Co-expression of a chimeric NKG2D receptor with membrane bound IL-15 enhances natural killer cell function and long term persistence in vitro and in vivo

Luxuan Buren, PhD*; Chao Guo, PhD*; DaoFeng Liu, PhD*; Sasha Lazetic*, James Trager, PhD*†
*Nkarta Inc, South San Francisco, CA, 94080, USA

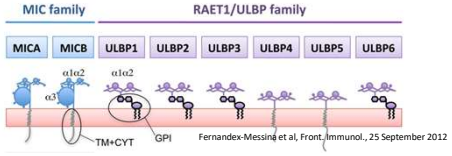
Abstract

Background: Chimeric antigen receptors 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, highly active and suitable for allogeneic use as they are not HLA-restricted and do not cause GVHD. A goal of NK cell engineering is to improve their *in vivo* persistence and recognition of cancer cells. Ligands of the natural killer group 2D (NKG2D) receptor are broadly expressed in solid tumor and hematological malignancies, making NKG2D an attractive target for NK cell engineering. This work was undertaken to demonstrate that NK activity and persistence can be elevated by simultaneous expression of chimeric constructs directing the expression of an activating NKG2D receptor (aNKr) and a membrane-bound form of IL-15 (mbIL-15).

Methods and Materials: NK cells were generated by co-culture of peripheral blood mononuclear cells (PBMC) with genetically modified irradiated K562 feeder cells. NK cells were transduced at a multiplicity of infection (MOI) of 1-2 with a bicistronic virus encoding an NKG2D aNKr and mbIL-15. NK expansion and NKG2D aNKr expression were evaluated using flow cytometry to detect the $CD56^+$ $CD3^-$ cell population and the elevation of NKG2D expression over endogenous levels. *In vitro* cytotoxicity of transduced NK cells was measured using flow cytometry. The *in vivo* activity of engineered NK cells was further assessed in a xenograft tumor model, using the osteosarcoma cell line U2OS engineered to express luciferase, with tumor growth measured using bioluminescence in NSG mice.

Results: NK cells were expanded for 7 days (40 to >100 fold) prior to transduction. Transduction increased NKG2D expression in NK cells by up to 30-fold (>70% transduction efficiency, N=8 donors) relative to the GFP control cells. NKG2D aNKr-mbIL15 NK cells could be maintained for up to 6 weeks in low IL-2 culture. aNKr-mbIL-15 expression significantly elevated cytotoxicity in NK cells against multiple tumor cell lines, inducing cell death of > 60% of target cells within 4 hours at a 1:1 effector to target (E:T) ratio. One infusion of transduced NK cells tumor-bearing NSG mice resulted in long-term anti-tumor responses. Moreover, co-expression of mbIL-15 significantly delayed tumor growth relative to that observed in cells expressing only the NKG2D aNKr.

Figure 1. Enhancing tumor recognition
NKG2D is a dominating activating receptor natively expressed on NK cells



NKG2D plays a major role in mediating anti-tumor immunity through the recognition of ligands on the tumor cell surface: NKG2D ligand upregulation has been demonstrated in leukemia, bladder, breast, colorectal, myeloma, ovarian & pancreatic cancers. NKG2D binds to 8 characterized ligands: MICA, MICB, ULBP1-6. Ligands are upregulated when cells are infected / stressed NKG2D ligands are upregulated by a variety of cancer therapies.

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

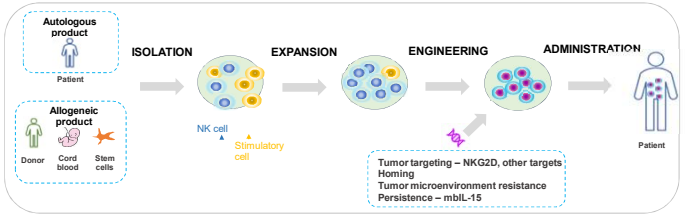


Figure 4. NKG2D NK receptor (aNKr) design

Schematic of retroviral vector encoding NKG2D-4-1BB-CD3ζand membrane bound IL-15

Figure 5. Elevated NKG2D aNKr expression and NK cytoxicity can persist weeks after transduction
(A) Mean fluorescence intensity (MFI) of NKG2D expression in expanded NK cells from 4 donors transduced with a retroviral vector containing GFP only or a vector encoding a NKG2D aNKr construct.
(B) percentage of cytotoxicity of mock GFP and NKG2D transduced NK cells against the acute lymphocytic leukemia (ALL) REH cell line 4 weeks post-transduction
(C) and (D) percentage of cytotoxicity of NKG2D transduced NK cells over GFP control against REH and the acute myelogenous leukemia (AML) HL60 cell line 14 days post-transduction

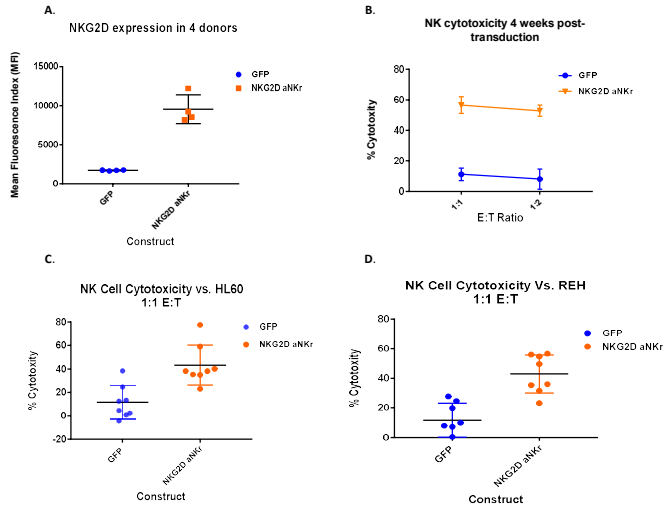
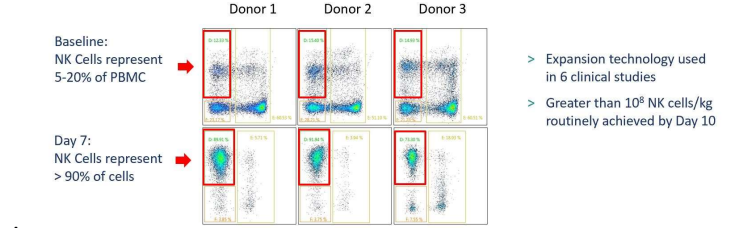


Figure 3. Nkarta NK cell platform: clinically validated expansion



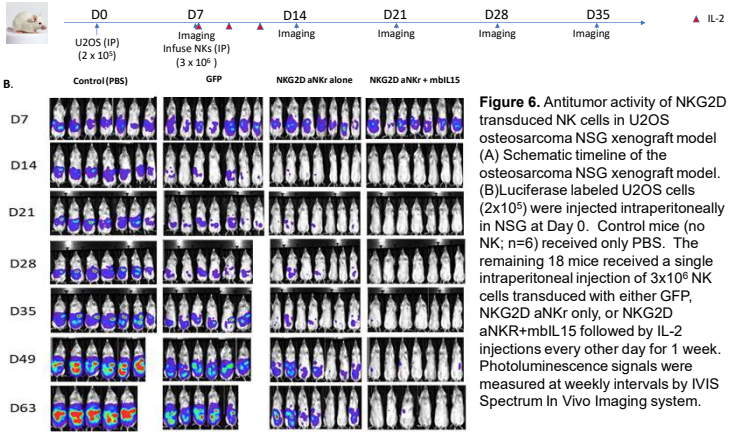


Figure 6. Antitumor activity of NKG2D transduced NK cells in U2OS osteosarcoma NSG xenograft model (A) Schematic timeline of the osteosarcoma NSG xenograft model. (B)Luciferase labeled U2OS cells ($2x10^5$) were injected intraperitoneally in NSG at Day 0. Control mice (no NK; n=6) received only PBS. The remaining 18 mice received a single intraperitoneal injection of $3x10^6$ NK cells transduced with either GFP, NKG2D aNKr only, or NKG2D aNKR+mbIL15 followed by IL-2 injections every other day for 1 week. Photoluminescence signals were measured at weekly intervals by IVIS Spectrum In Vivo Imaging system.

Conclusions

- Nkarta is developing next generation cellular immunotherapies for hematological malignancies and solid tumors.
- Nkarta's platform enables robust expansion of modified NK cells and substantial tumor killing.
- Co-expression of mbIL-15 and an NKG2D aNKr improves the persistence and potency of NK cells both in vitro and in vivo.
- NKG2D aNKr provides multiple opportunities to pair with existing standards of care in hematological malignancies and solid tumor settings.

†Contact

James Trager, PhD
Nkarta Inc
Email: jtrager@nkartatx.com
Website: www.nkartatx.com

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