SUPPLEMENTAL MATERIALS

Title: The safety and efficacy of SNK01 (autologous natural killer cells) in combination with cytotoxic chemotherapy and/or cetuximab after failure of prior tyrosine kinase inhibitor in non-small cell lung cancer: nonclinical mouse model and phase I/IIa clinical study

Myeong Geun Choi[1,2*], Gun Woo Son[3*], Mi Young Choi[3], Jae Seob Jung[4], Jin Kyung Rho[5], Wonjun Ji[1], Byeong Gon Yoon[3], Jong-Min Jo[3], Yong Man Kim[4], Dae-Hyun Ko[6], Jae Cheol Lee[7†], Chang-Min Choi[1,7†]

[1] Department of Pulmonary and Critical Care Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

[2] Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Mokdong Hospital, College of Medicine, Ewha Womans University, Seoul, Korea

[3] C&SR Inc., Uiwang, Korea

[4] NKMAX Co., Ltd., Seongnam, Korea

[5] Department of Biochemistry and Molecular Biology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

[6] Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

[7] Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

[*] Both authors equally contributed to this study

†**Corresponding Authors:**

Chang-Min Choi, MD, PhD

Department of Pulmonary and Critical Care Medicine, Asan Medical Center,

University of Ulsan College of Medicine, 88, Olympic-ro 43-gil, Songpa-gu,

Seoul 05505, South Korea

Tel.: 82-2-3010-5902

Fax: 82-2-3010-6968

E-mail: ccm@amc.seoul.kr

Jae Cheol Lee, MD, PhD.

Department of Oncology, Asan Medical Center,

University of Ulsan College of Medicine, 88, Olympic-ro 43-gil, Songpa-gu,

Seoul 05505, South Korea

Tel.: 82-2-3010-3208

Fax: 82-2-3010-6968

Email: jclee@amc.seoul.kr

SUPPLEMENTAL METHODS

1. Autologous natural killer (NK) cell isolation and expansion

The manufacturing and testing procedures adopted to produce *ex vivo* expanded NK cells for nonclinical and clinical uses were performed under good manufacturing practice conditions (NKMAX Co., Ltd., Seongnam, Korea). Peripheral blood mononuclear cells (PBMCs) were collected from the peripheral blood of the enrolled patients and then used for NK cell expansions as previously described with some modification. Briefly, the $CD56^+$ cells were isolated from PBMCs using CliniMACS CD56 microbeads (Miltenyi Biotech GmbH, NRW, Germany) according to the manufacturer's instructions. The isolated $CD56^+$ cells were then cultured in RPMI-1640 medium (WELGENE Inc.) supplemented with 10% FBS (Hyclone, Tauranga, New Zealand), 20 μg/mL gentamicin (GIBCO, Grand Island, NY), g-irradiated (100 Gy) KL-1 and LCL feeders, 500 IU/mL interleukin (IL)-2 (PROLEUKIN, Norvatis, Basel, Switzerland), and 50 ng/mL IL-21 (NKMAX Co.).

The growing NK cells were sub-cultured every 3-4 days using fresh RPMI-1640 medium containing IL-2. After 14 days of culture, the cells were harvested and subjected immediately to re-expansion by adding feeder cells in the presence of cytokines. Alternatively, they were cryopreserved for subsequent timely re-expansion as per the treatment schedule. The restimulated NK cells were sub-cultured every 3-4 days and cultured for an additional 17-18 days. The cells were harvested on either day 31 or 32 of total expansion excluding the cryopreservation period, then washed twice with phosphate-buffered saline (PBS; WELGENE Inc.) and once with Hartmann's solution (DAI HAN PHARM., Ansan, Korea), and formulated in 100 mL Hartmann's solution containing 1% human serum albumin (Albumin inj.; GREEN CROSS, Cheongju, Korea) and IL-2 (500 IU/mL) with the cell number of 2×10^9 cells.

The criteria for the release of final NK cell products included the absence of microbial

contamination (bacteria, fungus, virus, and mycoplasma), ≥ 80% viability in a trypan blue exclusion assay, ≥ 50% cytotoxicity against K562 target cells at the effector to target cell (E:T) ratio of 10:1, ≤ 0.5 EU/mL endotoxin level, and immune phenotyping via flow cytometric analysis proving the expression of the NK cell markers ($CD56^+/CD3^-$) (≥ 80%) and the absence of CD14, CD3, and CD20 (≤ 5% each). Only final NK cell products that met all the release criteria were shipped to the clinic at 2-8℃ and administered to the subjects via intravenous injection over a period of 45 ± 15 minute/100 mL (2×10^9 cells). The NK cell products used for animal experiments were prepared as excess from the clinical NK cells mentioned above, and the same criteria for release were applied.

2. Establishment of cell line-derived xenograft (CDX)-humanized mouse model

Human CD34+ transplanted humanized mice

Human umbilical cord blood-derived hematopoietic stem cells (HSCs) were purchased from Lonza Group, Ltd. (Walkersville, MD, USA). Four to five weeks old of fourteen NOD.Cg-$Prkdc^{scid}IL2rg^{tmlWjl}$/SzJ (NSG) mice were preconditioned with busulfan. The liquid busulfan was intraperitoneally (i.p.) injected into NSG mice (20mg/kg body weight) 24-48 hours prior to HSC transplantation. 24 and 48 hours later, 1×10^6 human CD34-positive HSCs in 100µl PBS were transplanted into the object's tail vein.

Humanized animal-based non-small cell lung cancer (NSCLC) xenograft tumor and CDX mice

To generate NSCLC xenograft tumor model, 1.5×10^6 osimertinib-resistant lung cancer cells (PC-9/OR) were implanted in the right intercostal of 12 weeks post-humanized NSG mice. All PC-9/OR cells were cellular DID-fluorescence labeling performed according to the manufacturer's (Vybrant™ DiD cell-labeling) protocols (Thermo Fisher Scientific, USA),

which procedure was cellular labeling for visualization prior to cell transplantation. For human xenograft tumor experiments, treatment started when implanted tumor reached a volume of 100 to 200mm^3. A control group of this experiment received an intravenous injection method of the saline vehicle. Cetuximab (0.4mg/kg) and NK cells (2×10^7/cells) were injected intravenously following the same method and volume described above.

Analysis of engraftment with flow cytometry

Mice peripheral blood from retro-orbital sinus were analyzed by 12 weeks after hCD34+ HSCs transplantation. Single-cell suspensions were prepared by standard procedure and were stained with the following antibodies: mouse CD45-anemonia majano cyan fluorescent protein (AmCyan), hCD45-allophycocyanin (APC), hCD3-allophycocyanin violet (APC-Vio), hCD4-pacific blue (Bio-Blue), hCD8-Phycoerythrin-Cyanine7 (PE-Cy7), hCD19-Phycoerythrin (PE), and hCD56 (fluorescein isothiocyanate [FITC], all antibodies were purchased from Miltenyi Biotec. One million events were acquired per sample and analyzed with BD FACSCanto™ Flow Cytometry System. FlowJo (software ver.10.8.1) packages were used for data analysis.

Immunohistochemistry (IHC)

The mice were sacrificed 1 day after the 5th treatment injection or on day 30 in the control group. The relationship between tumor cells and NK cells of NSCLC CDX-humanized mouse model was evaluated by IHC staining using cancer markers (EGFR/Vimentin) and NK cell markers (CD3/CD56), which had been cell developmental factors as tumor progression and NK cells. Tumor tissues harvested from humanized NSG mice were fixed with 4% paraformaldehyde for 24 hours and paraffin embedding process using an automated tissue processing machine (Leica, Biosystems). Then, 5μm serial sections were prepared and

analyzed for IHC. Paraffin-embedded using fully automated rotary microtome (Leica RM2255) and immune-histological processing were deparaffinized and rehydrated by xylene and ethanol preparation. Specimens were then washed and performed enzyme retrieval using 0.1% trypsin in PBS for 30min at 37℃. After Blocking with 1% goat anti-serum for 1hr, specimens were incubated with primary antibodies against monoclonal mouse and rabbit anti-human cancerous-specific antigen EGFR (1:200, Thermo, Cat no-MA5-13070), CD3(1:100, NOVUS, Cat no-NB600-1441), CD56(1:50, R&D system, Cat no-AF2408) or polyclonal rabbit anti-human vimentin (1:200, Genetex, Cat no-GTX85471), and tissues were incubated overnight at 4℃. All specimens were then incubated in the secondary antibodies, anti-rabbit/mouse/goat/chicken-Ig-Fluorescent conjugated Alexa flour 488, 550, and 594, for 2hs room temperature. For nuclear staining, whole mounting was done using a DAPI-conjugated mounting medium (VECTASHEILD, Burlingame, CA). Lastly, the expression of the markers was using a fluorescence-attached microscope.

3. Analysis of the characteristics of autologous natural killer cells from patients

Flow cytometry

NK cells from the initial and final doses of each patient were analyzed. The NK cells were suspended in cell staining buffer solution (1% bovine serum albumin/0.05% sodium azide in PBS) and incubated with the following antibodies in the dark at 4℃ for 20 minutes [CD56-FITC, CD3-PE, CD20-PerCP/Cy5.5, CD14-APC, CD16-APC, CD314 (NKG2D)-APC, CD159c (NKG2C)-APC, CD158a (KIR2DL1)-APC, CD184 (CXCR4)-PerCP/Cy5.5 (BD Biosciences, San Jose, CA), CD158b (KIR2LD2/DL3)-APC, CD158e(KIR3DL1)-APC (Miltenyi Biotec), CD337 (NKp30)-PerCP/Cy5.5, CD183 (CXCR3)-PerCP/Cy5.5, CD336 (NKp44)-PerCP/Cy5.5, CD335 (NKp46)-PerCP/Cy5.5, CD159a (NKG2A)-APC, CD244 (2B4)-PerCP/Cy5.5 (BioLegend, San Diego, CA)]. The NK cells were then washed twice and

re-suspended in cell staining buffer solution. Control antibody (isotype control) for each staining antibody was used to determine the background level of non-specific binding as a negative control. Samples were measured using a flow cytometer (FACSLyric™, BD Biosciences) and analyzed by FACSuite v1.2 software (BD Biosciences).

NK cells (1×10^6) were washed with cell staining buffer solution and re-suspended. The NK cells were stained with anti-CD56 antibodies that had been conjugated with Alexa Fluor® 488 for 20 minutes in dark at 4°C and then washed twice with cell staining buffer solution. The NK cells were fixed in dark at room temperature for 30 minutes using 100 μL of fixation buffer (BioLegend) to increase cell permeability. The NK cells were reacted with antibodies [IFN-γ-PE, TNF-α-PerCP/Cy5.5 (BioLegend)] in dark for another 20 minutes, measured using a flow cytometer, and analyzed by FACSuite v1.2 software.

Degranulation

For the NK cell degranulation assay, the NK cells from the first and final doses of each patient were incubated in media containing the anti-CD107a-APC antibody (BioLegend) or isotype control. Degranulation was induced by adding K562 target cells (E:T ratio=1:1) or phorbol 12-myristate 13-acetate plus ionomycin as a positive control, respectively (both from Sigma, St. Louis, MO). In the negative control, complete culture medium was added instead of degranulation stimuli. The NK cells were incubated for 2 hours, and subsequently monensin (Sigma) was added to the medium before the next 3-hour incubation to prevent the degradation of internalized CD107a. Then, the cells were washed and stained with the anti-CD56-FITC antibody and analyzed using the FACS flow cytometer (BD FACSLyric™).

Cytotoxicity assay

The cytotoxicity of the NK cells against the target cells (K562 and NSCLC cell lines) was

assessed using a fluorometric cytotoxicity assay. The cytotoxicity of NK cell against K562, PC9, and PC9/OR was analyzed for all doses of NK cells from each patient, and the cytotoxicity against HCC827 and HCC827/OR was measured for the first and final doses of NK cells from each patient. Each cell line was stained with 4 mM calcein-AM solution (Sigma-Aldrich, St. Louis, MO) for 30 minutes at 37℃ followed by washing with RPMI 1640 medium. The NK cells and target cells were mixed at the E:T ratio of 10:1, 3:1, 1:1, and 0.5:1 in 96-well U-bottom plates. After a 4-hour incubation in a humidified incubator with 5% of CO_2, 100 μL of supernatant was transferred to a new 96-well flat-bottom black plate. Fluorescence signal was measured using a SpectraMax M2 microplate reader (Molecular Devices, San Jose, CA), with excitation at 485 nm and emission detection at 525 nm. The percent specific lysis was calculated using the formula: (Test release - Spontaneous release)/(Maximum release - Spontaneous release) × 100.

Antibody-dependent cellular cytotoxicity (ADCC) assay

ADCC was assessed by a fluorometric cytotoxicity assay for NK cells from the first and final doses of each patient. The target cells were stained with calcein AM, and 1×10^4 cells were dispensed into each well of a U-bottom 96-well plate. Antibodies were added at a final concentration of 2.5 μg/mL, and NK cells were added at E:T ratios of 3:1, 1:1, and 0.5:1. After incubation at 37°C for 2 hours, the cytotoxicity against cancer cells was analyzed. For maximal release, the cells were lysed with 2% Triton X-100 without NK cells, while stained cancer cells were cultured without NK cells and used as a negative control. The cytotoxicity against cancer cells (expressed as percent of lysis) was calculated by the following formula: [(value obtained from the experimental group - value obtained from the negative control)/(value obtained from the positive control - value obtained from the negative control)] × 100 %.

SUPPLEMENTAL RESULTS

1. Characteristics of the NK cell products

To produce the *ex vivo* expanded NK cell products for nonclinical and clinical uses, the $CD56^+$ cells were isolated from PBMCs of 12 patients. These $CD56^+$ cells were cultured for 31 or 32 days in the presence of feeder cells and cytokines for expansion. The cells were harvested and then subjected to either re-expansion for the first dose or cryopreservation for subsequent re-expansion for the second to last doses. The fresh or cryopreserved cells were culture-expanded for 17-18 days in the presence of feeder cells and cytokines to generate clinical NK cells product (final product). In the freshly isolated $CD56^+$ cells from the peripheral blood of the enrolled patients, the proportion of NK cells ($CD56^+CD3^-$) varied among donors (77.82 ± 12.60%). However, the final products were mainly composed of NK cells (99.81 ± 0.22%) with a minimal contamination of $CD3^+$ T cells (0.15 ± 0.20%), $CD14^+$ monocytes (0.32 ± 0.10%), and $CD20^+$ B cells (0.01 ± 0.01%) (Supplemental Figure 3A). In the expansion culture, the NK cells were highly expanded (4,462,189 ± 2,063,228-fold) with high viability (98.00 ± 1.00%) (Supplemental Figure 3B–C), which was sufficient for multiple injections in all donors. The culture-expanded NK cells from all donors highly expressed activating receptors, including NKG2D (99.19 ± 1.28%), CD16 (91.02 ± 5.38%), NKp30 (93.14 ± 7.92%), NKp46 (84.34 ± 6.96%), CD244 (2B4) (99.60 ± 0.30%), inhibitory receptor NKG2A (83.01 ± 12.69%), and chemokine receptor CXCR3 (83.46 ± 7.92%), whereas the expression level of inhibitory receptors, CD158a (KIR2DL1; 11.45 ± 9.66%), CD158b (KIR2DL2/L3; 17.01 ± 9.90%), and CD158e (KIR2DL1; 17.88 ± 14.25%), was relatively low (Supplemental Figure 3D). When the cytotoxic activity of culture-expanded NK cells against the K562 cells was examined 1 day before injection day, the NK cell products from all patients exerted a strong cytotoxic activity against K562 even at a low E:T ratio of 0.5:1 (58.93 ± 5.23%) (Supplemental Figure 3E). In addition, NK cell degranulation

activity (CD107a expression) was upregulated when cocultured with K562 cells (44.08 ± 5.72%) (NK + K562) or treated with phorbol 12-myristate 13-acetate/ionomycin (91.72 ± 3.95%) (NK + P/I) (Supplemental Figure 3F). Furthermore, the expression level of IFN-g (17.40 ± 3.04%) and TNF-a (16.51 ± 2.48%) were significantly upregulated upon stimulation with K562 cells (Supplemental Figure 3G).

Previous experiment showed that epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) (gefitinib and osimertinib)-resistant NSCLC cell lines were more sensitive to NK cell-mediated killing compared to their parent cell lines (data not shown). Thus, we investigated if clinical NK cell products also have better cytotoxic activity against osimertinib-resistant NSCLC cell lines PC-9/OR and HCC827/OR than their corresponding parent cells PC-9 and HCC827 cells. The NK cell products showed higher cytotoxicity against osimertinib-resistant PC-9/OR and HCC827/OR than their parent cells PC-9 and HCC827, respectively (Supplemental Figure 4). Such results were observed across all NK cell -to-cancer cell ratios (E:T ratio) regardless of donors. After 4 hours of co-culture at an E:T ratio of 3:1, the average cytotoxicity of all NK cell products was 58.89 ± 9.19% against PC9 cells, 85.20 ± 6.33% against PC9/OR, 42.55 ± 10.88% against HCC827, and 66.32 ± 12.30% against HCC827/OR, confirming that osimertinib-resistant NSCLC cell lines are more sensitive NK cell-mediated killing.

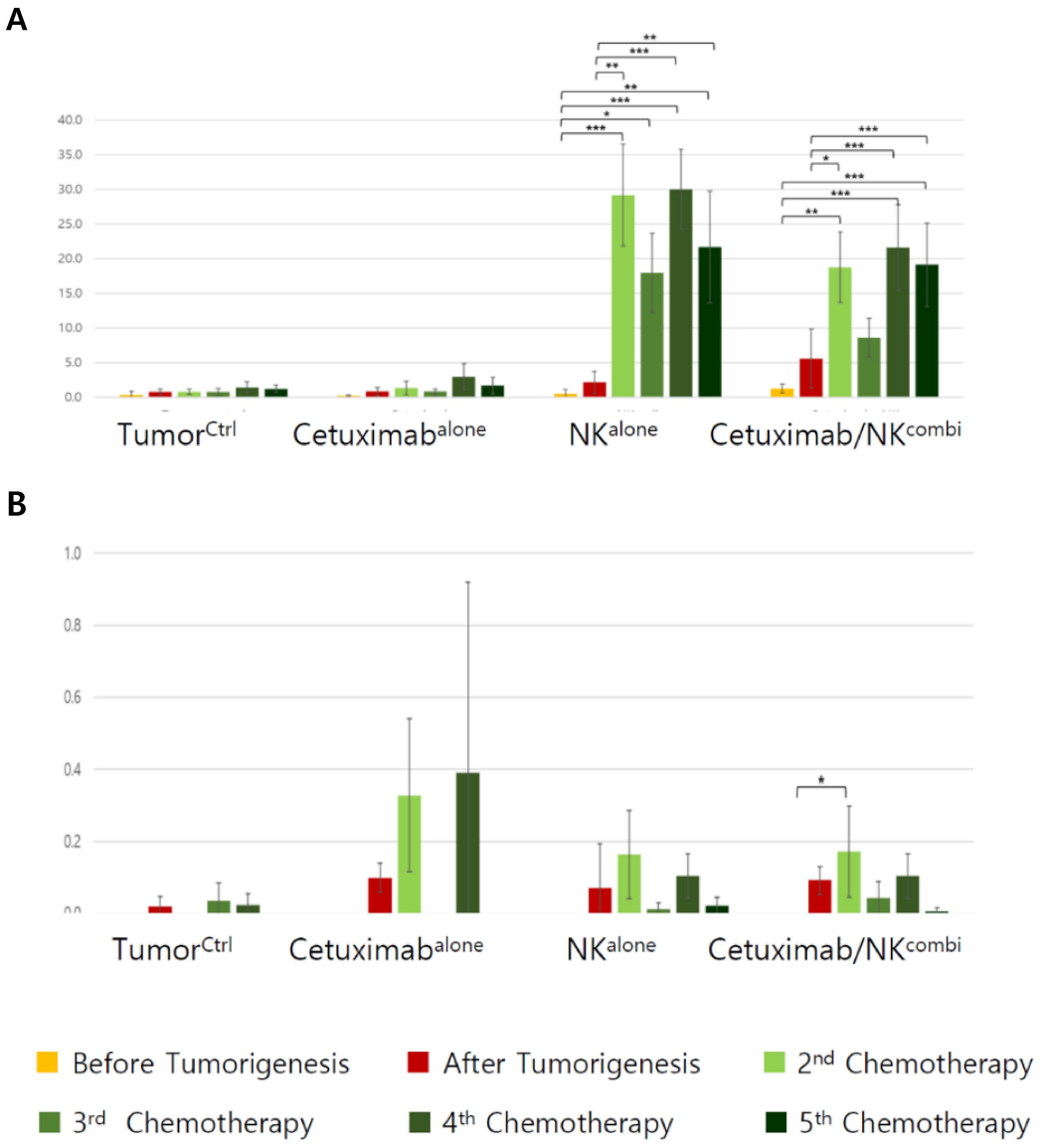
NK cells are well known to mediate ADCC through CD16 that was highly expressed on the NK cell products (Supplemental Figure 3D). Cetuximab exhibits anticancer effects by blocking the dimerization of EGFR through murine/human chimeric monoclonal Ab that binds to the extracellular domain III of EGFR and by inducing ADCC responses *in vivo*. Thus, we evaluated the ADCC effect of NK cell products against osimertinib-TKI-resistant NSCLC cells PC9/OR and HCC827/OR with cetuximab. Flow cytometric analysis showed that EGFR was highly expressed (over 99%) on all cell lines (data not shown). PC9/OR and its parent

cell PC9 were stained with calcein AM and co-cultured with NK cell products at an E:T ratio of 3:1 after treating with cetuximab for two hours. A control group treated with only media (NK) instead of cetuximab and a control group treated with isotype IgG antibodies (NK + IgG) exhibited similar cytotoxicity against PC9 and PC9/OR cells (Supplemental Figure 5A). However, compared to these two groups, the cytotoxicity of NK cell products was significantly increased against both cells by ADCC when they are pretreated with cetuximab. A similar increase in the cytotoxicity of the NK cell products in response to cetuximab was also observed in HCC827 and HCC827/OR cells (Supplemental Figure 5B). Therefore, combined use of NK cell product and cetuximab can further improve the cytotoxicity against $EGFR^+$ NSCLC cells via ADCC effect, irrespective of sensitivity to- EGFR-TKI.

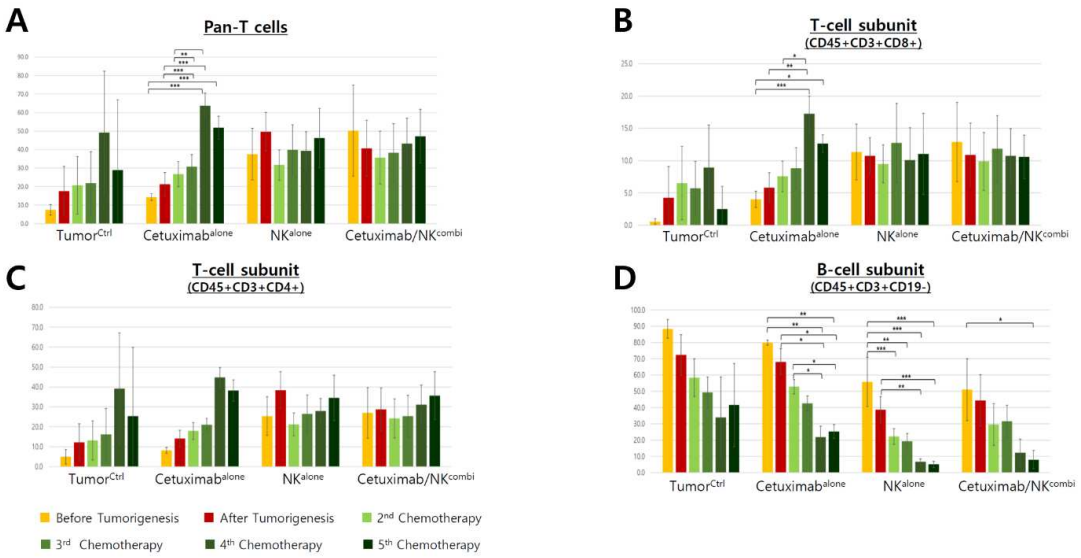
Overall, our study successfully generated a significant quantity of clinical-grade NK cell products with minimal contamination from other immune cells by employing *ex vivo* expansion techniques, utilizing two feeder cell types and cytokines. These NK cell products exhibited potent cytotoxic activity against osimertinib-resistant NSCLC cells through both natural killing activity and ADCC. The resulting NK cell products hold promise for multiple administration regimens in clinical settings, highlighting their potential as an effective therapy against resistant NSCLC.

SUPPLEMENTAL FIGURES

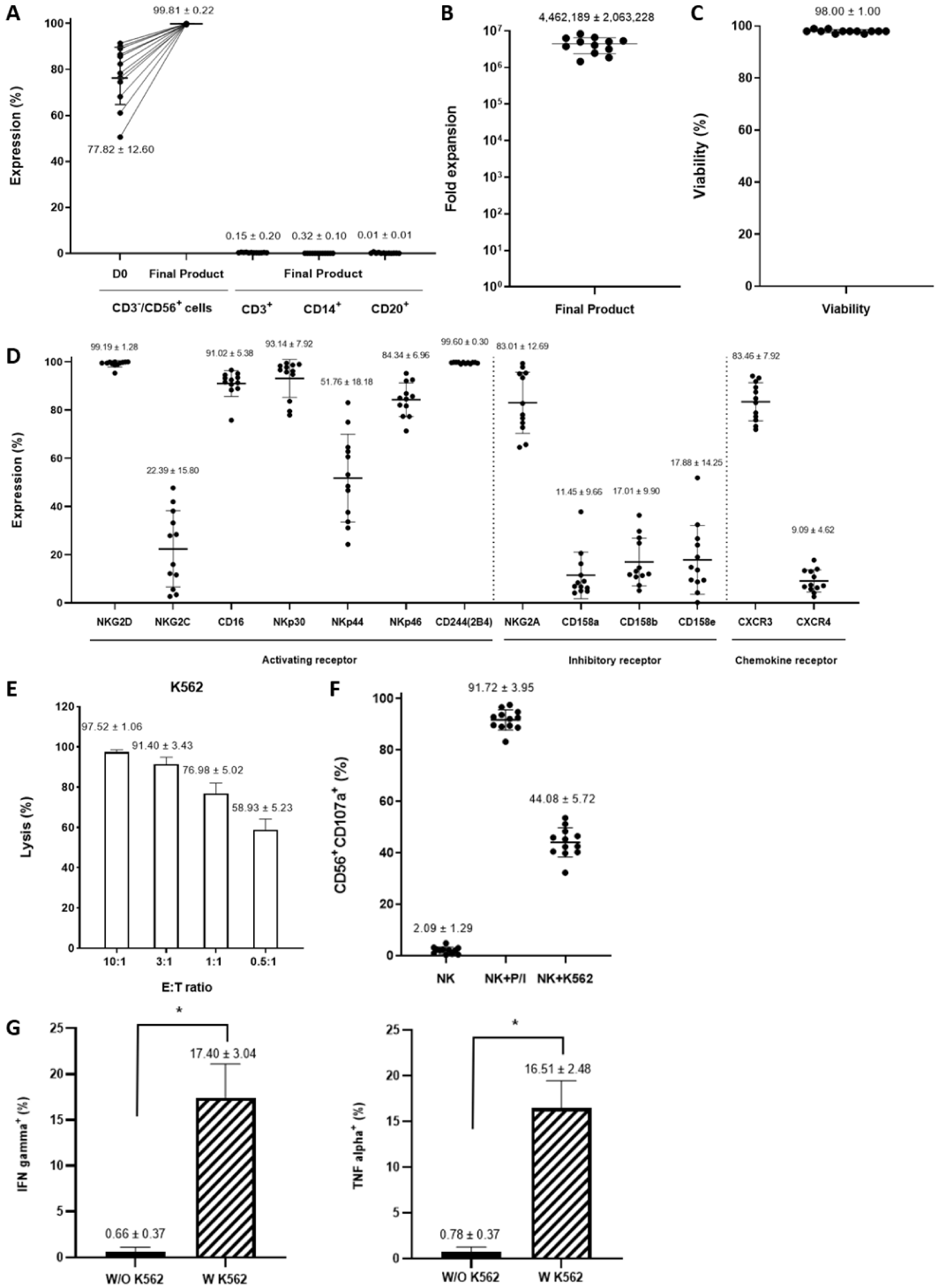
Supplemental Figure 1. Changes in human-specific CD3 positive/negative-dependent expression of NK cells during tumorigenesis and treatment in cell line-derived xenograft-humanized mice. (A) NK cells (CD45+/CD56+/CD3-). (B) NKT cells (CD45+/CD56+/CD3+).



Supplemental Figure 2. Changes in lymphocyte markers during tumorigenesis and treatment in cell line-derived xenograft-humanized mice. (A) Pan-T-cells (CD45+/CD3+). (B) T-cell subunit (CD45+/CD3+/CD8+). (C) T-cell subunit (CD45+/CD3+/CD4+). (D) B-cells (CD45+/CD3+/CD19-).



Supplemental Figure 3. Characteristics of expanded NK cells. (A) The percentages of $CD3^-CD56^+$ NK cells in freshly isolated cells using CliniMACS microbeads (D0) and culture-expanded cells (final product) as well as the percentages of $CD3^+$ T cells, $CD14^+$ monocytes, $CD20^+$ B cells in culture-expanded NK cells were analyzed using flow cytometry. (B) The fold expansion of the total cell population after expansion culture. (C) The viability of culture-expanded NK cells. (D) The expression levels of activating receptors, inhibitory receptors, and chemokine receptors were analyzed using flow cytometry in $CD56^+$ gated cells of final products. (E) The cytotoxicity activity of expanded NK cells against the leukemia K562 cell lines was measured using calcein-release assay at E:T ratios ranging from 10:1 to 0.5:1. (F) The degranulation activity of NK cells was assessed using flow cytometry by measuring the percentage of $CD56^+CD107a^+$ cells during co-incubation with K562 cells (NK+K562) at an E:T ratio of 1:1. Additionally, positive control samples were included by treating NK cells with phorbol 12-myristate 13-acetate/ionomycin (NK+P/I), while negative control samples were obtained without any treatment (NK). (G) Expression of cytokines (TNF-α and IFN-γ) in expanded NK cells were analyzed by intracellular staining after incubating with (W/K562) or without (W/O K562) K562 cells. The significant difference between W/K562 and W/O K562 was observed in terms of the expression of cytokines (TNF-α and IFN-γ) in expanded NK cells (*$p<0.005$). Each dot in A, B, C, D, and F represents the mean value of NK cells from each patient and the horizontal bars indicate the overall mean value from 12 donors.

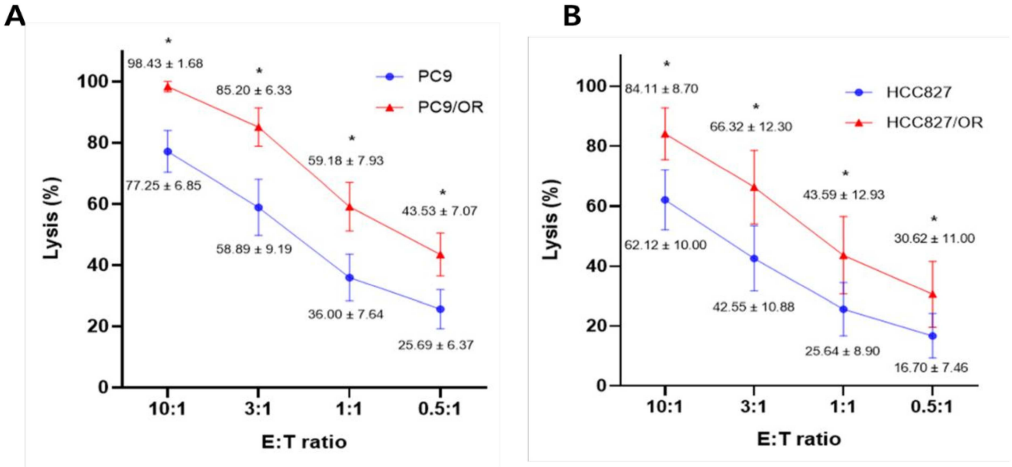

A
Expression (%)
99.81 ± 0.22
77.82 ± 12.60
0.15 ± 0.20
0.32 ± 0.10
0.01 ± 0.01
D0
Final Product
Final Product
CD3⁻/CD56⁺ cells
CD3⁺
CD14⁺
CD20⁺
B
Fold expansion
4,462,189 ± 2,063,228
Final Product
C
Viability (%)
98.00 ± 1.00
Viability
D
Expression (%)
99.19 ± 1.28
22.39 ± 15.80
91.02 ± 5.38
93.14 ± 7.92
51.76 ± 18.18
84.34 ± 6.96
99.60 ± 0.30
83.01 ± 12.69
11.45 ± 9.66
17.01 ± 9.90
17.88 ± 14.25
83.46 ± 7.92
9.09 ± 4.62
NKG2D
NKG2C
CD16
NKp30
NKp44
NKp46
CD244(2B4)
NKG2A
CD158a
CD158b
CD158e
CXCR3
CXCR4
Activating receptor
Inhibitory receptor
Chemokine receptor
E
K562
Lysis (%)
97.52 ± 1.06
91.40 ± 3.43
76.98 ± 5.02
58.93 ± 5.23
10:1
3:1
1:1
0.5:1
E:T ratio
F
CD56⁺CD107a⁺ (%)
91.72 ± 3.95
44.08 ± 5.72
2.09 ± 1.29
NK
NK+P/I
NK+K562
G
IFN gamma⁺ (%)
*
17.40 ± 3.04
0.66 ± 0.37
W/O K562
W K562
TNF alpha⁺ (%)
*
16.51 ± 2.48
0.78 ± 0.37
W/O K562
W K562

295

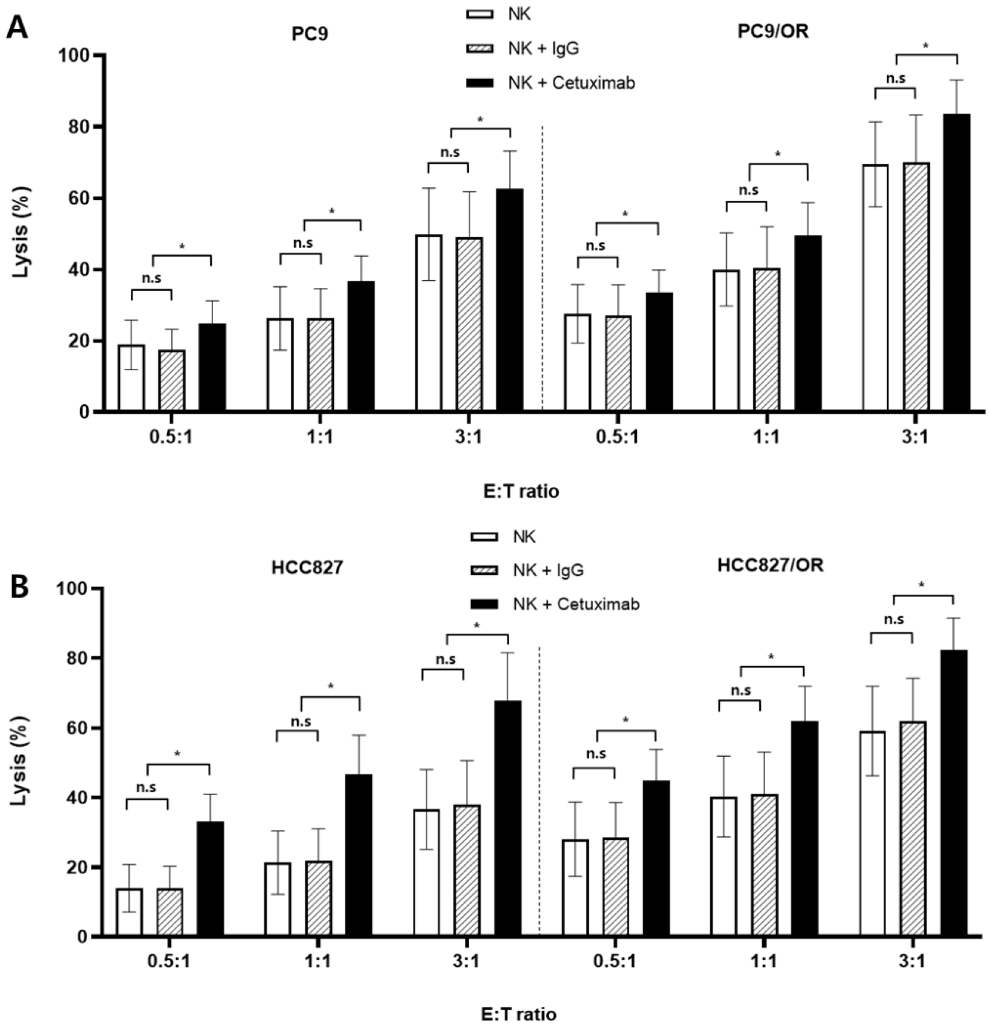
296

297

Supplemental Figure 4. Cytotoxicity activity of expanded NK cells toward osimertinib-resistant and their parental NSCLC cells. NK cell cytotoxicity against parental PC-9, and osimertinib-resistant PC-9 (PC9/OR) cells (A) as well as parental HCC827, and osimertinib-resistant HCC827 (HCC827/OR) cells (B) was evaluated using the calcein release assay after co-culturing for 4 hours with various E:T ratios ranging from 10:1, 3:1, 1:1 to 0.5:1). Data were shown as mean ± SD. One-way ANOVA with Tukey's post hoc analysis was applied for statistical analysis. *p<0.005, relative to each parental cell.

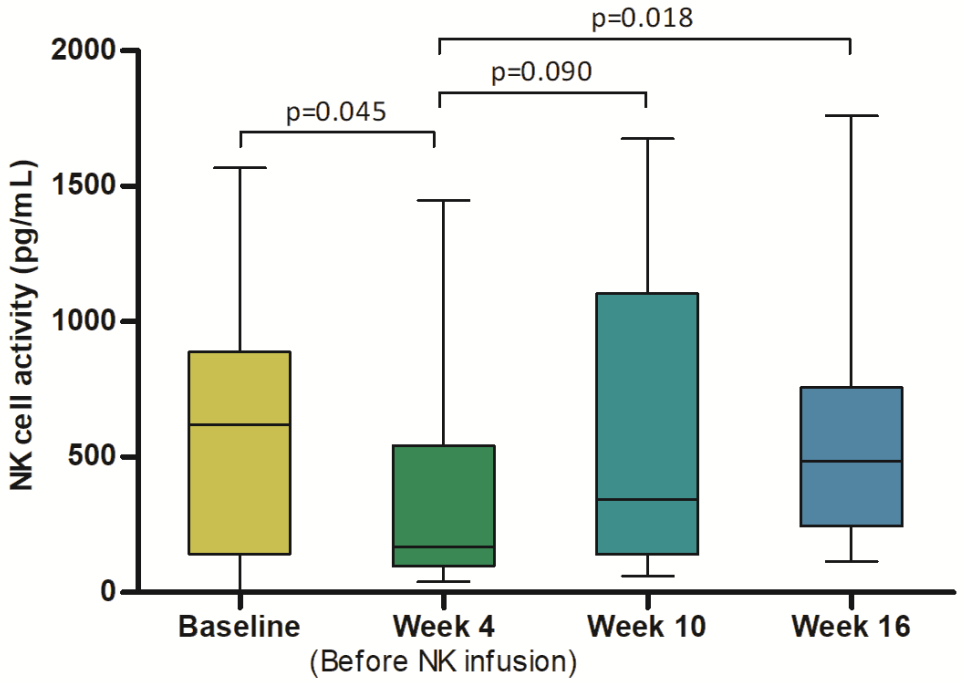


Supplemental Figure 5. Cetuximab-mediated ADCC activity of expanded NK cells against NSCLC cells. (A) Parental PC-9 and osimertinib-resistant PC-9 (PC9/OR) were co-cultured with NK cells for 2 hours at the E:T ratio of 0.5:1, 1:1 and 3:1 in the presence of media alone (NK), isotype control IgG antibody (NK + IgG) or cetuximab (NK + Cetuximab). (B) Cetuximab-mediated ADCC activity of expanded NK cells was measured in parental HCC827 and osimertinib-resistant HCC827 (HCC827/OR) at the indicated E:T ratio in the presence of media alone (NK), isotype control IgG antibody (NK + IgG) or cetuximab (NK + Cetuximab). The cytotoxic activity of NK cells against NSCLC cells was shown as mean ± SD. One-way ANOVA with Tukey's post hoc analysis was applied for statistical analysis. Asterisk indicates significant statistical difference relative to NK cell only (*p<0.005, n.s: not



significant).

Supplemental Figure 6. Changes in NK cell activity during treatment in patients with NSCLC receiving SNK01 (NK cells) in combination with either gemcitabine/carboplatin



or gemcitabine/carboplatin/cetuximab.

Supplemental Figure 7. Two representative cases in patients with NSCLC who received SNK01 in combination with gemcitabine/carboplatin/cetuximab.

(A) A patient in their 50s with adenocarcinoma received SNK01 in combination with cetuximab/gemcitabine/carboplatin as a second-line treatment after developing resistance to gefitinib. (B) A patient in their 50s, who had a recurrence (confirmed by biopsy) of adenocarcinoma 2 years after left upper lobectomy, received SNK01 in combination with cetuximab/gemcitabine/carboplatin as a third-line treatment after the failure of previous gefitinib and osimertinib treatments.