Expanded and engineered NK cells for the treatment of hepatocellular carcinoma

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nkarta THERAPEUTICS

Background

Natural killer (NK) cells offer an alternative to T cells for cellular immunotherapy, as they are not HLA-restricted, and are therefore suitable for allogeneic off-the-shelf use. Expanded NK cells can be engineered to express chimeric receptors to further improve their cytotoxicity against both hematopoietic and solid tumors. In healthy humans, a high proportion of intrahepatic lymphocytes are NK cells[1], and for cases of hepatocellular carcinoma (HCC), infiltration of $CD56^+$ NK cells positively correlates with cell apoptosis and patient survival[2]. Multiple ligands of the natural killer group 2D (NKG2D) receptor are highly expressed in HCC tissues, but at low levels on healthy tissues[3], making NKG2D an attractive candidate for NK cell engineering to target HCC. We aimed to demonstrate that engineering of NK cells with an NKG2D chimeric receptor (NKX101) can improve their cytotoxicity against HCC cells *in vitro*, enhance pro-inflammatory cytokine response, and control tumor burden in an *in vivo* NSG mouse model.

Methods

NK cells from peripheral blood mononuclear cells were expanded using co-culture with irradiated K562-mbIL15-41BBL stimulatory cells. NKX101 cells were generated by transduction with a bicistronic virus encoding NKG2D, an intracellular OX40 costimulatory domain, CD3ζ signaling domain, and membrane-bound IL-15, which supports prolonged cell survival and proliferation. NKX101 pro-inflammatory cytokine release in response to 24 hr co-culture with HCC cell lines at a 1:1 ratio was analyzed by Luminex. The *in vitro* cytotoxicity of NKX101 cells at 4, 24 and 48 hr endpoints was measured using a panel of HCC cell lines engineered to express luciferase. *In vivo* activity of NKX101 cells was evaluated using an NSG mouse xenograft tumor model in which intraperitoneal (IP) injection of 4 x 10^6 luciferase expressing SNU449 HCC cells was followed one week later with IP administration of 3 x 10^6 NKX101 or unmodified NK cells (NT NK), and tumor growth was measured using IVIS bioluminescence imaging. Combination with a sub-EC50 concentration of the kinase inhibitor sorafenib, an approved agent for HCC, was tested for additive cytotoxic effect at low E:T ratios of NKX101 *in vitro* at 48 hrs. Expression of NKG2D ligands was assessed by flow cytometry and analyzed for linear correlation with NKX101 cytotoxicity across a panel of HCC cell lines.

Results

We demonstrate that NKX101 has dramatically improved cytotoxicity versus NT NK cells against a panel of HCC cell lines in vitro. Co-culture of NKX101 with HCC cell lines was shown to enhance pro-inflammatory cytokine release. In an *in vivo* NSG mouse xenograft HCC model, NKX101 provided complete tumor clearance in 4/5 mice, in contrast to stable control by NT NKs. Combination with the kinase inhibitor sorafenib provided additive *in vitro* cytotoxicity with NKX101. High expression of NKG2D ligands was observed by flow cytometry, but no individual ligand demonstrated a correlation with NKX101 cytotoxicity.

Figure 1: Nkarta NK cell platform

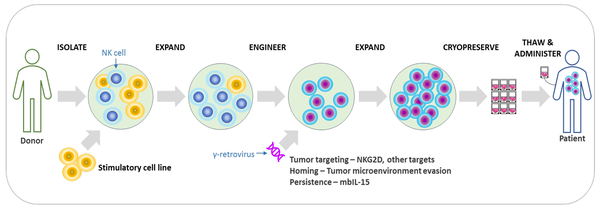

Figure 1: *Nkarta Natural Killer (NK) cell platform showing ex vivo cell expansion, activation and genetic modification procedures*

Figure 2: NKG2D and ligand family

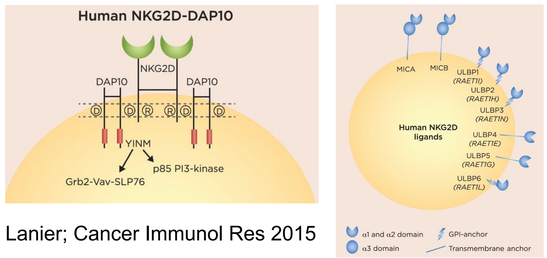

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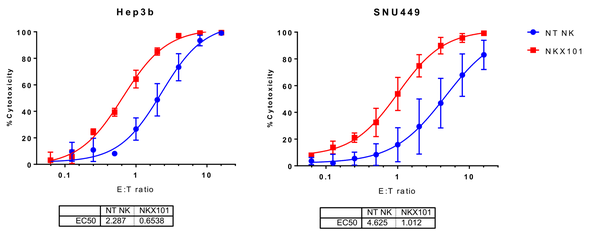
Figure 2: *NKG2D is an activating receptor expressed on NK cells and subsets of T cells that binds to a family of ligands which are upregulated on the surface of cells undergoing stress, excessive proliferation, viral infection, or transformation. This results in NK-mediated cytotoxicity of these abnormal cells and the secretion of cytokines and chemokines to enhance the immune response.*

Figure 3: NKX101 chimeric antigen receptor (CAR)

Figure 3: *NKX101 were generated by transduction of NK cells with a viral construct encoding a CAR including the extracellular domain of NKG2D, hinge and transmembrane regions of CD8, intracellular OX40 costimulatory domain and CD3ζ signaling domain. Membrane-bound IL-15 is co-expressed following a T2A self-cleaving peptide sequence.*

Figure 4: NKX101 enhanced cytotoxicity against HCC



	NT NK	NKX101
EC50	2.287	0.6538

	NT NK	NKX101
EC50	4.625	1.012

Figure 4: *In vitro cytotoxicity of NKX101 cells at 4 hr endpoint measured in 2 model HCC cell lines engineered to express luciferase. Averages shown for NKX101 and unmodified (NT NK) NK cells from 2 donors at day 21 post-expansion with K562-mbIL15-41BBL stimulatory cells. EC50 values calculated using Prism 4-parameter non-linear regression analysis.*

Figure 5: NKG2D ligand expression on human HCC

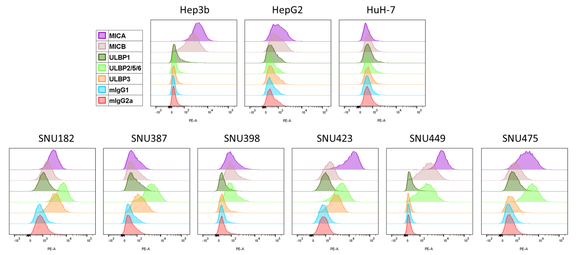


Figure 5: *Expression of NKG2D ligands across a panel of human HCC cell lines by flow cytometry.*

Figure 6: NKX101 cytotoxicity across HCC panel

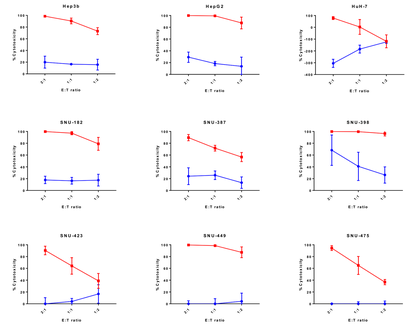


Figure 6: *In vitro cytotoxicity of NKX101 cells at 24 hr endpoint measured using a panel of HCC cell lines engineered to express luciferase. Averages shown for 2 donors at day 30 post-expansion.*

Figure 7: NKX101 anti-tumor efficacy in SNU449 xenograft model

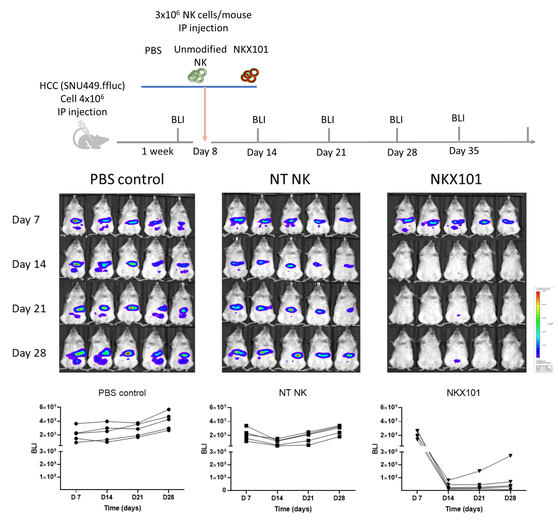


Figure 7: *In vivo activity of NKX101 cells in NSG mouse HCC xenograft tumor model. Intraperitoneal (IP) injection of 4 x 10^6 luciferase expressing SNU449 cells was followed one week later with IP injection of 3 x 10^6 NKX101 or NT NK, and tumor growth was measured using IVIS bioluminescence imaging.*

Figure 8: NKX101 sensitivity vs. NKG2DL expression

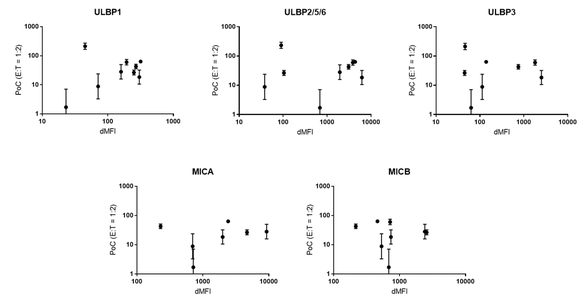


Figure 8: *NKX101 % cytotoxicity across a panel of HCC cell lines at an E:T ratio of 1:2 was analyzed for linear correlation with flow cytometry delta mean fluorescence intensity (dMFI) for individual NKG2D ligands. Values for dMFIs were calculated as target MFI – isotype MFI.*

Figure 9: NKX101 enhanced cytokine response to HCC

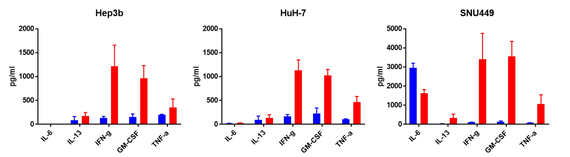


Figure 9: *Luminex analysis of NKX101 pro-inflammatory cytokine release in response to 24 hr co-culture with HCC cell lines at a 1:1 E:T ratio. Averages shown for 2 donors at day 30 post-expansion.*

Figure 10: NKX101 combination with sorafenib

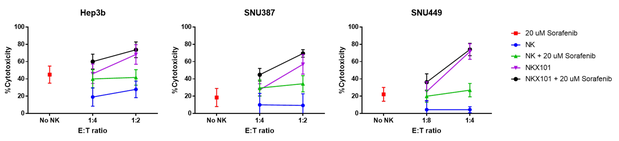


Figure 10: *Combination with a sub-EC50 concentration of the kinase inhibitor sorafenib, an approved treatment for HCC, was tested for additive cytotoxic effect at low E:T ratios of NKX101 or NT NK cells in vitro at 48 hrs. Averages shown for 2 donors at day 30 post-expansion.*

Conclusions

NKX101 demonstrates improved cytokine release and cytotoxicity against multiple HCC cell lines *in vitro,* shows clearance of tumor burden in an *in vivo* HCC xenograft model, and has potential for combination therapy with current HCC approved treatment sorafenib.

[1]Tian, Z et al. Natural killer cells in liver disease. Hepatology 2013, 57, 1654–1662
[2] Chew, V et al. Inflammatory tumor microenvironment is associated with superior survival in hepatocellular carcinoma patients. J Hepatol. 2010 52(3):370-9
[3] Kamimura H. Et al. Reduced NKG2D ligand expression in hepatocellular carcinoma correlates with early recurrence. J Hepatol. 2012 Feb;56(2):381-8

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