Expanded and engineered NK cells upregulate expression of activation and survival genes associated with increased cytotoxicity and persistence

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ABSTRACT

Background: Natural Killer (NK) cells expanded using irradiated K562 cells engineered to express membrane-bound IL-15 and 4-1BBL (K562-mbl15-4-1BBL) have been employed in the clinic [1]. Expansion upregulated activating NK cell receptors and increased NK cytotoxicity against tumor cells. Expanded NK cells can be engineered to express chimeric receptors to further improve their cytotoxicity. Chimeric receptors commonly incorporate an antigen binding domain, a costimulatory domain (i.e. 41BB, CD28) and a CD3ε signaling domain. Receptor structure can bias engineered cells to chronic activation and exhaustion [2,3] and can regulate metabolic pathways that influence cell phenotype and function. Characterization of NK cells expressing activating Chimeric Receptors (aCRs) may enable assessment of their clinical potency. We therefore evaluated the phenotypic characteristics of expanded and engineered NK cells and investigated signatures of activation or exhaustion upon antigen stimulation.

Material and Methods: NK cells from peripheral blood mononuclear cells were expanded on irradiated K562-mbl15-4-1BBL cells transduced with NKG2D based chimeras (NKG2D.aCR) and mbl15. The expression of relevant receptors on expanded and transduced NK cells was assessed by flow cytometry. To evaluate the impact of tonic aCR chimeric receptor signaling versus antigen-driven stimulation on NK activation, we analyzed gene expression after stimulation with either isotype or anti-NKG2D coated beads using a Nanostring immune function panel.

Results: Ex vivo expansion generates potent, activated NK cells with high expression of NKG2D, Nkp30, Nkp44, Nkp46, CD69, CD25, and DNAM-1 activation markers and decreased expression of KLRG1 and CD158b inhibitory receptors. Expanded NK cells also upregulated TIGIT and TIM-3 expression, markers associated with functional exhaustion. However, expanded NK cells retained enhanced tumor cytotoxicity and cytokine production. NKG2D.aCR construct expression induced tonic signaling in engineered NK cells characterized by the upregulation of genes involved in cell survival (Bcl-2), cell activation (CD25) and cell mediated cytotoxicity (TRAIL). NKG2D engagement further enhanced expression of anti-apoptotic genes, and genes encoding cytokines and chemokines required to control cell cytotoxicity (TNF-α, TNF-α, TNF-α and recruitment to tumor sites (XCL-1, CCL-3, CCL-4). Lastly, NKG2D stimulation of engineered cells led to a significant increase in the expression of TNF family members (41BB, Oxl40L, Oxl40, TRAIL). Increased 41BB, Oxl40L and TRAIL expression was confirmed by flow cytometry and correlated with enhanced cytotoxicity. Our data suggest that, upon stimulation, NKG2D.aCR cells are highly activated with greater longevity. Moreover, our data allow the identification of biomarker candidates that reflect NK.aCR potency.

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

Figure 2: A balance of signals delivered by activating and inhibitory receptors regulates the recognition of healthy and tumor cells by NK cells.

Figure 3: Importance of the NKG2D pathway in immune surveillance of cancer.
A. Human NKG2D complex

B. Schematic diagram showing all human NKG2D ligands identified to date

Figure 4: Characterization of K562-mbl15-41BBL- expanded NK cells on Day7 and Day12 post expansion

Figure 5: Nkarta NKG2D based chimeras (NKG2D.aCR)

Figure 6: Co-expression of mbl1-15 and NKG2D.aCR induces upregulation of genes associated with cell survival and activation

Figure 7: Increased activation and cytotoxicity of engineered NK cells upon NKG2D stimulation

CONCLUSIONS

- Nkarta’s expansion and transduction platform generates highly activated NKG2D aCR engineered NK cells with greater longevity.
- Investigating NKG2D.aCR gene signatures allows the identification of biomarker candidates that reflect NKG2D.aCR potency.
- NKG2D.aCR provides a robust approach to cancer therapy that can be combined with existing therapies, and has potential for allogeneic or autologous applications to ultimately improve outcomes for patients in hematological malignancies and solid tumor settings.

REFERENCES


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