HER2-specific highly scalable CAR NK cell (anti-HER2-CAR SNK02) exhibits a significantly enhanced antitumor activity against HER2-expressing tumors as an off-the-shelf allogeneic immune cell therapy.

[1]Yong-hee Rhee, [1]Hee-Sung Chae, [1]Minji Kim, [1]Seo-Gu Kang, [1]Joungmin Lee, [2]Yoon Mi Kang, [1]Jae Seob Jung, [2]Paul Y. Song, [1]Yong Man Kim, [1]Minchan Gil

[1]NKMAX Co., Ltd., Seongnam, Republic of Korea; [2]NKGen Biotech, Inc., Santa Ana, CA, USA

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BACKGROUND

Although anti-HER2 monoclonal antibodies (e.g., trastuzumab) are currently recognized as effective therapeutics in patients with HER2-overexpressing tumors, a significant number of patients demonstrate resistance to this treatment. Genetic modification of NK cells to express a HER2-specific chimeric antigen receptor (CAR) is likely a better therapeutic approach against HER2-positive solid tumors. SNK02 is a highly scalable, off-the-shelf allogeneic NK cell product in clinical development with high purity, cytotoxicity, and tumor site migration potential. In this study, we aimed to develop allogeneic anti-HER2-CAR NK cells (CAR-SNK02) using the SNK02 manufacturing platform and to test their antitumor activity against HER2-expressing cancers.

METHODS

The CAR-SNK02 cells were generated by ex vivo feeder-stimulated expansion of peripheral blood NK cells along with transduction of retrovirus expressing anti-HER2 CAR and membrane-bound IL-15 using the SNK02 manufacturing platform. The CAR-SNK02 cells were tested for their CAR expression, cytotoxicity, degranulation, cytokine production, and stability of cryopreserved form as well as for their antitumor activity in a xenograft mouse model of cancer in response to HER2-positive cancer cells.

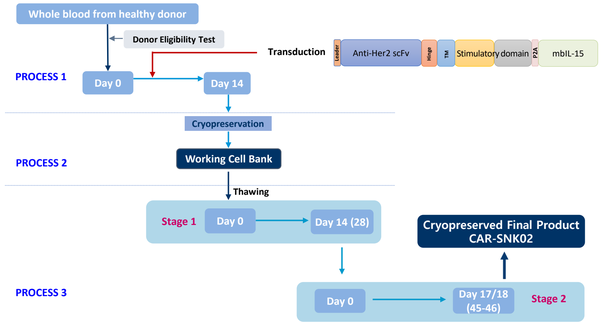


Figure 1. The production scheme of allogeneic CAR-SNK02.

RESULTS

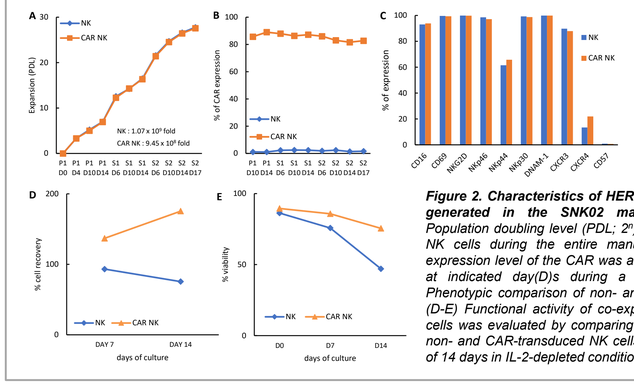


Figure 2. Characteristics of HER2 CAR-transduced NK cells generated in the SNK02 manufacturing platform. *(A) Population doubling level (PDL; 2^n)) of non- or CAR-transduced NK cells during the entire manufacturing period. (B) The expression level of the CAR was assessed using flow cytometry at indicated day(D)s during a 45-day culture period. (C) Phenotypic comparison of non- and CAR-transduced NK cells. (D-E) Functional activity of co-expressed mbIL-15 in CAR NK cells was evaluated by comparing cell recovery and viability of non- and CAR-transduced NK cells after their additional culture of 14 days in IL-2-depleted conditions.*

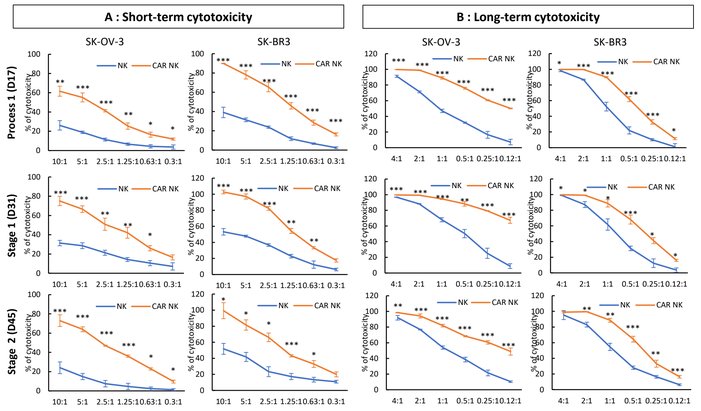


Figure 3. Comparison of cytotoxic activity between non- and CAR-transduced NK cells against HER2-positive cancer cells. *Short-term cytotoxicity (2hr, A) and long-term tumor suppressive activity (96hr, B) of non-transduced (NK) and CAR-transduced (CAR NK) NK cells against SK-OV-3 or SK-BR-3 were assessed. Significant statistical difference from cytotoxicity of non-transduced NK was shown at each E:T ratio (*p<0.05, **p<0.01, and ***p<0.005).*

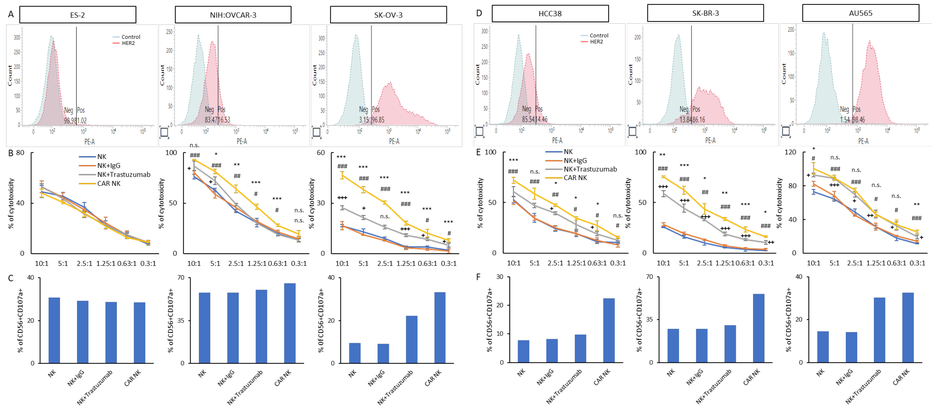
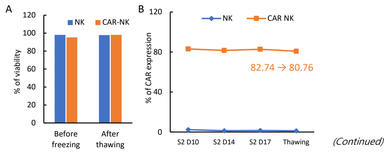


Figure 4. Comparison of cytotoxicity and the level of degranulation between CAR-transduced NK cells and NK cells treated with trastuzumab in response to HER2-positive ovarian cancer cells (A-C) and breast cancer cells (D-F). *Statistical significance was denoted as n.s. (not significant), #/+/*p<0.05 (#: CAR NK vs. NK, +: NK + trastuzumab vs. NK, *: CAR NK vs. NK), ##/++/**p<0.01, and ###/+++/***p<0.005.*



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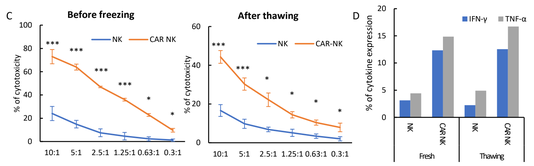

Figure 5. Viability, CAR expression level, and functional activity of CAR-transduced NK cells after cryopreservation. *(A-B) Viability and CAR expression level of non-transduced (NK) and CAR-transduced (CAR NK) NK cells before freezing and after thawing. (C) Cytotoxic activity of fresh (Before freezing) and cryopreserved (After thawing) NK cells against HER2-positive SK-OV-3 cells for 2 hours. Significant statistical difference from cytotoxicity of non-transduced NK was shown at each E:T ratio (*p<0.05, **p<0.01, and ***p<0.005). (D) Expression levels of IFN-γ and TNF-α in CAR-NK cells in response to SK-OV-3 cells.*

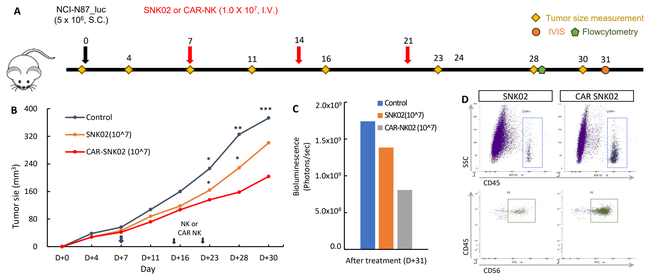

Figure 6. In vivo cytotoxic activity of CAR-SNK02 cells in a gastric cancer xenograft model. *(A) Experimental outline for the animal study. Three groups of NSG mice (5 mice per group) received s.c. injection of NCI-N87-Luc cancer cells (Day 0), followed by i.v. injection of PBS, SNK02 cells, or CAR SNK02 cells, as indicated. (B) Quantification of the tumor size on the indicated day. All results are mean ± SEM. Statistical significance was indicated by *<0.05, *<0.01, and ***p<0.005 from CAR SNK02. (C) Bioluminescent intensity in region of interest (ROI) at D+31 post-tumor inoculation. (D) Representative flow cytometry staining profiles of hCD45 gating on live cells (upper panel) and hCD56 gating on hCD45+ cells (lower panel) of mice blood infused with SNK02 or CAR SNK02 cells.*

CONCLUSIONS

- The CAR-SNK02 cells underwent a billion-fold expansion over a 45/46-day culture period, while maintaining persistent expressions of the CAR and functional IL-15, without phenotypic change of NK cells receptors in compared to non-transduced SNK02.
- The expanded CAR-SNK02 cells exhibited potent in vitro cytotoxic and degranulation activities in response to HER2-positive cancer cells. In addition, superior in vivo cytotoxicity of CAR-SNK02 cells was observed in a xenograft model of gastric cancer.
- Importantly, they effectively eliminated cancer cells with low HER2 expression levels, surpassing limitations of trastuzumab-induced antibody-dependent cellular cytotoxicity (ADCC) of NK cells.
- High expandability of the CAR-SNK02 cells, coupled with their enhanced antitumor activity against HER2-expressing cancer cells, highlights their promising potential as an off-the-shelf immune cell therapy.

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