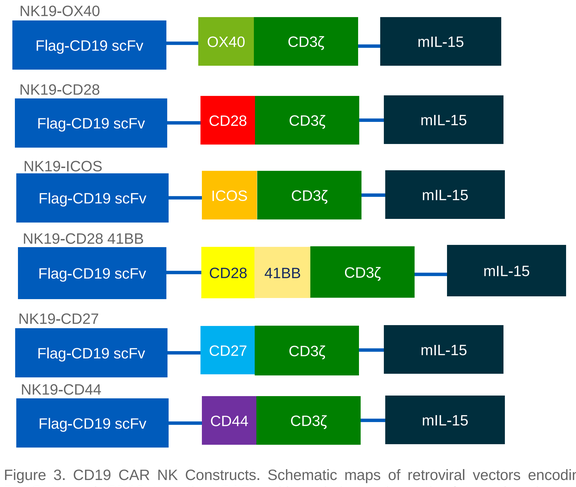

Figure 3. CD19 CAR NK Constructs. Schematic maps of retroviral vectors encoding FLAG tagged CD19 targeting CARs with variations of co-stimulatory domains and transmembrane domains co-expressed with membrane bound IL15.

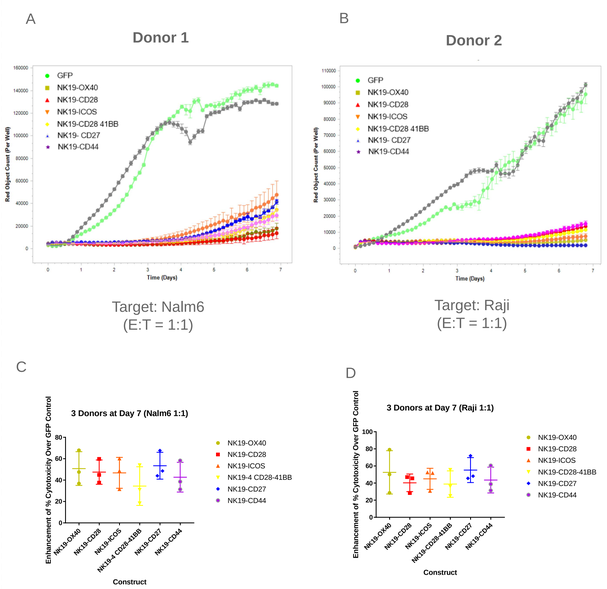

Figure 5. Elevated CD19 CAR NK cytotoxicity. (A) and (B) Percentage of cytotoxicity of mock GFP and CD19 CAR variant transduced NK cells against B cell precursor leukemia cell lines, Nalm6 and Raji via Incucyte S3 live cell analysis system. (C) and (D) Percent cytotoxicity of CD19 CAR variant transduced NK cells over GFP control against Nalm6 and Raji cell lines 7 days post transduction.

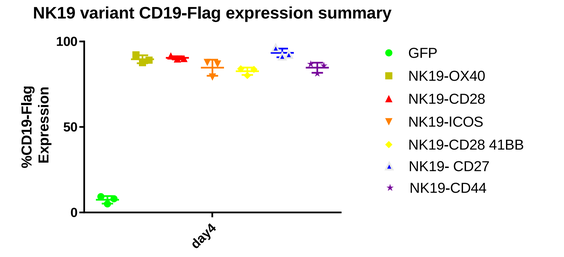

Figure 4. CD19 CAR expression of NK CAR constructs. FLAG-tagged CD19 CAR expression in expanded NK cells from 3 donors transduced with a retrovirus containing GFP only or encoding a CD19 CAR variant.

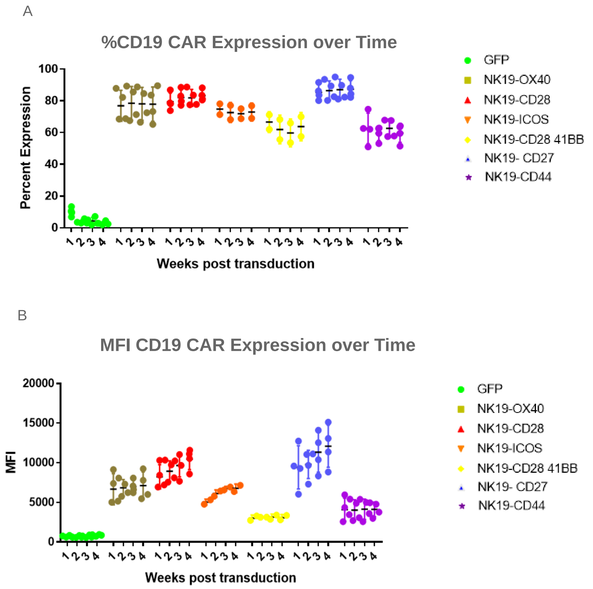

Figure 6. Expression of CD19 co-stimulatory domain variants is stable at least 4 weeks post transduction. (A) Percent FLAG tagged expression in expanded NK cells transduced with retrovirus containing GFP or encoding CD19 CAR NK variants up to 4 weeks post transduction. (B) Mean fluorescence intensity (MFI) of FLAG CD19 CAR expression in transduced NK cells and MFI measured up to 4 weeks post transduction.

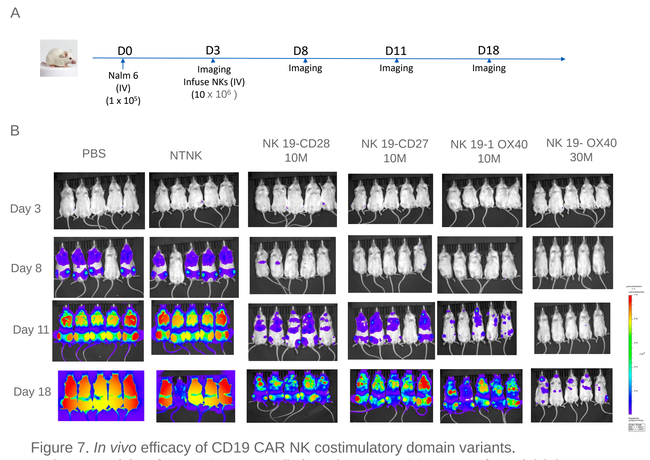

Figure 7. *In vivo* efficacy of CD19 CAR NK costimulatory domain variants. Antitumor activity of CD19 CAR NK cells in Nalm6 ALL NSG xenograft model (A) Schematic timeline of the ALL NSG xenograft model. (B) Luciferase labeled Nalm6 cells ($1x10^5$) were injected intravenously in NSG mice on Day 0. On Day 3, the following groups received $1x10^7$ NK cells: control mice (n=5, non-transduced NK, NTNK), NK19-OX40 (n=5, transduced with NK19-1), NK19-CD28 (n=5, transduced with NK19-CD28) and NK19-CD27 (n=5, transduced with NK19-CD27). Five mice also received a single intravenous injection of $3x10^7$ (n=5, transduced with NK19-OX40) NK cells. Photoluminescence signals were measured at weekly intervals by IVIS Spectrum In Vivo Imaging system.

Results:

CD19 CAR constructs containing various co-stimulatory domains were all expressed in expanded and transduced NK cells. CAR expression in multiple donor NK cells was typically between 60-90%. In cytotoxicity assays, CD19 CAR constructs containing the co-stimulatory domains of OX40, CD28 or CD27 generally exhibited the greatest cytotoxic activity against Nalm6 and Raji tumor cell lines *in vitro*. High expression of these three CD19 CAR constructs was also maintained over the course of 4 weeks in addition to supporting sustained NK cell proliferation and viability, indicating an increase in NK cell persistence. In comparing these constructs in an *in vivo* Nalm6 tumor model, all three constructs demonstrated greater anti-tumor activity relative to unmodified NK cells. The CD19 CAR construct containing the OX40 co-stimulatory domain showed moderately enhanced efficacy than the CD28 or CD27 variants *in vivo*.

Conclusion

Transduction of NK cells with CD19-OX40-CD3ζ CAR and mbIL15 increase their cytotoxic activity and persistence. Based on these data, further development of NK CD19 CAR for clinical use will be pursued.

References

1. Song et al, Blood, 2012 Jan 19
2. Imai et al, Leukemia, 2004 Apr; 18 (4):676-84
3. Chester et al, Fron. Immunol., 2015; 6:601

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