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

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 characterized for their CAR expression, cytotoxicity, degranulation, and cytokine production in response to HER2-positive cancer cells. The stability of CAR expression and cytotoxicity were also examined after freezing and thawing.

Results: The CAR-SNK02 cells underwent a billion-fold expansion over a 45-46 day culture period, through feeder cell and cytokine stimulation, while maintaining persistent high expression of the CAR in over 80% of the cells during expansion. Upon target cell stimulation, the expanded CAR-SNK02 cells exhibited potent anti-cancer activity against HER2-positive cancer cells, as well as increased cytokine production and high levels of degranulation. Moreover, the CAR-SNK02 cells maintained key phenotypic features of NK cells, including high expression of NK cell activating and chemokine receptors without signs of exhaustion,

indicating their preserved functionality. The CAR-SNK02 cells showed enhanced survival under IL-2-deprived conditions, confirming activity of IL-15. Furthermore, the NK cells maintained CAR expression and enhanced cytotoxicity against HE2-expressing cancer cells even after freezing and thawing. Importantly, they effectively eliminated cancer cells with low HER2 expression levels, surpassing limitations of trastuzumab-induced antibody-dependent cellular cytotoxicity (ADCC) of NK cells.

Conclusion: High expandability of the CAR-SNK02 cells, coupled with their enhanced antitumor activity against HER2-expressing cancer cells, even after freezing and thawing, highlights their promising potential as an off-the-shelf immune cell therapy. In addition, the SNK02 platform serves as a valuable foundation for further exploration and advancement of genetically engineered NK cell therapies. We are currently testing the antitumor activity of the CAR-SNK02 cells in a xenograft mouse model of cancer expressing HER2 for future translation into human trials.