NK Cells Engineered to Express a Bispecific CD123/NKG2D Chimeric Antigen Receptor (CAR) and IL-15 As Off-the-Shelf Therapy for Acute Myeloid Leukemia

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nkarta THERAPEUTICS

Background

Natural killer (NK) cells have an emerging role in cellular immunotherapy for patients with acute myeloid leukemia (AML). NK cells are highly active and suitable for allogeneic use as they are not HLA-restricted and do not cause GVHD. We sought to demonstrate that engineering of NK cells can improve their ability to recognize and kill AML cells and control tumor burden. Natural killer group 2D (NKG2D) is a transmembrane protein belonging to the CD94/NKG2 family of C-type lectin-like receptors. Ligands of NKG2D (NKG2D-L) are highly expressed in AML tissues but weakly on healthy tissues, making NKG2D an attractive target for NK cell engineering. The interleukin-3 receptor alpha chain (CD123) has also been identified as a potential immunotherapeutic target for high risk AML patients due to its overexpression in AML blasts and leukemia stem cells compared with normal hematopoietic stem cells. We therefore developed a CD123-NKG2D tandem CAR construct that contains human CD123-specific single-chain variable fragment (scFv) and NKG2D. We co-expressed this chimera in NK cells with membrane-bound IL-15 to support prolonged cell survival and proliferation.

Results

Retroviral transduction of NK cells with tandem CAR (n=6) resulted in 68.4% and 37.8% median CD123 and NKG2D CAR positivity respectively when measured 14 days post-transduction (Figure 4). In a 4 hour cytotoxicity assay, the CD123-NKG2D tandem CAR transduced NK cells were no more effective than NKG2D-CAR alone in killing the CD123^{low} AML cell line HL60, but showed better cytotoxicity than Mock (GFP)-transduced NK cells, indicating that the tandem CAR can trigger robust cytotoxicity through NKG2D (figure 5). CD123-NKG2D tandem CAR NK cells were effective at killing the CD123^{+} THP-1 AML cell line (figure 6a), exhibiting significantly greater cytotoxicity than Mock (GFP)-transduced NK cells, or NK cells expressing either CD123-CAR (Figure 6a); in a kinetically resolved cytotoxicity assay, the tandem CAR was more effective than the NKG2D CAR against THP-1 (figure 6b). In addition, the cytotoxicity of CD123-NKG2D tandem CAR transduced NK cells was efficiently blocked by either CD123 peptides (Figure 7) or anti-NKG2D antibodies (Figure 5b), further demonstrating the ability of the tandem receptor to be triggered by both component binding moieties. Studies to test the *in vivo* anti-tumor efficacy of CD123-NKG2D tandem CAR transduced NK cells in an NSG mouse model of THP-1 are under way.

Figure 1. Nkarta Natural Killer((NK) cell platform showing ex vivo natural killer (NK) cell activation and expansion procedures

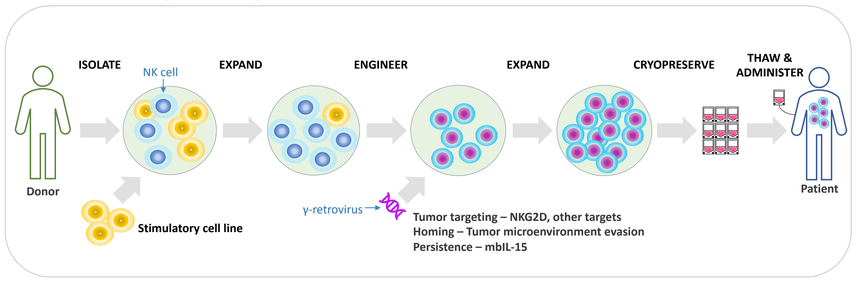
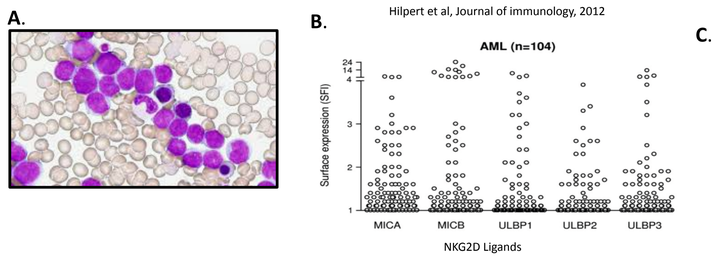


Figure 2. Targeting AML with CAR-NK cells and CD123-NKG2D-CAR design

(A) Bone marrow aspirate showing AML (B) SFI levels of the various NKG2DL obtained with each single patient of the different leukemia entities. (C) Primary patient AML samples express CD123. (D) CD123-NKG2D-CAR design



Schematic of retroviral vector encoding CD123-NKG2D-CD3ζ and membrane bound IL-15

Figure 3. NKG2D-CAR (NKX101) shows dose-dependent efficacy in an AML model

Antitumor activity of NKG2D transduced NK cells in THP-1 AML NSG xenograft model (A) Schematic timeline of the AML NSG xenograft model. (B) Luciferase labeled THP-1 cells ($2x10^5$) were injected intravenously in NSG at Day 0. Control mice (GFP; n=5) received $5x10^6$ NK cells. The remaining 15 mice received a single intravenous injection of $5x10^6$, $10x10^6$ and $20x10^6$ NK cells, transduced with NKG2D-CAR 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.

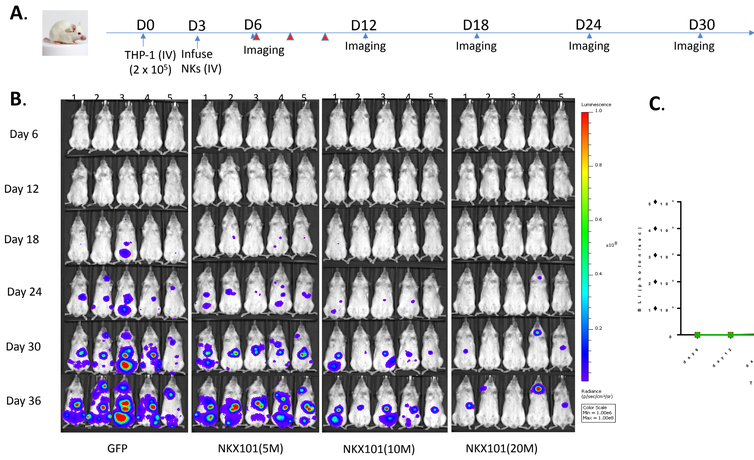


Figure 4. CD123/NKG2D bispecifics can be expressed at high levels in NK cells.

(A) CD123 expression and (B) NKG2D expression in expanded NK cells from 6 donors transduced with a retroviral vector containing GFP only or a vector encoding a CAR construct.

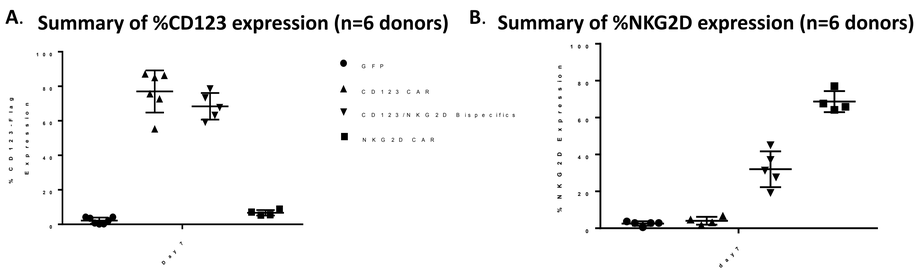


Figure 5. Gain of function and loss of function studies demonstrate that bispecific constructs can fire through NKG2D (A) Results of 4-hour cytotoxicity assays performed by against the CD123^{low} HL60 cell line. (B) A significant difference ($P < 0.05$) between lysis in the presence and absence of NKG2D blockade is indicated by an asterisk.

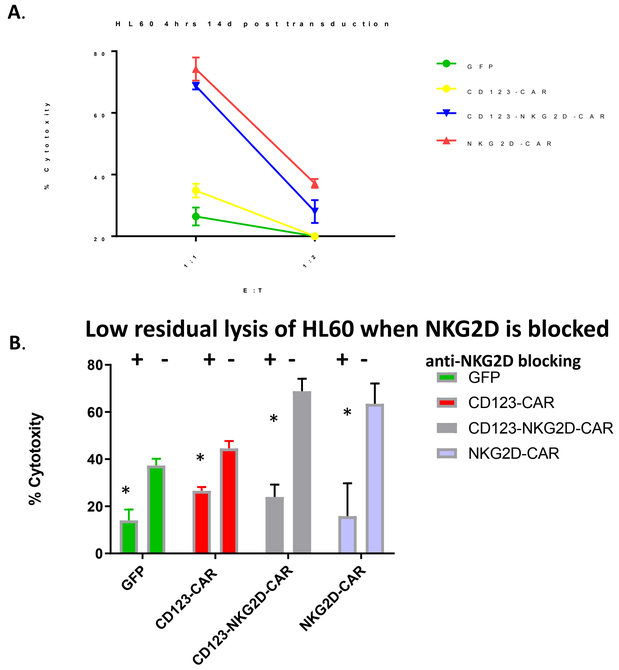


Figure 6. CD123-NKG2D bispecific CAR confers best durable cytotoxicity of NK cells for at least 2 weeks after NK transduction. Cytotoxicity of NK-CARs and GFP-transduced NK cells against the AML cell line 2 weeks post-transduction. (A) 4 hour cytotoxicity assay. (B) *In vitro* cytotoxicity assay was monitored for up to 4 days in culture with IncuCyte® live-cell imaging. E:T ratio is 1:8.

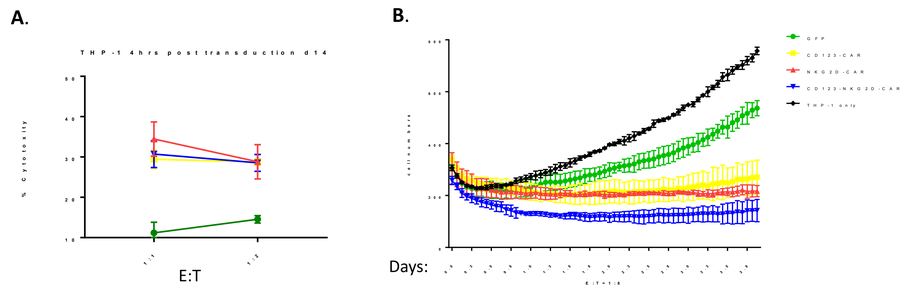
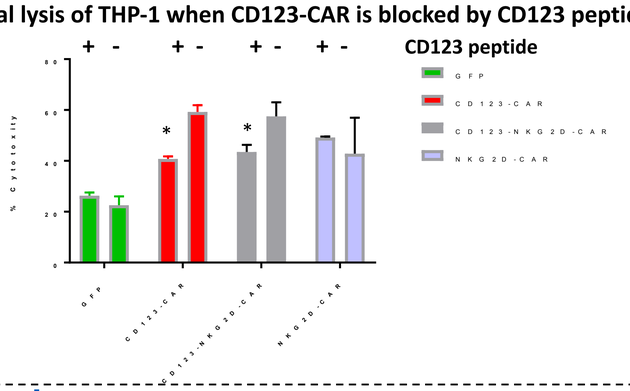


Figure 7. Selective cytotoxicity against CD123high THP-1 line demonstrates that bispecific constructs can fire through CD123. A significant difference ($P < 0.05$) between lysis in the presence and absence of CD123 peptide is indicated by an asterisk.



Conclusion

In summary, our data demonstrate the capacity of CD123-NKG2D tandem CAR NK cells to kill both CD123^{+} and NKG2D-L^{+} targets and support the clinical use of tandem CAR NK cells for patients with AML.

References

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