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Treatment of Alzheimer's Disease subjects with expanded non-genetically modified autologous natural killer cells (SNK01): a phase I study

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Abstract

Background The importance of natural killer (NK) cells of the innate immune system in neurodegenerative disease has largely been overlooked despite studies demonstrating their ability to reduce neuroinflammation (thought to be mediated by the elimination of activated T cells, degradation of protein aggregates and secretion of anti-inflammatory cytokines). SNK01 is an autologous non-genetically modified NK cell product showing increased activity in vitro. We hypothesized that SNK01 can be safely infused to reduce neuroinflammation in Alzheimer's Disease (AD) patients.

Methods SNK01 was produced and characterized for its ability to eliminate activated T cells, degrade protein aggregates and secrete anti-inflammatory cytokines. In this phase 1 study, SNK01 was administered intravenously every three weeks for a total of 4 treatments using a 3 + 3 dose escalation design (1, 2 and 4×10^9 cells) in subjects with either mild, moderate, or severe AD (median CDR-SB 10.0). Cognitive assessments and cerebrospinal fluid biomarkers associated with protein aggregation, neurodegeneration and neuroinflammation including amyloid-β42 and 42/40, α-synuclein, total Tau, pTau217 and pTau181, neurofilament light, GFAP and YKL-40 analyses were performed at baseline, at 1 and 12 weeks after the last dose. The primary endpoint was safety; secondary endpoints included changes in cognitive assessments and biomarker levels.

Results In preclinical in vitro studies, SNK01 were able to uptake and degrade the protein aggregates of amyloid-β and α-synuclein, produce anti-inflammatory cytokines and eliminate activated T cells. In the phase 1 clinical study, eleven subjects were enrolled (10 evaluable). No drug-related adverse events were observed. Despite 70% of subjects being treated at relatively low doses of SNK01 (1 and 2×10^9 cells), 50–70% of all enrolled subjects had stable/improved CDR-SB, ADAS-Cog and/or MMSE scores and 90% had stable/improved ADCOMS at one-week after the last dose. SNK01 also appeared to have beneficial effects on protein aggregate levels and neuroinflammatory biomarkers in the cerebrospinal fluid, with decreases in pTau181 and GFAP appearing to be dose-dependent.

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Conclusions SNK01 was well tolerated and appeared to have clinical activity in AD while also having beneficial effects on cerebrospinal fluid protein and neuroinflammatory biomarker levels. A larger trial with a higher dosing/duration has been initiated in the USA in 2023.

Trial registration www.clinicaltrials.gov NCT04678453, date of registration: 2020–12-22.

Keywords Natural killer cell, Alzheimer's Disease, Biomarkers

Background

Alzheimer's disease (AD) is a slow-progressing neurodegenerative disease that begins many years before symptoms emerge. The causes of AD are still unknown, and it remains a devastating neurodegenerative disease. The accumulation of amyloid-β peptides (Aβ) into extracellular plaques and the aggregation of abnormal tau protein within neurons as neurofibrillary tangles (NFTs), along with chronic neuroinflammation, are the defining hallmarks of AD [61]. Important strides in the areas of anatomy, genetics, and immunology led to the concept of the "neuroimmune axis of AD" deciphering the roles of the innate and adaptive immune systems to mitigate or cause neurodegeneration, as well as the participation of drainage systems [27]. Chronic inflammation mediated by the immune system plays an important part in the development and progression of AD; the inflammation drives toward synaptic loss and neurodegeneration and synergizes with the Aβ and NFT cascades [37].

Immune cells are important for maintaining brain homeostasis and for removing wastes and dead cells, all of which are essential for preventing AD [54]. Since immune function is known to decline with age, so are these normal cleaning mechanisms necessary for keeping the brain tissue healthy. Natural killer (NK) cells are an important component of the innate immune system. Constituting approximately 10 to 20% of the white blood cells in a healthy body, they provide a first line of defense against infections and cancer [66]. NK cells function through several mechanisms. NK cell stimulation and effector function depend upon the integration of signals derived from two distinct types of receptors—activating and inhibitory—that bind to ligands on target cells [9] forming the NK cell–target cell "zipper" [58]. NK cell effector function is activated when there is a positive balance towards the activating NK cell receptors rather than inhibitory signals from the target cell [7]. Although the number of NK cells remains unchanged between subjects with AD and healthy controls of the same age [46], multiple studies have shown a difference in the activity of NK cells. While some studies showed increased NK activity in AD patients, [52], other investigations have found that activity is reduced in subjects with AD [4, 23], which suggests that NK cells function abnormally in the brains of subjects with the disease.

The role of NK cells in the central nervous system (CNS) is just beginning to be understood. For example, different lines of research point towards a neuroprotective effect (e.g. secretion of interferon-gamma [IFN-γ] with stimulation of protective microglia, suppression of proinflammatory microglia, synthesis of interleukin-10 [IL-10] leading to the inactivation of inflammatory cells, and their ability to discard neurotoxic aggregates such as α-synuclein [α-syn]), or a neurotoxic effect (e.g. recruitment of inflammatory cells, release of cytotoxic molecules and depletion of Tregs) all depending on the context of the neurologic disease and the NK cell phenotype [6]. NK cells play a crucial role in shaping the adaptive immune response by selectively eliminating activated autologous CD4 + T cells, without affecting resting ones [40]. Moreover, the cytokines IL-2, IL-15, IL-12 + IL-18, and IFN-α enhance the killing properties of NK cells [40]. Therefore, NK cells appear to participate in normal immunological brain homeostasis.

FDA-approved Alzheimer's drugs so far have been focused on improving cognitive function and relieving symptoms and can potentially improve the overall quality of life for individuals and their caregivers, but there is still no permanent cure. Therefore, there is an enormous unmet need to develop therapies targeting different pathological aspects. The development of drugs against AD has, for the past 20 years, focused mainly on targeting the proteins found in brain plaques, like Aβ. We have developed a novel NK cell manufacturing technology that enables large-scale ex vivo production and expansion of NK cells (SNK01). Expanded NK cells are an autologous biological product using the patient's own cells that are cultivated and expanded outside the body and then reinfused into the same patient. The NK cell expansion process produces activated, fully functional non-genetically modified NK cells with both immunoregulatory and cytotoxic potential. SNK01 is being developed as a single agent for the treatment of neurodegenerative disorders such as AD.

This study provides in vitro evidence to support the potential of NK cell treatment of AD. Furthermore, the clinical evidence described herein of a phase 1 study which sought to determine the safety and preliminary efficacy of subjects with mild to severe AD, provide clinical evidence of the potential ability of NK cells to reduce

neuroinflammation and potentially affect cognitive performance.

Methods

Preparation of SNK01 cells for use in preclinical and clinical studies

All the manufacturing and testing procedures used to produce ex vivo expanded NK cells for clinical use were performed under good manufacturing practice conditions (preclinical studies: NKMAX Co, Ltd, Seongnam, Republic of Korea; clinical study: NKGen Biotech Inc., Santa Ana, CA). Peripheral blood mononuclear cells (PBMCs) were collected from the peripheral blood of healthy subjects (for preclinical studies) or AD subjects (for the clinical study) and then used for NK cell expansions as described previously with some technical adjustments [29]. The PBMCs were either used immediately or cryopreserved and stored for batch production by suspending the cells in a freezing medium consisting of 90% fetal bovine serum (FBS; HyClone, Tauranga, New Zealand) and 10% Dimethylsulfoxide (DMSO; Miltenyi Biotech, Bergisch Gladbach, Germany). The cell suspension was initially stored at -70 °C overnight or processed using a controlled-rate freezer (CRF) before being transferred to liquid nitrogen storage at ≤ -130 °C until required for use. The $CD56^+$ cells were then isolated from PBMCs using CliniMACS CD56 microbeads (Miltenyi Biotech GmbH, Bergisch-Gladbach and Teterow, Germany) according to the manufacturer's instructions. The isolated $CD56^+$ cells were then cultured in RPMI-1640 medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (FBS, Hyclone, Tauranga, New Zealand), 20 μg/mL gentamicin (GIBCO, Grand Island, NY), γ-irradiated (100 Gy) KL-1 and LCL feeders, 500 IU/mL interleukin IL-2 (rhIL-2, PROLEUKIN; Clinigen Healthcare Ltd, Burton-on-Trent, UK), and 50 ng/mL IL-21 (NKMAX Co. Ltd., Seongnam, Korea). The growing NK cells were sub-cultured every 3–4 days using fresh RPMI-1640 medium containing 10% FBS, 20 μg/mL Gentamicin and 500 IU/mL IL-2. After 17–18 days of culture, the cells were harvested, washed twice with phosphate-buffered saline (PBS; Hyclone) and once with Hartmann's Solution (Lactated Ringer's Injection USP; Baxter, Marion, NC), and formulated in 100 mL Hartmann's Solution containing 1% human serum albumin (20%; Octapharma AB, Stockholm, Sweden) and IL-2 (500 IU/mL) with the cell number of 2×10^9 cells(2×10^7 cells/mL). The autologous NK cells produced by these procedures were named as SNK01. The criteria for the release of the clinical NK cell product included the absence of microbial contamination (bacteria, fungus, 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 45 ± 15 min/100 mL (2×10^9 cells); the cells were to be used within 42 h from the time of manufacture. SNK01 cells for preclinical use were prepared as described above from the PBMCs of healthy donors.

Preclinical studies

Preparation of protein aggregates

The amyloid-β42 (Aβ42) peptide was purchased from GenScript (Piscataway, NJ; No. RP10017) and dissolved according to the manufacturer's protocol. To prepare Aβ aggregates, Aβ-(1–42) proteins were diluted to phenol red-free RPMI 1640 medium (Welgene Inc., Gyeongsansi, Korea) and assembled by incubating at a concentration of 100 μM at 37 °C for 24 h under quiescent conditions. Recombinant human α-synuclein (α-syn) 1–140 were obtained from NKMAX Co., Ltd. (Seongnam-si, Korea; No. SNA2001L). In vitro aggregation was performed by diluting recombinant α-syn monomers to 1 mg/mL in sterile PBS, then incubating at 37 °C with constant agitation at 1,000 rpm for 7 days and the aggregation was confirmed by a thioflavin T assay [8].

Internalization and degradation of protein aggregates by microglial and SNK01 cells

To investigate the internalization of Aβ aggregates in SNK01 cells, SNK01 cells were plated at 1×10^7 cells per well in a 6-well dish and incubated with Aβ concentrations ranging from 0—10 μM for 1 h, or with 5 μM Aβ for durations ranging from 0 to 24 h. Human microglia clone 3 (HMC3) cell line (ATCC, Manassas, VA; No. CRL-3304) was plated at 5×10^5 cells per well in a 6-well dish and allowed to settle for 24 h. The following day, HMC3 cells were exposed to 5 μM of Aβ aggregates for 1 to 3 h, followed by 3 washes with phosphate-buffered saline (PBS), and then harvested.

To assess the internalization of α-syn aggregates in SNK01 cells, SNK01 cells were plated at 1×10^7 cells per well in a 6-well dish and incubated with α-syn aggregates concentrations ranging from 0 to 10 μg/ml for 1 h, or with 5 μg/ml α-syn aggregates for durations ranging from 0 to 24 h. HMC3 cells were plated at 5×10^5 cells per well in a 6-well dish and allowed to settle for 24 h. The next day, HMC3 cells were exposed to 5 μg/ml of α-syn aggregates for 1–3 h, followed by three washes and harvested.

For the degradation assay, SNK01 cells and HMC cells were incubated with 5 μM Aβ or 5 μg/mL of α-syn

aggregate for 1 h, followed by 3 washes in PBS. Subsequently, cells were further incubated in fresh media for up to 48 h and harvested at indicated times for Western blot analysis. In the degradation inhibition experiment, SNK01 cells were treated with 5 μM Aβ aggregates for 1 h, washed three times with PBS, and then incubated in fresh medium with or without 25 μM chloroquine (CQ) for 6 and 24 h.

Western blot analysis

Cells were harvested, washed with PBS, and lysed with lysis buffer (GenDEPOT, Katy, TX) supplemented with 1×protease inhibitor cocktail and 1% Triton X-100 (Sigma-Aldrich, Saint Louis, MO) for 30 min at 4 °C with agitation. The lysate was centrifuged at 12,400 g for 5 min at 4 °C and the supernatant was collected and transferred to a new tube and mixed with 4×Laemmli sample buffer (Soluble fraction; Bio-Rad, Hercules, CA). The remaining pellet was further washed with ice-cold PBS, resuspended in 1×Laemmli sample buffer, and sonicated prior to being loaded (Insoluble fraction). The protein concentration was determined using the Pierce™ BCA protein assay kit following manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). Each sample was separated by 12% SDS-PAGE gels (Bio-Rad) with Xpert Prestained Protein Marker Ladder (GenDEPOT), transferred to polyvinylidene fluoride (PVDF) membranes (Cytiva, Marlborough, MA) and proved with anti-α-syn (Abcam, Cambridge, UK), anti-Aβ (Santa Cruz Biotechnology, Dallas, TX) or anti-β-actin antibody (Cell Signaling Technology, Danvers, MA) followed by anti-mouse IgG or anti-rabbit IgG secondary antibody (Thermo Fisher Scientific) conjugated to horse-radish peroxidase (HRP; Jackson ImmunoResearch, West Grove, PA). The detected bands were visualized using ECL reagent (Cytiva) and quantified using ImageJ (National Institute of Health, Bethesda, MD).

NK cell cytotoxicity assay

Erythroleukemia cell line K562 (ATCC; No. CCL-243) was used as a target cell to determine NK cell cytotoxicity. The target cells were stained with calcein acetoxymethyl ester (calcein AM; Sigma-Aldrich) by suspending in 4 μM of serum-free calcein AM at a cell number of 1×10^6 cells/mL and then incubated for 30 min at 37 °C incubator with 5% CO_2 with occasional shaking. The stained target cells were washed and re-suspended in RPMI 1640 medium containing 10% FBS at 1×10^5 cells/mL. The SNK01 cells were suspended in RPMI 1640 medium with 10% FBS and serially diluted directly within the wells of a U-bottom 96-well plate. The initial cell density of 1×10^5 cells was halved at each subsequent well until reaching a final cell density of 0.03×10^5 cells per 100 μL (quadruplicate at each cell density). Subsequently, stained cancer cells at a concentration of 1×10^4 cells/100 μL were plated into each well of a U-bottom 96-well plate containing effector cells diluted sequentially in 100 μL, with quadruplicates per effector concentration. Stained target cells treated with 2% Triton X-100 without NK cells were used as a positive control (maximum release), while stained cancer cells were cultured without NK cells and used as a negative control (spontaneous release). The plate inoculated with NK cells and the target cells was centrifuged for 5 min at 1,000 rpm and cultured for 2 to 4 h in a 37℃ incubator with 5% CO_2. After the culture, the plate was centrifuged for 5 min at 1,000 rpm to pellet the cells and debris, and 100 μL of the supernatant was moved to a 96 well black plate (SPL Life Sciences, Pocheon-si, Korea), and fluorescence was measured at an excitation wavelength of 485 nm and emission wavelength of 525 nm using a Synerge H1 microplate reader (BioTek, Winooski, VT). The cytotoxicity against target cells (cytotoxicity, 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%.

Flow cytometry

a) Cell surface staining

SNK01 cells or T cells were suspended in cell staining buffer solution (1% bovine serum albumin/0.05% sodium azide in PBS) and left in dark at 4℃ for 20 min to react with antibodies [CD319 (CRACC)-APC, CD226 (DNAM-1)-PerCP/Cy5.5, CD335 (NKp46)-PerCP/Cy5.5, CD337 (NKp30)-PerCP/Cy5.5, CD3-PE, CD69-PerCP/Cy5.5 (BioLegend, San Diego, CA), CD159c (NKG2C)-PE (R&D Systems, Minneapolis, MN), CD244 (2B4)-PE, CD314 (NKG2D)-APC, CD336(NKp44)-BV421, CD16-APC, CD56-Alexa Fluor® 488, or CD25-APC (BD Biosciences, Franklin Lakes, NJ)]. The cells were washed twice and then 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 v1.5 software (BD Biosciences).

b) Intracellular staining

SNK01 cells (1×10^6) were washed with cell staining buffer solution and re-suspended. The cells were stained with anti-CD56-Alexa Fluor® 488 antibody for 20 min in the dark at 4℃ and washed twice with cell staining buffer solution. Subsequently, cells were fixed in the dark at room temperature for 30 min using 100 μL of fixation buffer (BioLegend) to increase cell permeability. The cells

were reacted with antibodies [IFN γ-PE, TNF α-PerCP/Cy5.5 (BioLegend)] in the dark at 4℃ for another 20 min, measured using a flow cytometer, and analyzed by FACSuite v1.5 software.

c) Analysis of cell viability

SNK01 cells were washed twice with PBS and suspended with annexin V binding buffer. 7-AAD (BioLegend) was added to cells and incubated for 20 min at room temperature in the dark. Following the addition of annexin V binding buffer, cell viability was measured and analyzed by flow cytometry.

Degranulation assay

CD107a is known as lysosome-associated membrane glycoprotein 1 (LAMP1) and expressed on the surface of NK cells following activation and degranulation. To measure the degree of degranulation, SNK01 cells were suspended at a concentration of 1×10^6 cells per 100 µL of RPMI 1640 medium containing 10% FBS in 1.5 mL tubes, and 5 µL of anti-CD107a antibody (BioLegend) conjugated with APC fluorescence was added. Since CD107a is externalized to the cell surface of NK cells immediately upon degranulation and then quickly internalized, the anti-CD107a antibody was maintained in the medium throughout the stimulation period. Degranulation of SNK01 cells was induced by adding K562 or T cells as target cells (1×10^6 cells/well; E:T ratio = 1:1). For the negative control, culture medium was used instead of a stimulus for degranulation. The SNK01 cells were incubated at 37 °C for 2 h and then cultured for an additional 3 h after adding a 1 µM solution of monensin (Sigma-Aldrich), a protein transporter inhibitor. The cells were washed and stained with anti-CD56 antibodies conjugated with Alexa Fluor® 488. Finally, cells were analyzed for CD56 and CD107a expression by flow cytometry.

NK cell-mediated killing of activated T cells

T cells were enriched using CD3 MicroBeads (Miltenyi Biotec, Bergisch-Gladbach, Germany) following the manufacturer's instructions. Following enrichment, the isolated CD3 + T cells were suspended in RPMI-1640 media supplemented with 20 µg/mL gentamicin and 10% FBS. The cells were plated at 1×10^6 cells per well in a 6-well dish and activated with phorbol myristate acetate (PMA, 5 ng/mL) and ionomycin (250 ng/mL) for 48 h at 37℃ in a 5% CO_2 incubator. Recombinant human IL-2 at a concentration of 10 IU/mL was added to all groups to ensure survival during the incubation period. After incubation, the cells were harvested and utilized in subsequent experiments. Cytotoxicity of SNK01 cells against activated T cells was evaluated by flow cytometric cytotoxicity assay. Activated T cells were labeled with CellTrace™ Violet Cell Proliferation Kit (CTV; Thermo Fisher Scientific) and co-cultured with SNK01 cells at the ratio of 1:1 for 4 h at 37 °C in 5% CO_2 incubator. Control group was incubated without SNK01 cells. The percentage of specific lysis was determined according to the formula: % specific lysis = 100-[(average number of CTV + cells in wells with SNK01 co-culture)/(average number of CTV + cells in wells with target cells only) × 100].

Enzyme-linked immunosorbent assay (ELISA)

SNK01 cells were plated at a density of 2×10^6 cells/mL in a 24-well plate. Cell-conditioned media were collected by centrifugation at 1,500 rpm for 5 min after a 48-h incubation of unstimulated SNK01 cells or SNK01 cells stimulated with target cells at a 1:1 ratio for 3 h. The collected media were then stored at -80 °C until analysis. Concentrations of TGF-β1 and IL-10 in the supernatant were determined using the Human IL-10 (Abcam) and Human transforming growth factor (TGF)-β 1 ELISA Kits (R&D Systems), respectively, following the manufacturer's instructions.

Immunofluorescence staining

To confirm the colocalization of Aβ aggregates and LAMP1 in SNK01 cells, the cells were treated with 5 µM Aβ for 3 h and washed 3 times with PBS. The cells were fixed with 4% paraformaldehyde (PFA) for 20 min, followed by 3 washes with PBS containing 0.1% bovine serum albumin (BSA; Sigma-Aldrich). SNK01 cells were blocked with PBS containing 0.3% Triton X-100 and 1% BSA, then incubated for 2 h with anti-CD56 (Novus Biologicals, Centennial, CO) and anti-Aβ (Santa Cruz) antibodies diluted in PBS containing 0.03% Triton X-100 and 1% BSA. After 3 washes with PBS containing 0.1% BSA, the cells were incubated with Cy3- and Alexa-488-conjugated secondary antibodies (Abcam) for 30 min at 4 °C. The cells were then washed 3 times with PBS containing 0.1% BSA and incubated with APC-conjugated anti-LAMP1 antibody (eBioscience, San Diego, CA) for 1 h at 4 °C. After final washes with PBS containing 0.1% BSA, the stained cells were mounted on slides using VECTASHIELD® Antifade Mounting Medium with DAPI (Vector Laboratories, Newark, CA) and analyzed using a super-resolution confocal laser scanning microscope (SR-CLSM, STELLARIS 8; Leica, Wetzlar, Hesse, Germany).

Phase 1 clinical study

Study design

This study was a single-center, open-label, phase 1 study to evaluate the safety, tolerability and exploratory efficacy of SNK01 in subjects stages 3, 4, 5 and 6 AD (Study SNK01-MX04; NCT04678453). Fifteen subjects were screened at Hospital Angeles, Tijuana, Mexico from

January 6, 2021 to August 25, 2023, of which 11 enrolled and four failed screening. Using a 3 + 3 design to identify the maximum tolerated dose (MTD) and/or the recommended phase 2 dose (RP2D), three dose cohorts (1×10^9 cells, 2×10^9 cells, and 4×10^9 cells) were enrolled, each receiving a total of four doses at three-week intervals.

Safety and efficacy assessments (see below) were performed at baseline, one week (Week 11) after the fourth dose, and 12 weeks (Week 22) after the fourth dose. This study was approved by the research ethics committee (REC) and regulatory body of Mexico (COFEPRIS), and followed consensus ethical principles derived from international guidelines, including the *Declaration of Helsinki – Ethical Principles For Medical Research Involving Human Subjects*, as well as applicable International Council for Harmonization (ICH) Guidelines, Good Clinical Practice (GCP) Guidelines, and laws and regulations.

Participants

Subjects were eligible for enrolment if they were 55 to 85 years of age, in the past six months had structural evidence on magnetic resonance imaging (MRI) of the brain for a diagnosis of AD, including hippocampal volume loss and/or overall loss of cerebral volume, had positive evidence for diagnosis with AD by positron emission tomography (PET) with fluorodeoxyglucose (FDG) within the past six months, met the following basic clinical criteria, as determined by the investigator: spontaneous memory error, or presenting abnormal memory function in early screening. Written informed consent was obtained from enrolled subjects or their legally authorized representative.

Subjects were not eligible to participate if they had 1) any medical or neurological disease that could be a contributing cause of cognitive impairment of the subject or that would have imitated AD, 2) no evidence of AD, 3) presence of CNS hemorrhage or infarction with clinical impairment, 4) a history of cerebrovascular accident or transient ischemic attack, or unexplainable loss of consciousness within 12 months, 5) a history of psychiatric disorders or any other disorders impacting cognitive function, 6) a history of seizure episodes in the past three years, 7) any endocrine disorder not managed optimally, including diabetes mellitus, 8) a history of unstable angina, myocardial infarction, chronic heart failure, or other medical conditions requiring acute management within 12 months prior to screening, including history of cardiovascular, renal, hepatic, gastrointestinal, neurological, or metabolic disease within the past 6 months, 9) presence of kidney or liver failure, 10) an infection with human immunodeficiency virus (HIV), hepatitis B, hepatitis C, or any other infection or active systemic disease, 11) current treatment with anticoagulants (except aspirin at or below a prophylactic dose) or who were taking any dose of current medications for AD, like memantine or acetylcholinesterase inhibitors exceeding the normal recommended dose range, 12) any contraindication for performing required imaging or lumbar spinal taps.

Test methods

After signing consent and verifying eligibility, the enrolled subjects provided approximately 300–350 mL of peripheral blood in order to obtain the NK cells to produce the SNK01 product. If the initial amount of blood was inadequate to produce enough doses, additional blood draws were performed. Each blood draw was sufficient to be able to produce 1–4 doses of SNK01.

The safety of study subjects was monitored throughout the study on an ongoing basis. The Safety Review Committee (SRC) reviewed safety information, including dose-limiting toxicities (DLTs), for each dose cohort as well as previous cohorts, if applicable, and determined whether dose escalation would occur and the next dose level to be studied. The SRC could consider the totality of clinical evidence including safety and preliminary efficacy data in its review. The SRC was comprised of the Sponsor Medical Monitor or designee and the Principal Investigator. All reports generated by the SRC were submitted to the REC for review and approval of dose escalation.

The primary objective of this study was to evaluate the safety and tolerability of SNK01 in subjects with AD. In order to evaluate this, measurements of the incidence and severity of DLTs (Cohorts 1–3) and other AEs (Cohorts 1–3) (graded according to NCI-CTCAE v 5.0 and cytokine release syndrome [CRS] revised grading system [31]), vital signs, clinical laboratory tests and physical examinations were performed.

The secondary objective was to evaluate the preliminary efficacy of SNK01. In order to evaluate this, clinical evaluation of cognitive abilities and biomarkers for AD were performed. The following cognitive scales were used: Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), Mini-Mental State Examination (MMSE), Clinical Dementia Rating: Sum of Boxes (CDR-SB) as well as the AD composite score (ADCOMS), which is a composite measure composed of clinically sensitive items from the ADAS-Cog, the MMSE, and the CDR-SB scales using weighting factors as shown by Wang et al. (2016; see Table 2 in that reference). The following CSF biomarkers associated with abnormal protein aggregation, neurodegeneration and neuroinflammation were measured: Amyloid-beta 42 (Aβ42), Aβ42/Aβ40 ratio, total Tau, pTau181, pTau217, neurofilament light (NfL), glial fibrillary acidic protein (GFAP) and YKL-40 (Chitinase 3-like I). CSF samples were collected from

each subject by lumbar puncture with atraumatic spinal needle using gravity drip method at baseline, one week (Week 11) and 12 weeks (Week 22) after the fourth dose. Approximately 12–15 mL of CSF is collected into low-bind polypropylene tubes to prevent peptide adhesion. The initial 1.5–2 mL CSF collected were discarded to eliminate the excessive red cell contamination. The CSF was processed within one hour of collection by centrifuge at 2000xg for 10 min at 4℃. The supernatant of the CSF was aliquoted into 0.5 mL low-bind polypropylene microcentrifuge tube of 400 µL each and was snap freeze in dry ice and immediately transport to the testing lab for longer term stored at -80℃ until analysis. The quantification of the markers was conducted using Meso Scale Discovery (MSD) multiplexed sandwich immunoassays. The multiplexed assays use electrochemiluminescent labels that are conjugated to detection antibodies. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface which allow the recruitment of the detection antibodies. Electricity is then applied to the electrodes leading to light emission by the conjugated labels. Light intensity is then measured to quantify analytes in the sample.

Data analysis

As this was a phase 1 study, the following planned analyses were performed: Subject disposition and analysis sets; Demographics and baseline characteristics; Treatment exposure; Safety variables; and Efficacy variables. The All-enrolled population was defined as all subjects who signed the consent form and successfully passed screening. The All-enrolled population was used in disposition outputs and/or outputs related to subjects' eligibility. The Intention-to-Treat (ITT) population was defined as all enrolled subjects who were assigned to a study cohort. This population was used for all efficacy analyses. The assigned treatment was used for analysis based on the ITT population. The Safety population was defined as all subjects who received at least one dose of SNK01 treatment. This population was used for all safety analyses. Actual treatment received was used for analysis based on the Safety population. The DLT population was defined as all subjects who received at least one dose of SNK01 treatment and: 1.) experienced a DLT, or 2.) completed the observation period for DLT (21 days post first SNK01 infusion at Week 1). This population was used for the primary endpoint and partial safety analyses within the Dose Escalation stage. Actual treatment received was used for analysis based on the DLT population.

Descriptive statistics on the actual values and changes from baseline for the ADAS-Cog, MMSE, CDR-SB, and ADCOMS are presented for each cohort (Baseline, Week 11, and Week 22). These were conducted for each score/domain and final score/domain. The baseline for each questionnaire was defined as the last non-missing assessment prior to the first dose of SNK01. Analysis of these efficacy variables was based on the ITT population. The values of the CSF biomarkers are also presented for each cohort at baseline (only observed values), at Week 11, and at Week 22, where available. The results were treated as exploratory and compared to baseline values.

Results

Elucidating the mechanism of action

SNK01 cells internalize and degrade Aβ aggregates

NK cells have been shown to halt the propagation of extracellular α-syn aggregates in the CNS by using NK cells' innate immune response to act as efficient scavengers [20]. We hypothesized that SNK01, a product of activated and expanded autologous NK cells in vitro, can also internalize and degrade Aβ aggregates in vitro. To investigate the ability of NK cells to internalize and degrade Aβ aggregates, SNK01 cells were treated with varying concentrations of Aβ aggregates for 1 h (Fig. 1A) or 5 µM of Aβ aggregates for various time periods, up to 24 h (Fig. 1B). Following incubation, cells were washed, lysed, and subjected to Western blot analysis. Interestingly, the results revealed that the amount of internalized Aβ aggregates was increased in a concentration-dependent manner in a fixed 1-h treatment (Fig. 1A). Additionally, the results showed that SNK01 cells efficiently internalized Aβ in a time-dependent manner (Fig. 1B). Subsequently, to assess the degradation of internalized Aβ aggregates, SNK01 cells were incubated with 5 µM of Aβ aggregates for 1 h for internalization and washed three times with PBS to remove the Aβ aggregates that remained in media. Cells were then further incubated for 48 h and the remaining Aβ proteins in cells were visualized by Western blotting at each time point. Interestingly, the amount of internalized Aβ aggregates gradually decreased until 24 h (Fig. 1C). This result suggests that SNK01 cells can internalize Aβ aggregates and then degrade them intracellularly.

To confirm the internalization of Aβ aggregate in SNK01 cell, immunocytochemistry was performed with SNK01 cell co-incubated with 5 µM Aβ aggregate for 3 h. Our results showed that internalized Aβ was localized in the cytoplasm of NK cells and co-localized with LAMP1, suggesting that internalization of Aβ aggregate and association with the lysosomal pathways (Supplementary figure S1A). Partial inhibition of Aβ aggregate degradation by the lysosomal inhibitor CQ also implies that degradation occurred intracellularly (Supplementary Figure S1B and C).

One of the major cell types to clear the harmful Aβ aggregates is microglial cells in the CNS [41]. The HMC3

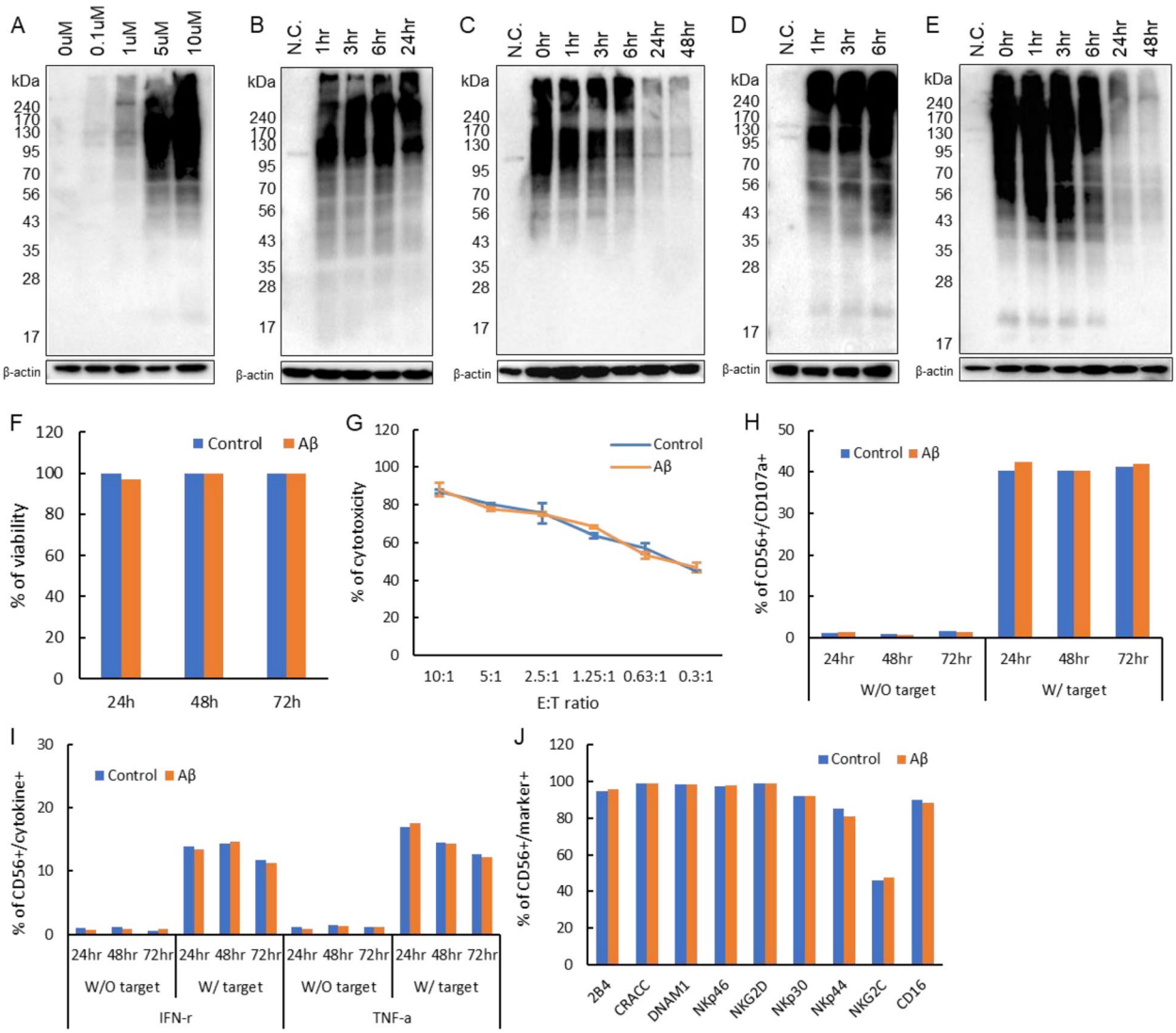

Fig. 1 Uptake and degradation of extracellular amyloid β (Aβ) aggregates by SNK01. **A**-**E** Intracellular levels of Aβ aggregates in SNK01 and HMC3 cells were analyzed by Western blot analysis. **A** SNK01 cells were treated with varying concentrations of Aβ aggregates for 1 h and harvested. **B** SNK01 cells were treated with 5 μM of Aβ aggregates and harvested at 1, 3, 6, 9, and 24 h after treatment. **C** SNK01 cells were treated with 5 μM Aβ aggregates for 1 h and washed 3 times with PBS. Then SNK01 cells were incubated in fresh medium for up to 48 h and harvested at 1, 3, 6, 24, and 48 h after wash. **D** HMC3 cells were treated with 5 μM of Aβ aggregates for 1, 3, and 6 h. N.C: negative control without treatment with Aβ aggregates. **E** HMC3 cells were treated with 5 μM Aβ aggregates for 1 h and washed 3 times with PBS. Then cells were incubated further in fresh medium for up to 48 h and harvested at 1, 3, 6, 24, and 48 h after wash. **F**-**J** SNK01 cells were incubated with 5 μM of Aβ aggregates for 24, 48, and 72 h and analyzed for viability (**F**), cytotoxicity at 72-h after treatment (**G**), degranulation (**H**), intracellular expression of IFN-γ and TNF-α (**I**), and surface expression of various activating NK receptors at 72-h after treatment (**J**)

cell is a human microglial cell line displaying phagocytic activity for Aβ aggregates [1, 19, 49]. Western blot analysis also revealed uptake and degradation of Aβ aggregate in HMC3 in identical experimental conditions with SNK01 cells (Fig. 1D and E). By comparing levels of Aβ aggregate uptake and degradation in both cells, our results suggest that the clearance ability of SNK01 cells could be comparable to that of the microglial cell.

To investigate whether Aβ aggregates affect the viability and characteristics of SNK01 cells, we examined viability (Fig. 1F), cytotoxicity (Fig. 1G), degranulation activity (Fig. 1H), IFN-γ and TNF-α secretion (Fig. 1I), and surface expression of various NK cell activating receptors (Fig. 1J) at 24, 48, and 72 h after treatment

with 5 μM Aβ aggregates. The results showed that treatment of Aβ aggregates did not change viability, natural killing activity, and other characteristics of SNK01 cells.

Overall, these results indicate that SNK01 cells can internalize and degrade Aβ aggregates without a decrease in their viability, killing activity, and activating receptor expression.

SNK01 internalize and degrade α-synuclein aggregates

Misfolded α-syn apart from its presence in Parkinson's Disease (PD) is also found in more than 50% of brains from autopsy-confirmed AD patients [55]. Depletion of NK cells in a murine model of PD led to an exacerbation of clinical neurological deficits and histology-confirmed robust α-syn inclusions [20]. NK cells' neuroprotective effects in the CNS may be related to their ability to discard neurotoxic aggregates such as α-syn [6]. To investigate whether SNK01 cells retained the ability to internalize and degrade α-syn aggregates as previously reported for NK cells [20], SNK01 cells were incubated with α-syn aggregates and washed, and the internalized α-syn aggregates were subjected to Western blot analysis. The results showed that the amount of internalized α-syn aggregates was increased in a concentration-dependent manner up to 10 μg/mL α-syn aggregates treatment in fixed 1-h treatment (Fig. 2A). Additionally, α-syn aggregates were not detected in NK cells at 0 h without co-incubation with 5 μg/mL α-syn aggregates but were detected after co-incubation and peaked at 1 h, followed by a decrease (Fig. 2B). Subsequently, to access the degradation of internalized a-syn aggregates, SNK01 cells were incubated with 5 μg/mL α-syn aggregates for 1 h for internalization and washed three times with PBS. Then, cells were further incubated for 48 h, and remaining α-syn proteins were subjected to Western blotting at each time point. The amount of internalized α-syn aggregates gradually decreased and was rarely detectable as of the 6 h-incubation (Fig. 2C). These results showed that SNK01 cells not only efficiently internalized α-syn species but also rapidly degraded them.

Microglia have been shown to internalize and degrade α-syn, both in vivo and in vitro [16]. In our study, Western blot analysis also revealed uptake and degradation of α-syn aggregates in HMC3 in identical experimental conditions with SNK01 cells (Fig. 2D and E). By comparing the level of α-syn aggregate uptake and degradation in both cells, we suggest that the clearance ability of SNK01 cells could be comparable to microglial cells.

To investigate whether α-syn aggregates affect the viability and characteristics of SNK01 cells, we examined viability (Fig. 2F), cytotoxicity (Fig. 2G), degranulation activity (Fig. 2H), IFN-γ and TNF-α secretion (Fig. 2I), and surface expression of various NK cell activating receptors (Fig. 2J) at 24, 48, and 72 h after treatment of 5 μg/mL α-syn aggregates. The results showed that treatment of α-syn aggregates did not change viability, natural killing activity, and other characteristics of SNK01 cells.

Overall, these results indicate that SNK01 cells can internalize and degrade α-syn aggregates without a decrease in their viability, killing activity, and activating receptor expression.

SNK01 express immune modulatory cytokines

NK cells' beneficial effects in the CNS may also be related to their ability to secrete cytokines, leading to the inactivation of inflammatory cells [6]. To determine whether SNK01 can produce immunoregulatory cytokines, the levels of IL-10 and TGF-β1 in conditioned media of SNK01 were analyzed using ELISA. SNK01 cells were plated at a density of 2×10^6 cells/mL, and the conditioned media was collected after a 2-day incubation of unstimulated SNK01 cells or SNK01 cells stimulated with K562 target cells for 3 h before harvest (Fig. 3). IL-10 and TGF-β1 were detected in the supernatant of SNK01 cells in both conditions. However, there was no significant difference in levels of IL-10 between unstimulated SNK01 cells and SNK01 cells stimulated by K562 cells. The level of TGF-β1 increased slightly, by less than 10%, upon stimulation with target cells. These findings suggest that SNK01 cells constitutively produce IL-10 and TGF-β1 regardless of the activation status of NK cells.

SNK01 kill activated T cells but not resting T cells

Aberrant activation of CD4 + and CD8 + T cells is involved in AD-associated neuroinflammation. Although their role is not fully understood, T cells may regulate the inflammatory response observed in AD patients. The permeability of the blood–brain barrier (BBB) to peripheral T cells increases as does the expression of chemokine receptors (e.g. C–C motif chemokine receptor type2 [CCR2]; C–C motif chemokine receptor type 5 [CCR5]; and C-X-C motif chemokine receptor 2 [CXCR2]) on these T cells allowing their trafficking into the brain [63]. Elevated peripheral T helper cells (Th1, secreting INF-γ) and T helper 17 cells (Th17, releasing proinflammatory factors, such as FasL, IL-17, and IL-22) have been associated with neuroinflammation in AD. Moreover, a positive correlation between CD3 + T cells and Tau (but not amyloid) pathology has been observed, whereas T cell activation can be modulated by several AD risk factors including pTau and Aβ [63].

It has been reported that $CD56^{dim}$ and $CD56^{bright}$ NK cells can eliminate activated autologous CD4 + T cells (not resting cells) via recognition of upregulated ligands for NKG2D, LFA-1, TRAIL and NKp46 [40]. It has also been reported that activated autologous or allogeneic

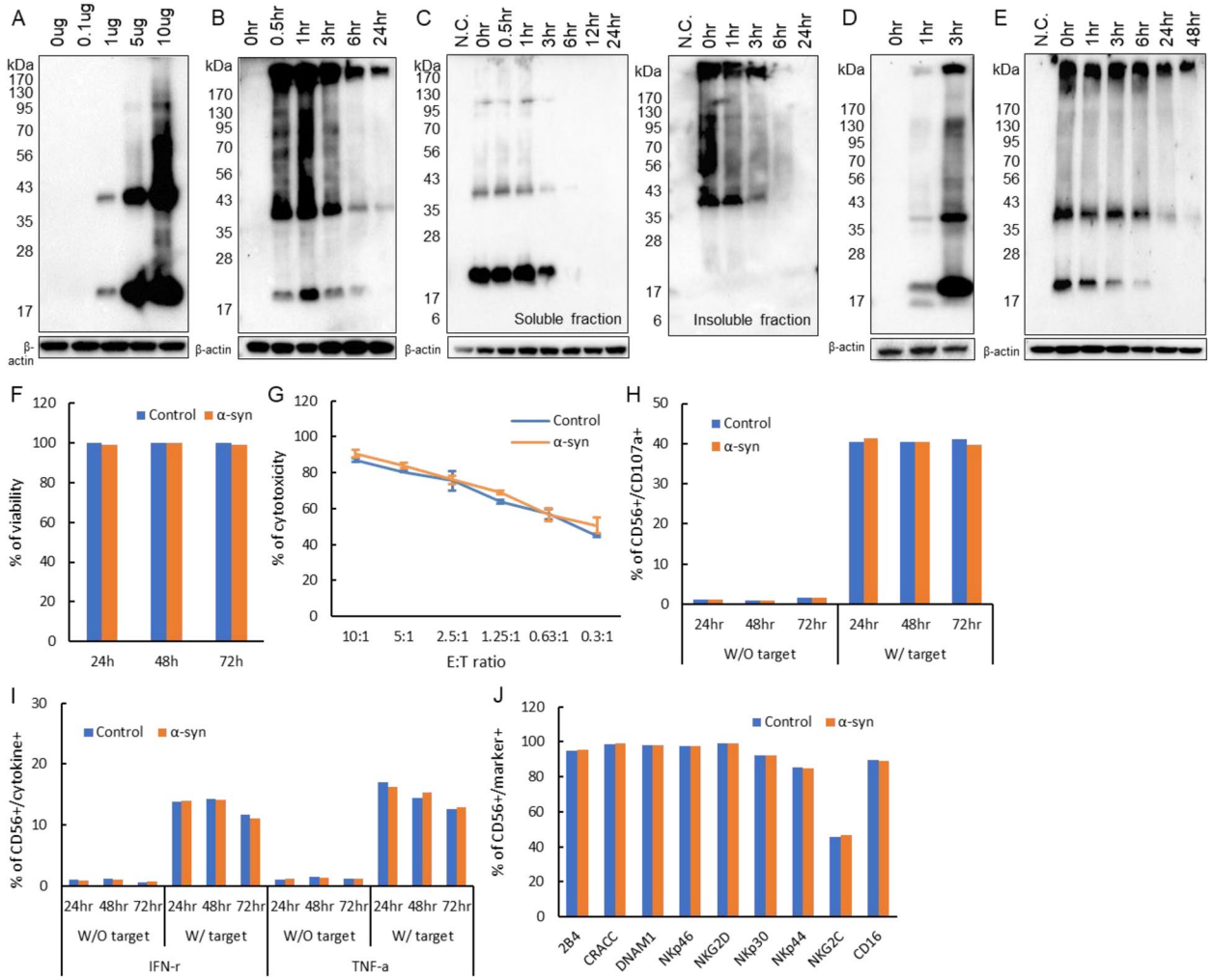

Fig. 2 Uptake and degradation of extracellular α-synuclein (α-syn) aggregates by SNK01. **A**-**E** Intracellular levels of α-syn aggregates in SNK01 and HMC3 cells were analyzed by Western blot analysis. **A** SNK01 cells were treated with varying concentrations of α-syn aggregates for 1 h and harvested. **B** SNK01 cells were treated with 5 μg/mL of α-syn aggregates and harvested at 0.5, 1, 3, 6, and 24 h after treatment. **C** SNK01 cells were treated with 5 μg/mL of α-syn aggregates for 1 h and washed 3 times with PBS. Then SNK01 cells were incubated in fresh medium for up to 24 h and harvested at 0.5, 1, 3, 6, 12, and 24 h after wash. Left panel: soluble fraction with lysis buffer with 1% triton X-100, Right panel: insoluble fraction with lysis buffer with 1% triton X-100. **D** HMC3 cells were treated with 5 μg/ml of α-syn aggregates for 1 and 3 h. N.C: negative control without treatment with α-syn aggregates. **E** HMC3 cells were treated with 5 μg/mL of α-syn aggregates for 1 h and washed 3 times with PBS. Then cells were incubated further in fresh medium for up to 48 h and harvested at 1, 3, 6, 24, and 48 h after wash. **F**-**J** SNK01 cells were incubated with 5 μg/mL of α-syn aggregates for 24, 48, and 72 h and analyzed for viability (**F**), cytotoxicity at 72-h after treatment (**G**), degranulation (**H**), intracellular expression of IFN-γ and TNF-α (**I**), and surface expression of various activating NK receptors at 72-h after treatment (**J**)

T cells can be killed by NK cells through the NKG2D/NKG2DL interaction, with a minor but significant participation of DNAM-1[5]. To test the effect of NK cells on T cells, we evaluated the ability of SNK01 to kill activated T cells in vitro. The activation status of T cells was confirmed by flow cytometric analysis of CD69 and CD25 expression. CD69 is generally regarded as the earliest activation cell surface maker on leukocytes and plays a role in the proliferation and survival of activated T lymphocytes. As a membrane receptor, CD69 is rapidly and transiently expressed on the activated lymphocytes, but not on the resting lymphocytes [18,48]. CD25, the alpha chain of the trimeric IL-2 receptor, is also considered one of the most prominent cellular activation markers. While CD69 takes the lead as the earliest activation marker, CD25 follows as a moderately late marker and is considered the most important marker of cellular response activation [30]. Our results demonstrated that T cells stimulated with PMA/ionomycin (P/I) upregulated the expression of CD69 and CD25 up to 99.79% and 99.81%,

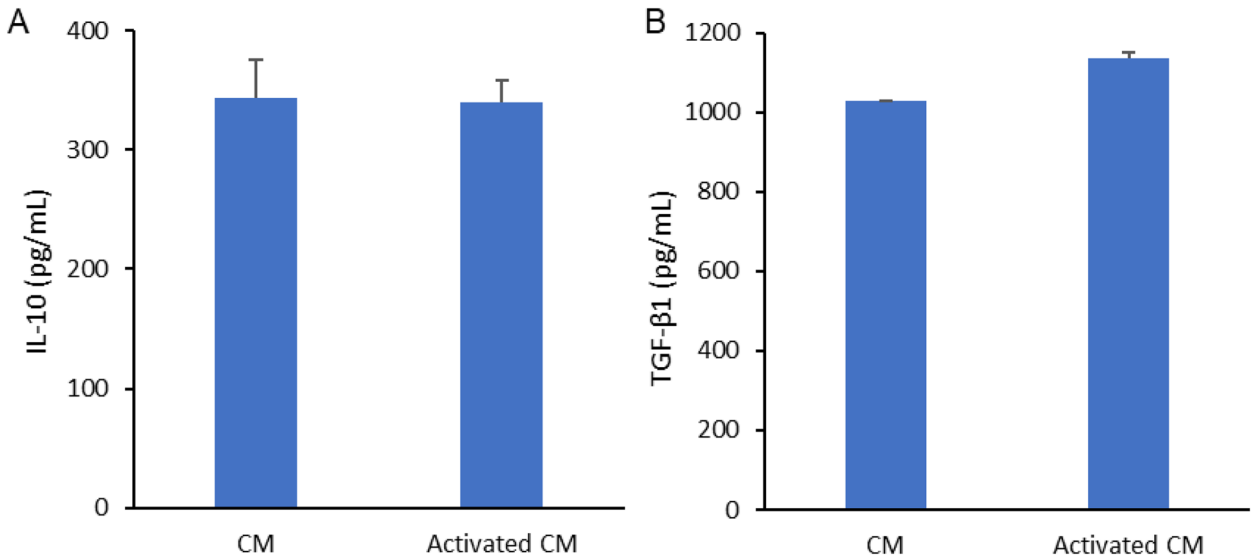

Fig. 3 Level of interleukin 10 (IL-10) and TGF-β1 in conditioned media (CM) of SNK01 cells. The production of IL-10 (**A**) and TGF-β1 (**B**) by SNK01 cells for 2 days cultivation after stimulation with or without K562 target cells for 3 h before harvest at a 1:1 E:T ratio was investigated by ELISA. CM and Activated CM indicate culture supernatant of SNK01 cells treated without (CM) or with target cells (Activated CM)

respectively, while the expression of CD69 and CD25 were 52.74% and 26.73%, respectively, in unstimulated T cells (Fig. 4A). This confirms that PMA/ionomycin effectively induced the activation of T cells.

As previously mentioned, various in vitro and in vivo studies have consistently demonstrated the ability of NK cells to directly eliminate activated T cells. Direct elimination of effector $CD4^+$ T cells by NK cells has been reported in mouse models of chronic inflammation [21] and graft-versus-host disease [47]. Similarly, human NK cells have been reported to be capable of killing activated T cells [14, 40]. Furthermore, in vitro experiments that examined the sensitivity of T cells to NK cell-mediated killing revealed that while resting T cells exhibited resistance to lysis by NK cells, recently primed T cells were susceptible [45]. Activated or unstimulated control T cells were then co-cultured with SNK01 cells at 1:1 E:T ratio for 4 h in a cytotoxicity assay. In the cytotoxicity assay, activated T cells (38%) were found to be 2.71-fold more susceptible to SNK01-mediated cytotoxicity compared to unstimulated T cells (14%) (Fig. 4B).

NK cell activation triggers cytotoxicity through the release of specialized granules (degranulation) and the production of IFN-γ. The degranulation of SNK01 cells was evaluated by measuring the cell surface expression of CD107a during co-culture with T cells. Additionally, IFN-γ expression was assessed by intracellular staining. As depicted in Fig. 4C and D, SNK01 cells exhibited degranulation and IFN-γ production in response to activated T cells, but not in response to unstimulated control T cells. This suggests that SNK01 cells possess the ability to effectively eliminate activated T cells.

Clinical Study

Characteristics of clinical SNK01 product

Prior to NK cell expansion, the average percentage of CD3-CD56 + population in the isolated CD56 + cells from PBMC was (63.31 ± 21.52%) (Fig. 5A). However, following expansion of 17–18 days with γ-irradiated KL-1 and LCL feeders in the presence of interleukin (IL)-2 and IL-21, the average proportion of CD3-CD56 + NK cells was significantly increased (98.74 ± 0.90%) in the final SNK01 products with a low proportion of CD3 + T cells (0.66 ± 0.79%), CD20 + B cells (0.003 ± 0.007%), and CD14 + monocytes (0.08 ± 0.05%). In the expansion culture, the NK cells were efficiently expanded (1,790 ± 1,080-fold) (Fig. 5B) with high viability (97% ± 1.16%) (data not shown), which were sufficient for multiple injections in each donor. When the cytotoxic activity of SNK01 cells was examined 1 day before injection day (16–17 days of culture) against the K562 cells, the expanded NK cells from all patients exerted a strong cytotoxic activity against K562 even at a low E:T ratio of 0.5:1 (49.17 ± 6.72%) (Fig. 5C).

Patient characteristics

A total of fifteen subjects were screened for the study of which 11 subjects were enrolled; four subjects were enrolled in Cohort 1 (1×10^9 cells), four subjects in Cohort 2 (2×10^9 cells), and three in Cohort 3 (4×10^9 cells). No subject withdrew from the study, however, one

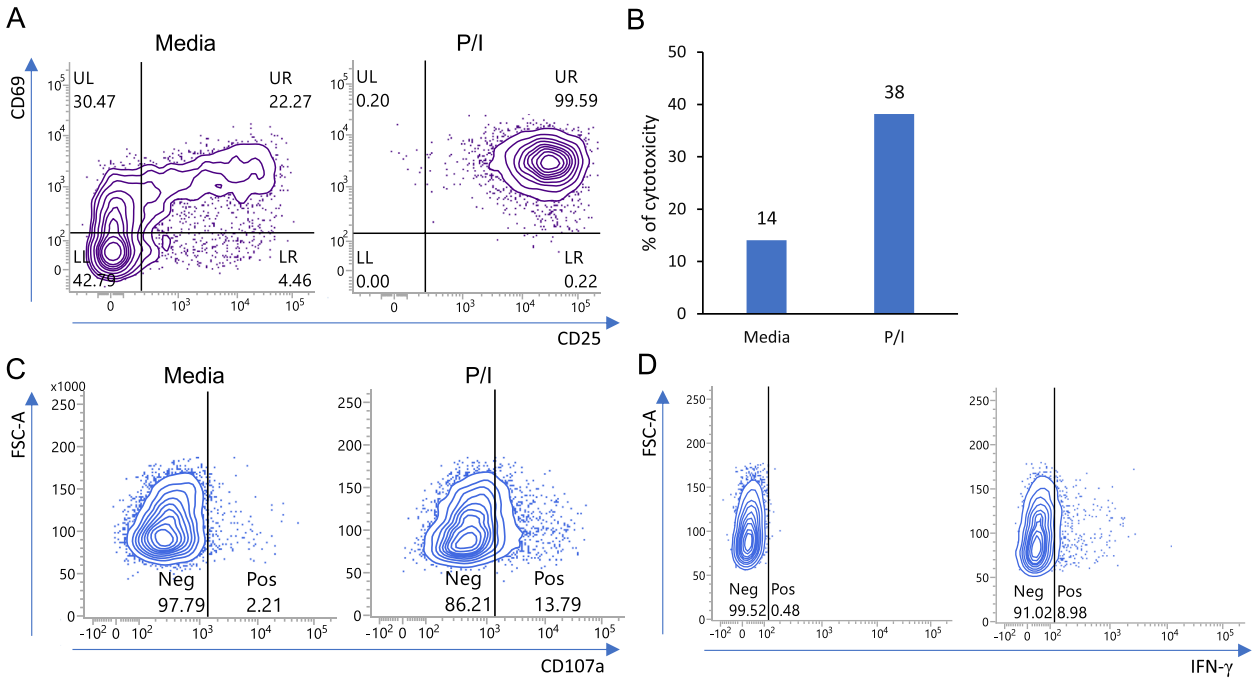

Fig. 4 Cytotoxic activity and degranulation activity of SNK01 against stimulated T cell. **A** Human T lymphocytes were cultured with (P/I) or without (Media) stimulation by PMA (5 ng/mL)/ionomycin (250 ng) (P/I) for 48 h. The cells were stained with PE-CD3, PerCP/Cy5.5-CD69, and APC-CD25 antibodies and then analyzed by flow cytometry. Contour plots represent percentages of the $CD25^+$ $CD69^+$ cells on $CD3^+$ gated T cells. **B** Cytotoxic activity of SNK01 against T cells was assessed by flow cytometry. T cells labeled with CTV were activated either with (P/I) or without (Media) PMA and ionomycin, and then co-incubated with SNK01 cells at an E:T ratio of 1:1 for 4 h. **C**-**D** Degranulation activity and IFN-γ expression of SNK01 cells against T cells. T cells were activated either with (P/I) or without (Media) PMA and ionomycin and then mixed with SNK01 cells at an E:T ratio of 1:1. The expression of CD107a (C) or IFN-γ (D) in CD56 + population was assessed by flow cytometry after 5- or 3- hour incubation, respectively

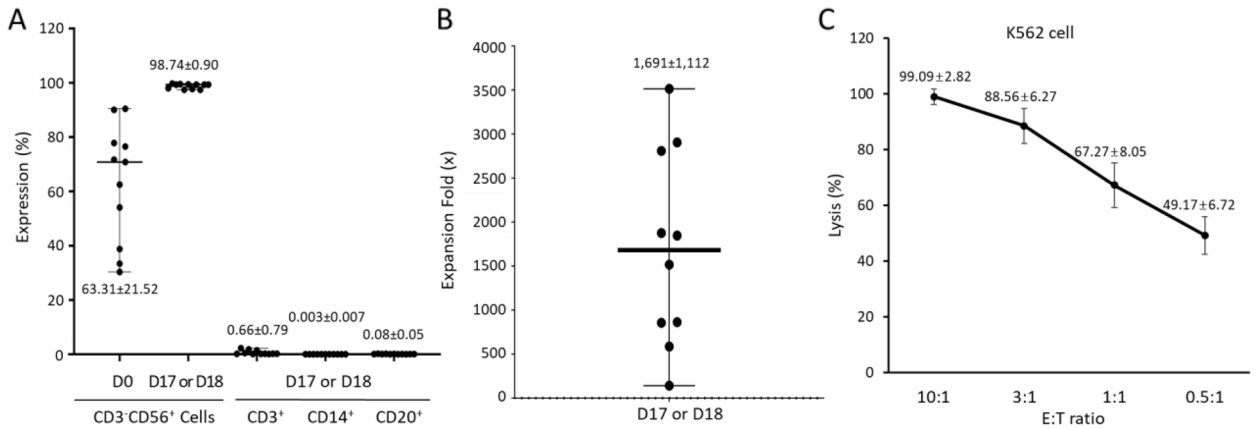

Fig. 5 Characteristics of SNK01 cells. **A** The percentages of $CD3^-CD56^+$ NK cells, CD3 + T cells, $CD20^+$ B cells, and $CD14^+$ monocytes were analyzed flow cytometrically on freshly isolated cells from PBMC using CliniMACS CD56 microbeads (D0; before expansion) and expanded NK cells for 17–18 days of culture (D17-18). **B** The fold expansion of the total cell population after 17–18 days of culture (D17-18). **C** The cytotoxic activity of expanded NK cells against the K562 was measured via calcein-release assay at E:T ratios of 10:1 to 0.5:1 in triplicate. Dots and horizontal bars represent the mean value of each patient from 4 cultures for clinical trial and mean ± SD from all patients, respectively

died before the completion of the study (one subject in Cohort 2; unrelated to study drug; multiple organ dysfunction syndrome). One subject in Cohort 3 (subject 012) did not complete the study. The subject did not attend the end-of-study visit in person due to travel burden. The clinical evaluation questionnaires were

completed remotely, but other study procedures were not performed. All subjects enrolled in the study comprised the Safety, ITT, and DLT populations. The characteristics of the subjects enrolled in the study are provided in Table 1.

All subjects presented with Dementia of the Alzheimer's Type: five (5) enrolled subjects presented with mild, three (3) with moderate, and three (3) with severe AD based on the CDR-SB. By cohort, Cohort 1 included two (2) subjects with mild and two (2) subjects with moderate AD. Cohort 2 included two (2) subjects with mild and two (2) subjects with severe AD. Cohort 3 included one (1) subject with mild, one (1) subject with moderate, and one (1) subject with severe AD. All 11 subjects had an MRI and an FDG-PET assessment performed within the last 6 months or during screening. In all cases, there was positive evidence supporting AD diagnosis and ruling out vascular disease or presence of Lewy body disease. In addition, baseline CSF and plasma biomarkers for the 10 enrolled evaluable subjects, including the CSF Aβ42/Aβ40 ratio, Aβ42, t-Tau, p-Tau 181, and plasma p-Tau 217 aligned with profiles typically seen in classical AD patients. These biomarkers revealed decreased levels of CSF Aβ42 and Aβ42/Aβ40, along with increased levels of CSF t-Tau and p-Tau 181, and with increased levels of plasma p-Tau 217 compared to healthy controls (healthy controls biomarker ranges were obtained from published studies). For concomitant medications, 90.9% of subjects received donepezil, 27.3% memantine, 18.2% rivastigmine, and 9.1% each of the following: citalopram, sertraline, mirtazapine, piracetam, trazodone, and vortioxetine.

Throughout the study, peripheral blood collection was performed 2–3 times per patient in order to produce the required doses of SNK01. All 11 subjects enrolled received at least 1 treatment infusion of SNK01. A total of nine subjects received all four scheduled treatment infusions. Three out of four (3/4) subjects in Cohort 1 and Cohort 2 received all four of the planned number of treatments with SNK01. Out of those who did not receive all four treatments, one subject received three (subject 003 in Cohort 1) and one received two treatments (subject 009 in Cohort 2). Subject 003 did not receive the intended dose at Week 4, and subject 009 received two treatment infusions and died subsequently. All three subjects recruited on the highest dose tested (Cohort 3) were administered the full four doses of SNK01.

Safety assessment

The purpose of this study was to assess the safety and tolerability of SNK01 at different doses in an AD population. The observation period for DLTs was 21 days following the administration of the first SNK01 infusion at a given dose level. Each cohort was also monitored for delayed DLTs. There were no DLTs at any of the doses administered. All subjects were followed for toxicities and adverse events (AEs) during the study and for 12 weeks post-final infusion (Week 22), until death, or discontinuation from the study, whichever occurred first. Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1.

The proportion of subjects who experienced at least one AE was comparable in all cohorts as follows: 75% (3) of subjects in Cohort 1 experienced at least one AE; 100% (4) of subjects in Cohort 2 experienced at least one AE; 100% (3) of subjects in Cohort 3 experienced at least one AE; 90.9% (10) of subjects in all cohorts experienced at least one AE. The proportion of subjects who experienced at least one treatment-emergent adverse event (TEAE) was 75%, 100%, and 66.7% of subjects in Cohorts 1, 2, and 3, respectively. Overall, 81.8% (9/11 subjects) experienced TEAEs on study. The most common TEAEs experienced by subjects were puncture site pain (63.6% [7 subjects]), vessel puncture pain (36.4% [4 subjects]), anemia (36.4% [4 subjects]), and hypertension (18.2% [2 subjects]).

The most frequent TEAEs by System Organ Class (SOC) ($\geq$25% of subjects in any cohort) were as follows: General disorders and administration site conditions (75% [3 subjects], 100% [4 subjects], and 66.7% [2 subjects] in Cohort 1, Cohort 2, and Cohort 3, respectively); Blood and lymphatic system disorders (50.0% [2 subjects] in Cohort 2 and 66.7% [2 subjects] Cohort 3); Infections and infestations (25.0% [1 subject] in Cohort 1 and Cohort 2, each); Vascular disorders (50.0% [2 subjects] in Cohort 2); Nervous system disorders (25.0% [1 subject] in Cohort 2 and 33.3% [1 subject] in Cohort 3); Skin and subcutaneous tissue disorders (25.0% [1 subject] in Cohort 2]).

Only one subject experienced Grade 3–5 AEs (Cohort 2, subject 009), which included multiple organ dysfunction syndrome, sepsis, skin ulcer, and decubitus ulcer. This subject had a fatal event of grade 5, due to multiple organ dysfunction syndrome. The subject was on study for 3.1 weeks and had received two infusions of 2×10^9 cells. The subject died 5 days after the second infusion was administered. None of the events experienced by this subject were deemed related to SNK01. The investigator cited the underlying diabetes mellitus as the alternative etiology. The subject's medical history included mostly controlled diabetes mellitus type 2 (with no ketoacidosis events requiring insulin or changes to oral medications (metformin 500 mg BID) while on study), hypertension, and hypercholesterolemia. Given the association of cardiovascular disease with diabetes mellitus, hypertension,

Table 1 Summary of demographic and baseline characteristics – ITT Population

Parameter	Category/ Statistics	Cohort 1 (*N* = 4)	Cohort 2 (*N* = 4)	Cohort 3 (*N* = 3)	All Patients (*N* = 11)
Age (years)	n	4	4	3	11
	Mean	73.0	76.5	79.3	76.0
	SD	11.40	6.14	5.51	7.96
	Median	78.0	75.5	79.0	79.0
	Min	56	71	74	56
	Max	80	84	85	85
Sex[(a)]	Male, n (%)	1 (25.0)	2 (50.0)	2 (66.7)	5 (45.5)
	Female, n (%)	3 (75.0)	2 (50.0)	1 (33.3)	6 (54.5)
Ethnicity	Hispanic/Latino, n (%)	4 (100)	4 (100)	2 (66.7)	10 (90.9)
	Not Hispanic/Latino, n (%)	0	0	1 (33.3)	1 (9.1)
Race	American Indian or Alaska Native, n (%)	0	0	0	0
	Asian, n (%)	0	0	0	0
	Black or African American, n (%)	0	0	0	0
	Native Hawaiian or Other Pacific, n (%)	0	0	0	0
	White, n (%)	4 (100)	4 (100)	3 (100)	11 (100)
	Other, n (%)	0	0	0	0
CDR-SB score	Median	10.0	11.25	10.0	10.0
	Min	4.5	4.0	6.0	4.0
	Max	13.0	18.0	18.0	18.0
ADAS-Cog score	Median	39.5	33.0	42.0	38.0
	Min	34.0	28.0	37.0	28.0
	Max	43.0	77.0	75.0	77.0
MMSE score	Median	13.5	16.5	14.0	14.0
	Min	13.0	3.0	2.0	2.0
	Max	23.0	21.0	21.0	23.0
ADCOMS score	Median	1.047	1.160	1.054	1.054
	Min	0.700	0.616	0.755	0.615
	Max	1.330	1.701	1.718	1.718
Biomarkers[(b)]					
CSF Aβ42 (pg/mL)	Median	324.16	278.33	352.91	323.23
	Min	198.39	247.54	262.72	198.39
	Max	473.37	503.58	368.57	503.58
CSF Aβ42/40 ratio	Median	0.0429	0.0457	0.042	0.0437
	Min	0.0404	0.0256	0.0379	0.0256
	Max	0.0642	0.056	0.0484	0.0642
CSF t-Tau (pg/mL)	Median	1043.51	830.59	703.72	767.16
	Min	435.47	653.23	702.56	435.47
	Max	1540.21	953.29	1418.55	1540.21
CSF p-Tau 181 (pg/mL)	Median	78.2	51.68	57.21	54.72
	Min	44.48	47.72	48.6	44.48
	Max	177	67.66	137.3	177
Plasma p-Tau217 (pg/mL)	Median	31.48	13.63	17.07	20.5
	Min	17.22	10.28	12.32	10.28
	Max	83.08	26.08	43.41	83.08

SD Standard Deviation

N Number of subjects in analysis population

Percentages are based on N

(a)Percentages are based on Female subjects in the analysis population

(b)Subject 009 (cohort 2) values are not included

and hypercholesterolemia, the patient's medical history serves as the likely alternative etiology for the event.

The majority of TEAEs were of grade 1 severity, clearly related to the procedures linked to blood and CSF collection and were transient in nature. No interruption of infusion or treatment with other medications were required. The principal investigator evaluated these as being clinically insignificant. There were two events of grade 2 severity (localized edema and urinary tract infection). A summary of TEAEs by severity is provided in Table 2. There were no serious adverse events related to SNK01. None of the TEAEs were thought to be related to SNK01. There were no TEAEs leading to SNK01 treatment discontinuation.

Efficacy assessment

To assess the clinical significance of differences in outcomes as measured using the ADAS-Cog, CDR-SB and MMSE scales, the minimal clinically important differences (MCIDs) by AD stage (mild versus moderate/severe), as suggested by Andrews and colleagues [3], were used. In a retrospective analysis of the National Alzheimer's Coordinating Center Uniform Data Set between 09/2005 and 09/2016, the authors correlated the clinicians' assessment of meaningful decline with changes in the cognitive and functional questionnaire scores to derive the MCID. These MCIDs were used to interpret the changes observed in this study for the MMSE, ADAS-Cog and CDR-SB scores. The ADCOMS is a composite measure composed of clinically sensitive items from the ADAS-Cog, the MMSE, and the CDR-SB scales [59]. The ADCOMS scale is thought to be sensitive to changes in cognition during the predementia and early stages of AD. ADCOMS value ranges can help distinguish the AD subject's severity stage. This was highlighted in a retrospective data analysis study by Tahami Monfared et al. [53] who calculated ADCOMS scores for subjects who were enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI) between the years 2003 and 2018. ADCOMS scores were derived for participants at an initial visit and a 24-month follow-up time point, and the change in ADCOMS values was examined. For this

Table 2 Summary of treatment-emergent adverse events by Severity and by Treatment Cohort – Safety Population

System Organ Class (SOC) Preferred Term (PT)	Cohort 1 *N* = 4		Cohort 2 *N* = 4		Cohort 3 *N* = 3		All subjects *N* = 11	
	Gr ≤ 2 n (%)	Gr ≥ 3 n (%)	Gr ≤ 2 n (%)	Gr ≥ 3 n (%)	Gr ≤ 2 n (%)	Gr ≥ 3 n (%)	Gr ≤ 2 n (%)	Gr ≥ 3 n (%)
Subjects with at least one TEAE	3 (75.0)	0	4 (100)	1 (25.0)	2 (66.7)	0	9 (81.8)	1 (9.1)
General disorders and administration site conditions	3 (75.0)	0	4 (100)	1 (25.0)	2 (66.7)	0	9 (81.8)	1 (9.1)
Puncture site pain	2 (50.0)	0	3 (75.0)	0	2 (66.7)	0	7 (63.6)	0
Vessel puncture site pain	0	0	3 (75.0)	0	1 (33.3)	0	4 (36.4)	0
Localised oedema	1 (25.0)	0	0	0	0	0	1 (9.1)	0
Multiple organ dysfunction syndrome	0	0	0	1 (25.0)	0	0	0	1 (9.1)
Blood and lymphatic system disorders	0	0	2 (50.0)	0	2 (66.7)	0	4 (36.4)	0
Anaemia	0	0	2 (50.0)	0	2 (66.7)	0	4 (36.4)	0
Vascular disorders	0	0	2 (50.0)	0	0	0	2 (18.2)	0
Hypertension	0	0	2 (50.0)	0	0	0	2 (18.2)	0
Nervous system disorders	0	0	1 (25.0)	0	1 (33.3)	0	2 (18.2)	0
Lethargy	0	0	1 (25.0)	0	0	0	1 (9.1)	0
Somnolence	0	0	1 (25.0)	0	0	0	1 (9.1)	0
Headache	0	0	0	0	1 (33.3)	0	1 (9.1)	
Infections and infestations	1 (25.0)	0	0	1 (25.0)	0	0	1 (9.1)	1 (9.1)
Urinary tract infection	1 (25.0)	0	0	0	0	0	1 (9.1)	0
Sepsis	0	0	0	1 (25.0)	0	0	0	1 (9.1)
Skin and subcutaneous tissue disorders	0	0	0	1 (25.0)	0	0	0	1 (9.1)
Decubitus ulcer	0	0	0	1 (25.0)	0	0	0	1 (9.1)
Skin ulcer	0	0	0	1 (25.0)	0	0	0	1 (9.1)

N Number of subjects in the analysis population

Percentages are based on N. TEAEs are defined as AEs not present before the start of SNK01-related procedures, or AEs present before and worsened after the first session of SNK01 treatment. If a partially missing date or time of onset allows the possibility that an AE may be a TEAE it will be assumed that it is a TEAE. SOCs and Preferred Terms (PTs) are sorted in descending order for All Subjects, Cohort 1, Cohort 2, and Cohort 3 respectively. Subjects are counted once within each level of summarization (System Organ Class and Preferred Term)

study, the derived composite score was calculated using weighting factors, as provided by Wang et al. [59]. Higher ADCOMS scores were indicative of greater impairment.

Questionnaires were administered at the beginning of the study (Week 1), one week after the last dose (Week 11), and at the end-of-study visit, 12 weeks after the last dose (Week 22). A total of 9 out of 11 subjects completed the questionnaires for all three time points. Two subjects in Cohort 2 did not complete the questionnaires for all time points (subject 009 died shortly after the second treatment infusion, and subject 006 was not assessable for week 22). This subject presented with somnolence and lethargy (unrelated to study treatment) at the visit and evaluations were suspended.

For the CDR-SB questionnaire, baseline mean sum of boxes scores were 10.55 for all subjects (range: 4.0–18.0; median: 10.0), 9.38 for Cohort 1 (range: 4.5–13.0; median: 10.00), 11.13 for Cohort 2 (range: 4.0–18.0; median: 11.25) and 11.33 for Cohort 3 (range: 6.0–18.0; median: 10.00). At Week 11 (1 week after the last treatment infusion), the mean sum of boxes scores was 9.75 for all subjects (range: 3.5–18.0; median: 7.25), 9.63 for Cohort 1 (range: 3.5–15.0; median: 10.00), 9.67 for Cohort 2 (range: 5.0–18.0; median: 6.0) and 10.00 for Cohort 3 (range: 5.5–18.0; median: 6.50). At end of study (Week 22, or early termination), the mean sum of boxes scores was 9.89 for all subjects (range: 4.0–18.0; median: 7.5), 10.75 for Cohort 1 (range: 4.0–16.0; median: 11.50), 7.00 for Cohort 2 (range: 6.5–7.5; median: 7.00) and 10.67 for Cohort 3 (range: 7.0–18.0; median: 7.00).

When evaluating individual subject responses, the following MCIDs were used to denote a decline in cognition and function: a change of $\geq +2$ for mild AD subjects; a change of $\geq +2$ for moderate/severe AD subjects. At Week 11, data was available for 10/11 subjects. Of these, 7/10 (70%) subjects showed an improvement or remained stable in the CDR-SB (3 subjects in Cohort 1, 1 subject in Cohort 2, and 3 subjects in Cohort 3). One subject in Cohort 3 (subject 014) showed a clear improvement in score at Week 11. The rest of the subjects evaluated (3) showed an increase in the sum of scores of 2.0 points and, thus, were considered to decline in function.

At Week 22, a point at which the subjects had been off of study treatment for 12 weeks, data was available for 9/11 subjects. Of these, 6/9 (66.7%) subjects had stable or improved their sum of boxes scores compared to baseline (≤ 2 point difference from baseline). Amongst the subjects with stable or improved scores, 3 subjects had mild AD, 2 subjects had moderate and 1 subject had severe AD at baseline, where the 1 subject with moderate AD improved most (subject 014); see Table 3 for individual subject results and Fig. 6 for the change from baseline for individual subjects. One subject who was considered stable at Week 11 showed an overall decline at Week 22 compared to baseline (subject 004 in Cohort 1). Three subjects were deemed to have declined at the last time point evaluated (change from baseline ≥ 2).

For the ADAS-Cog (12 item) questionnaire, baseline mean scores were 43.73 for all subjects (range: 28.0–77.0; median: 38.0), 39.0 for Cohort 1 (range: 34.0–43.0; median: 39.5), 42.8 for Cohort 2 (range: 28.0–77.0; median: 33.0) and 51.3 for Cohort 3 (range: 37.0–75.0; median: 42.0). At Week 11 (1 week after the last treatment infusion), the mean scores were 42.1 for all subjects (range: 28.0–78.0; median: 34.5), 38.3 for Cohort 1 (range: 29.0–44.0; median: 40.0), 41.3 for Cohort 2

Table 3 CDR-SB Results by Subject and Visit– ITT Population

Cohort	Subject	AD Severity Stage	Baseline	Week 11/ Visit 5		Week 22/ EOS	
			Score	Score	CFB	Score	CFB
1	**002**	moderate	13	15	2	16	3
	003	moderate	12	12	0	12	0
	004	mild	8	8	0	11	3
	005	mild	4.5	3.5	-1	4	-0.5
2	**006**	severe	16	18	2	np	
	007	mild	6.5	5	-1.5	7.5	1
	009	severe	18	np		np	
	011	mild	4	6	2	6.5	2.5
3	**012**	severe	18	18	0	18	0
	014	moderate	10	5.5	-4.5	7	-3
	015	mild	6	6.5	0.5	7	1
Medians (IQR)			10 (6.0,16.0)	7.25 (5.4,15.8)	0.0 (-1.1, 2.0)	7.5 (6.8,14.0)	1.0 (-0.3, 2.8)

CFB change from baseline, *np* not performed, *IQR* interquartile range

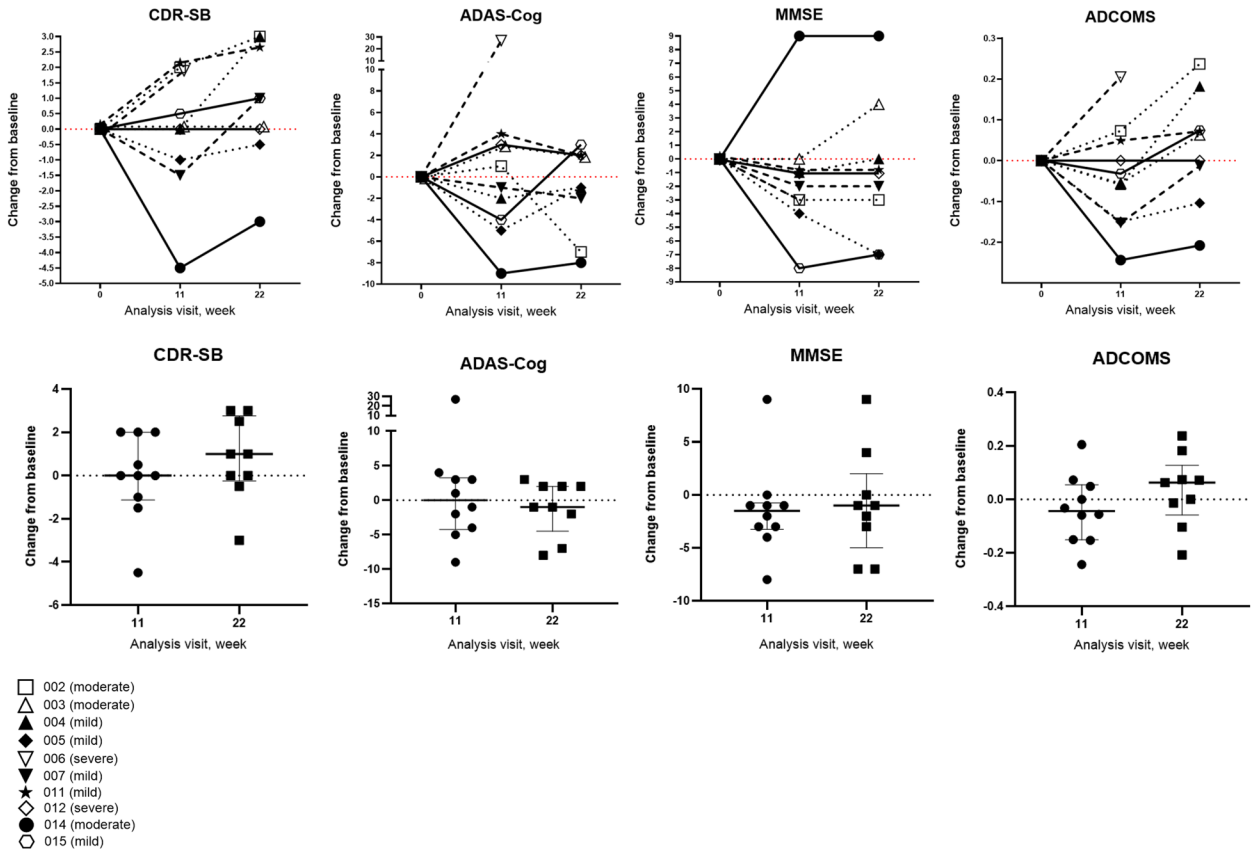

Fig. 6 Cognitive Assessment Changes from Baseline Score. Cognitive assessments measured at Baseline, at Week 11 (1 week after the last treatment infusion), and at the end of the study (Week 22, or early termination), expressed as a change from baseline scores for each assessment scale. Top panels: individual subject plots. Dotted lines are subjects in Cohort 1 (1×10^9 cells), dashed lines are subjects in Cohort 2 (2×10^9 cells) and solid lines are subjects in Cohort 3 (4×10^9 cells). Subject 6 did not complete the Week 22 assessment. Bottom panels: medians with interquartile ranges

(range: 28.0–64.0; median: 32.0) and 48.0 for Cohort 3 (range: 33.0–78.0; median: 33.0). At end of study (Week 22, or early termination), for those subjects for whom scores were available, the mean scores were 39.7 for all subjects (range: 27.0–77.0; median: 36.0), 37.3 for Cohort 1 (range: 33.0–43.0; median: 36.5), 28.5 for Cohort 2 (range: 27.0–30.0; median: 28.5) and 50.3 for Cohort 3 (range: 34.0–77.0; median: 40.0).

When evaluating individual subject responses, the following MCIDs were used to denote a cognitive decline: a change of $\geq +3$ for mild AD subjects; a change of $\geq +4$ for moderate/severe AD subjects. At Week 11, data was available for 10/11 subjects. Of these, 3/10 (33.3%) subjects showed an improvement in the overall ADAS-Cog score of at least 4 points (1 subject in Cohort 1, and 2 subjects in Cohort 3). Five subjects (50%) were stable at Week 11 (3 in Cohort 1, 1 in Cohort 2, 1 in Cohort 3). The remaining two subjects showed a decline at Week 11 of more than 3 or 4 points. At Week 22, data was available for 9/11 subjects. Of these, 2/9 (22.2%) subjects improved their final score compared to baseline (1 in Cohort 1 and 1 in Cohort 3) and 6/9 (66.7%) could be said to remain stable (3 in Cohort 1, 2 in Cohort 2 and 1 in Cohort 3). Amongst the subjects with stable or improved scores, 4 subjects had mild AD, 3 subjects had moderate and 1 subject had severe AD at study start, where the 2 subjects with moderate AD improved most (subjects 002 and 014); see Table 4 for individual subject results and Fig. 6 for the change from baseline for individual subjects. One subject (015) who appeared improved at week 11 showed a subsequent worsening in the score at week 22 and one subject clearly declined at week 11 (subject 006).

For the MMSE questionnaire (MMSE-2:SV), baseline mean MMSE total raw scores were 14.27 for all subjects (range: 2.0–23.0; median: 14.0), 15.8 for Cohort 1 (range: 13.0–23.0; median: 13.5), 14.3 for Cohort 2 (range: 3.0–21.0; median:16.5) and 12.3 for Cohort 3 (range: 2.0–21.0; median: 14.0). At Week 11 (1 week after the last treatment infusion), the mean scores were 13.9 for all subjects (range: 1.0–22.0; median: 13.0), 13.8 for Cohort 1 (range: 11–19; median: 12.50), 16.0 for Cohort 2 (range: 11–20; median: 17.0) and 12.3 for Cohort 3 (range: 1.0–23.0;

Table 4 ADAS-Cog Questionnaire Final Score[a] results by subject and visit– ITT population

Cohort	Subject	AD Severity Stage	Baseline	Week 11/ Visit 5		Week 22/ EOS	
			Score	Score	CFB	Score	CFB
1	**002**	moderate	43	44	1	36	-7
	003	moderate	41	44	3	43	2
	004	mild	38	36	-2	37	-1
	005	mild	34	29	-5	33	-1
2	**006**	severe	37	64	27	np	
	007	mild	29	28	-1	27	-2
	009	severe	77	np		np	
	011	mild	28	32	4	30	2
3	**012**	severe	75	78	3	77	2
	014	moderate	42	33	-9	34	-8
	015	mild	37	33	-4	40	3
Medians (IQR)			38.0 (24.0,43.0)	34.5 (31.3,49.0)	0.0 (-4.3, 3.3)	36.0 (31.5,41.5)	-1.0 (-4.5, 2.0)

CFB Change from baseline, *np* not performed, *IQR* Interquartile range

[a] This study utilized ADAS-Cog 12 item, and the total score ranges from 0 to 80, where higher scores signify more significant cognitive impairment

median: 13.0). At end of study (Week 22, or early termination), for those subjects for whom scores were available, the mean scores were 14.67 for all subjects (range: 1.0–23.0; median: 16.0), 14.3 for Cohort 1 (range: 11.0–17.0; median: 14.5), 18.5 for Cohort 2 (range:17.0–20.0; median: 18.5) and 12.7 for Cohort 3 (range: 1.0–23.0; median: 14.0).

When evaluating individual subject responses, the following MCIDs were used to denote a decline: a change of ≤ -2 for mild AD subjects; a change of ≤ -3 for moderate/severe AD subjects. At Week 11, data was available for 10/11 subjects. Of these, 5/10 (50%) subjects (2 subjects in Cohort 1, 1, subject in Cohort 2, 2 subjects in Cohort 3) were deemed to be stable or improved (change from baseline ≤ -1), with one subject in Cohort 3 showing an improved (+8) total raw score. Five subjects showed a decline in MMSE total raw score. At Week 22, data was available for 9/11 subjects. Of these, 5/9 (55.5%) subjects were deemed to remain stable or improved in their MMSE final raw scores compared to baseline. These were the same subjects that were stable/improved at Week 11. Amongst the subjects with stable or improved scores, 2 subjects had mild AD, 2 subjects had moderate and 1 subject had severe AD at baseline, where the 2 subjects with moderate AD improved most by Week 22; see Table 5. for individual subject results and Fig. 6 for the change from baseline for individual subjects.

For the composite ADCOMS score in all subjects at baseline, the mean was 1.117 (range: 0.615–1.718; median: 1.054). For subjects classified (using CDR-SB) as presenting with mild AD, ADCOMS values were between 0.615 and 0.901, moderate AD subjects had values between 1.054 and 1.330, while subjects with severe AD had ADCOMS values between 1.460 and 1.718. By Cohort, the mean ADCOMS values at baseline were 1.031 for Cohort 1 (range: 0.700–1.330; median: 1.047), 1.159 for Cohort 2 (range: 0.615–1.701; median: 1.160), and 1.176 for Cohort 3 (range: 0.755–1.718; median: 1.054). At Week 11 (1 week after the last treatment infusion), mean ADCOMS values were 0.983 for Cohort 1 (range: 0.550–1.403; median: 0.990), 1.012 for Cohort 2 (range: 0.664–1.665; median:0.706) and 1.084 for Cohort 3 (range: 0.723–1.718; median: 0.810). At end of study (Week 22, or early termination), for those subjects for whom scores were available, mean ADCOMS values were 1.126 for Cohort 1 (range: 0.596–1.567; median: 1.170), 0.767 for Cohort 2 (range: 0.687–0.846; median: 0.766) and 1.131 for Cohort 3 (range: 0.829–1.718; median: 846).

The ADCOMS cut-off for meaningful change has not been defined in this subject population over a 22-week time period. Tahami Monfared et al. [53] showed that changes in the ADCOMS score are sensitive to the state of cognition of an individual. As proposed by this group, a change in ADCOMS score of 0.1 can be considered a clinically meaningful change. The following can then be said about the subjects in this study: At Week 11, data was available for 10/11 subjects. Of these, 3/10 (30%) of subjects showed an improved change score of ≥ 0.100,two subjects with mild AD (1 from Cohort 1, and 1 from Cohort 2) and one subject with moderate AD (Cohort 3). Six subjects (60%) appeared to be stable (change score < 0.100) and one subject from Cohort 2 showed a clear increase in

Table 5 MMSE Total Raw Score by Subject and Visit– ITT Population

Cohort	Subject	AD Severity Stage	Baseline	Week 11/ Visit 5		Week 22/ EOS	
			Score	Score	CFB	Score	CFB
1	**002**	moderate	14	11	-3	11	-3
	003	moderate	13	13	0	17	4
	004	mild	13	12	-1	13	0
	005	mild	23	19	-4	16	-7
2	**006**	severe	14	11	-3	np	
	007	mild	19	17	-2	17	-2
	009	severe	3	np		np	
	011	mild	21	20	-1	20	-1
3	**012**	severe	2	1	-1	1	-1
	014	moderate	14	23	9	23	9
	015	mild	21	13	-8	14	-7
Medians (IQR)			14.0 (13.0,21.0)	13.0 (11.0,19.3)	-1.5 (-3.3,-0.8)	16.0 (12.0,18.5)	-1.0 (-5.0, 2.0)

CFB: change from baseline; np: not performed; IQR: interquartile range

the ADCOMS value of 0.205. At Week 22, data was available for 9/11 subjects. Of these, 2/9 (22.2%) still showed an improved change score of ≥ 0.100; one subject with mild AD (1 from Cohort 1) and one subject with moderate AD (Cohort 3). These two subjects were the same subjects that showed improvements at week 11. Five subjects (55.6%) appeared to be stable (change score < 0.100) and one subject from Cohort 1, who appeared stable at week 11, showed a clear increase in the ADCOMS value of 0.237 compared to baseline; see Table 6 for individual subject results and Fig. 6 for the change from baseline for individual subjects.

The subject with the most improved scores at week 11 and week 22 was subject 014 (Cohort 3), who presented with moderate AD at baseline. This subject had an ADCOMS value of 1.054 at baseline, which dropped to 0.810 at week 11 and 0.846 at week 22, which is well within the ADCOMS scores noted in the subjects characterized as mild (range: 0.615–0.901) at study onset.

Biomarkers

Biomarkers in the CSF were measured at baseline, Week 11 and Week 22. In addition to protein biomarkers (Aβ42, Aβ42/40, total Tau, pTau181, pTau217, α-synuclein), neuroinflammation and neuronal damage biomarkers (GFAP, YKL-40, NfL) were also analyzed.

Studies have suggested a close relationship between α-synuclein and AD. A-synuclein may directly interact

Table 6 ADCOMS by Subject and Visit– ITT Population

Cohort	Subject	AD Severity Stage	Baseline	Week 11/ Visit 5		Week 22/ EOS	
			Score	Score	CFB	Score	CFB
1	**002**	moderate	1.330	1.403	0.073	1.567	0.237
	003	moderate	1.193	1.137	-0.056	1.256	0.063
	004	mild	0.901	0.842	-0.059	1.083	0.182
	005	mild	0.700	0.550	-0.150	0.596	-0.104
2	**006**	severe	1.460	1.665	0.205	np	
	007	mild	0.859	0.706	-0.153	0.846	-0.013
	009	severe	1.701	np		np	
	011	mild	0.615	0.664	0.049	0.687	0.072
3	**012**	severe	1.718	1.718	0	1.718	0
	014	moderate	1.054	0.810	-0.244	0.846	-0.208
	015	mild	0.755	0.723	-0.032	0.829	0.074
Medians (IQR)			1.054 (0.755, 1.460)	0.826 (0.696, 1.469)	-0.044 (-0.151, 0.055)	0.846 (0.758, 1.412)	0.063 (-0.059, 0.128)

CFB Change from baseline, *np* not performed, *IQR* Interquartile range

with Aβ and tau causing aggregation and exacerbating cognitive decline. In AD, α-syn and Tau levels in CSF were both elevated and revealed a high positive correlation [51]. In our study, 60–70% of subjects showed decreases in α-synuclein and pTau181 levels (at Week 11, at Week 22, or at both time points) which are potentially associated with less of a disease burden (see Fig. 7), whereas positive changes (increases for Aβ or decreases for Tau) for CSF levels of Aβ-42, Aβ42/Aβ40 ratio, total Tau, and pTau217 were seen in 40–50% of subjects. These findings were observed fairly evenly across the three cohorts (Cohorts 1, 2, and 3). Although it is not known what the clinically relevant changes of these protein biomarkers are in this population given the lack of a placebo group, the changes observed seem to support an effect of SNK01 on protein aggregates in the CNS.

Glial fibrillary acidic protein (GFAP) is an intermediate filament structural protein involved in cytoskeleton assembly and integrity, expressed in high abundance in activated glial cells. Neuronal stress, caused by either disease or injury, evokes astrocyte activation as a response, including hypertrophy, proliferation, and increased GFAP expression. GFAP is a marker of reactive astrogliosis that increases CSF and blood of individuals with AD [22]. YKL-40 (Chitinase 3-like I) is elevated in the brain and CSF in several neurological and neurodegenerative diseases associated with inflammatory processes. YKL-40 quantification might have a potential for application in the evaluation of therapeutic intervention in dementias with a neuroinflammatory component [34]. In our study, decreases from baseline values in the neuroinflammatory markers GFAP and YKL-40 were also observed in 60% of subjects at Week 11 and at the end of study (Week 22). Similar to findings with the structural protein biomarkers above, these changes were observed in all cohorts relatively evenly. Although it is not known what the clinically relevant changes in any of these biomarkers are in this

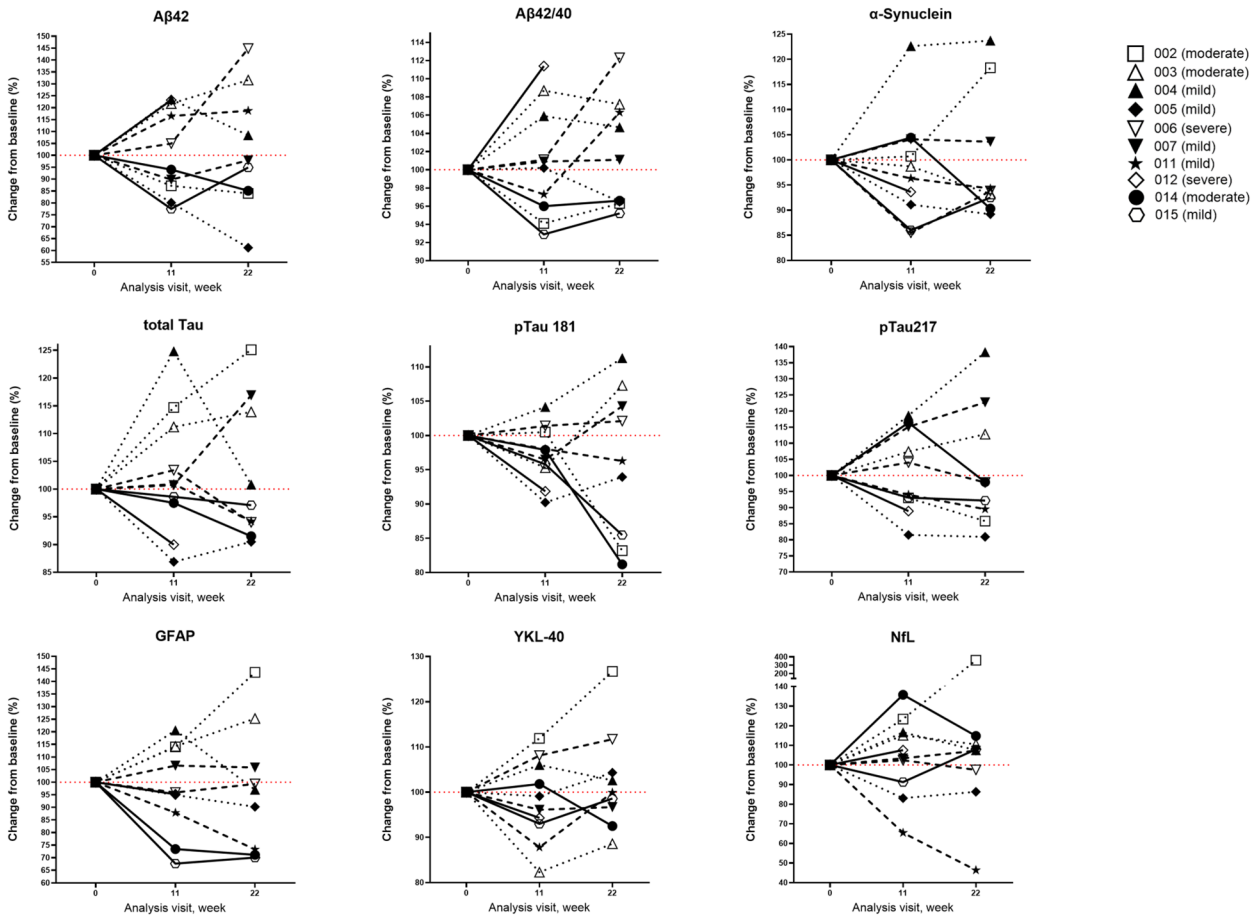

Fig. 7 CSF Biomarkers Percent Changes from Baseline. Biomarkers in the CSF, measured at Baseline, at Week 11 (1 week after the last treatment infusion), and at the end of the study (Week 22, or early termination), expressed as a percentage of baseline values. Dotted lines are subjects in Cohort 1 (1×10^9 cells), dashed lines are subjects in Cohort 2 (2×10^9 cells) and solid lines are subjects in Cohort 3 (4×10^9 cells). Subject 12 did not complete the Week 22 assessment

Table 7 Relationship of Biomarker level changes to changes in ADCOMS scores at week 11 compared to baseline

Cohort	Subject	AD Severity Stage	ADCOMS score CFB	Biomarkers CFB								
				Aβ-42	Aβ-42/40	Tau (total)	Tau (pT181)	Tau (pT217)	α-Synuclein	GFAP	YKL-40	NfL
1	**002**	moderate	N	D	D	I	I	D	I	I	I	I
	003	moderate	N	I	I	I	D	I	D	I	D	I
	004	mild	N	I	I	I	I	I	I	I	I	I
	005	mild	D	D	D	D	D	D	D	D	D	D
2	**006**	severe	I	I	I	I	I	I	D	D	I	I
	007	mild	D	D	D	I	D	I	I	I	D	I
	011	mild	N	I	D	I	D	D	D	D	D	D
3	**012**	severe	N	I	I	D	D	D	D	D	D	I
	014	moderate	D	D	D	D	D	I	I	D	I	I
	015	mild	N	D	D	D	D	D	D	D	D	D
Percent of subjects with stable/ decrease in ADCOMS with positive[a] biomarker changes				44.4	33.3	44.4	77.8	55.6	55.6	55.6	66.7	33.3

CFB Change from baseline, *N* no change from baseline, *I* Increase from baseline, *D* Decrease from baseline

[a] A positive biomarker change is an increase for Aβ-42, Aβ42/Aβ40 ratio and a decrease for all other markers

population given the lack of a placebo group, the changes observed seem to support a reduced or stable inflammatory state following treatment with SNK01.

At week 11, when relating the biomarker changes from baseline (increased or decreased, without relating this to clinical relevance) to the composite ADCOMS scores for each individual subject (using a change of 0.1 in ADCOMS score as clinically relevant), Table 7 shows the proportion of subjects where the changes in ADCOMS may also be reflected in changes in several of the biomarkers. Of those subjects with stable or decreased ADCOMS at week 11, a high proportion have decreased pTau181, and YKL-40 and a little more than half have decreased pTau217, α-synuclein and GFAP. At week 22, as shown in Table 8, subjects who remain stable or continue to have decreased ADCOMS scores 12 weeks after the last dose, appear to continue to have less neuroinflammation (GFAP/YKL-40) and lower tau/synuclein burden, with no apparent effect on Aβ-42 or Aβ42/Aβ40 ratio.

Discussion

The mechanism of action of NK cells in neurodegenerative diseases is likely multifactorial and is not yet clearly understood. Our in vitro work, and the work of others, has started to shed some light on the potential involvement of NK cells in the homeostatic control of inflammation in the CNS and in the clearing of protein aggregates found in AD. It is speculated that NK cells may be beneficial in the AD brain via inhibition of neuroinflammation by stimulating protective microglia, suppressing proinflammatory microglia, inactivating inflammatory cells, removing neurotoxic aggregates, and killing activated T cells.

Microglial hyperactivation has been established as a significant factor associated with the pathogenesis of neurodegenerative disorders. Anti-inflammatory cytokines may play a role in regulating microglial hyperactivation. In vitro studies have demonstrated that IL-10 plays a pivotal role in attenuating this hyperactivation, particularly through the inhibition of proinflammatory cytokine production by microglia and other glial cells [35]. In the CNS, IL-10 is mainly produced by astrocytes, microglia, and neurons, and it is capable of attenuating the expression of pro-inflammatory cytokines such as IL-1β, IL-12, TNF-α, adhesion molecules [60]. Recent studies indicate that the loss of IL-10 can result in increased Tau hyperphosphorylation, neurotoxicity, and IL-6 overexpression in mice when exposed to lipopolysaccharide (LPS)[62]. Therefore, IL-10 is of paramount importance in targeting therapy for neurodegenerative diseases. IL-10 produced by various immune cells, including T and B lymphocytes, macrophages, and certain non-hematopoietic cells, assumes a central role in immunoregulation and anti-inflammatory responses. It is also produced by both human and mouse NK cells in response to diverse microbial stimuli [10, 17, 32]. NK cells' secretion of IL-10 may lead to the inactivation of inflammatory cells and thus to a balancing of the ratio of protective microglia to proinflammatory microglia in CNS disorders [6].

Similarly, TGF-β (which can be secreted by NK cells) has been suggested to have a similar role as an anti-inflammatory cytokine, and in the AD brain, appears to have an immunomodulatory and neuroprotective action [28]. TGF-β regulates a wide variety of cellular responses, including cell growth arrest, apoptosis, cell differentiation, motility, invasion, extracellular matrix production, tissue fibrosis, angiogenesis, and immune function. Notably, TGF-β deficiency in mice can result in the loss of microglia and the absence of the typical ramified morphology of microglia, indicating that TGF-β plays a crucial role in microglial function [12]. In addition, TGF-β regulates microglial homeostatic molecular and functional signatures in the brain [39]. Moreover, TGF-β1 is a neurotrophic factor responsible for the initiation and maintenance of neuronal differentiation and synaptic plasticity. Deficiencies in TGF-β1 signaling are linked to Aβ pathology and the formation of neurofibrillary tangles in AD animal models, suggesting the potential benefit of restoring TGF-β1 signaling as a strategy to decelerate the neurodegenerative process in AD [13]. Therefore, TGF-β may provide insights into microglial biology and the possibility of targeting microglia for the treatment of AD.

As mentioned previously, the pathological characteristics of AD present extracellular accumulation of Aβ peptides that aggregate into plaques and intraneuronal hyper-phosphorylation of the Tau protein which generates NFTs [44]. In the CNS, $CD56^{+}$ NK cells were increased in the cerebrospinal fluid (CSF) of AD patients and positively correlated with Aβ levels [11]. A recent study reported that NK cell depletion can alleviate neuroinflammation, loss of neural progenitors and cognitive deficits in triple transgenic (3×Tg-AD) mice, although the Aβ deposits were not significantly altered [67], suggesting an inhibitory role of NK cells in CNS inflammation and neurodegeneration in AD.

A-syn is a neuronal protein that regulates synaptic vesicle trafficking and subsequent neurotransmitter release. However, insoluble α-syn aggregates accumulate as inclusions in Lewy bodies in Parkinson's disease (PD) and other synucleinopathies. A-syn is also involved in the pathophysiology of AD [55]. Lewy-related pathology (LRP), primarily comprised of α-syn, is present in a majority of autopsied AD brains, and higher levels of α-syn in the cerebrospinal fluid (CSF) of patients with mild cognitive impairment (MCI) and AD have been

Table 8 Relationship of biomarker level changes to changes in ADCOMS scores at week 22 compared to baseline

Cohort	Subject	AD Severity Stage	ADCOMS score CFB	Biomarkers CFB								
				Aβ-42	Aβ-42/40	Tau (total)	Tau (pT181)	Tau (pT217)	α-Synuclein	GFAP	YKL-40	NfL
1	**002**	moderate	I	D	D	I	D	D	I	I	I	I
	003	moderate	N	I	I	I	I	I	D	I	D	I
	004	mild	I	I	I	I	I	I	I	D	I	I
	005	mild	D	D	D	D	D	D	D	D	I	D
2	**006**	severe										
	007	mild	N	D	I	I	I	I	I	I	D	I
	011	mild	N	I	I	D	D	D	D	D	D	D
3	**012**	severe										
	014	moderate	D	D	D	D	D	D	D	D	D	I
	015	mild	N	D	D	D	D	D	D	D	D	I
Percent of subjects with stable/ decrease in ADCOMS with positive[a] biomarker changes				33.3	50.0	66.7	66.7	66.7	83.3	66.7	83.3	33.3

CFB Change from baseline, *N* No change from baseline, *I* Increase from baseline, *D* Decrease from baseline

[a] A positive biomarker change is an increase for Aβ-42, Aβ42/Aβ40 ratio and a decrease for all other markers

No ADCOMS available for subject 006 and no biomarkers available for subject 012 at week 22

linked to cognitive decline [56]. Recent studies also suggest that the asymptomatic accumulation of Aβ plaques is associated with higher CSF α-syn levels in subjects at risk of sporadic AD and in individuals carrying autosomal dominant AD mutations,experimental evidence has further linked α-syn mainly to Tau hyperphosphorylation, but also to the pathological actions of Aβ and the APOEε4 allele, the latter being a major genetic risk factor for both AD and dementia with Lewy bodies [55]. Earls and colleagues have recently reported that human NK cells can efficiently internalize and degrade α-syn aggregates via the endosomal/lysosomal pathway [20]. This has been proposed as a protective mechanism of NK cells against α-synucleinopathy by removing harmful α-synuclein aggregates from the patient's brain. In a murine model of AD [26], murine-expanded NK cells injected into these mice resulted in decreased Aβ deposits, and reduced escape latency in the Morris test compared to controls, with microglial cells from the treated group showing an upregulation of genes related to phagocytic activity.

The chronic deposit of neuronal harmful protein aggregates has been found to elicit a pro-inflammatory dysregulation with increased T cell autoreactivity to amyloid [38], Tau [15] or synuclein [33] proteins. The inflammation induces the recruitment of peripheral NK cells to the CNS via chemokines produced by neurons (CX3CL1) and microglia (CCL2 and CXCL10) [50]. In the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS), NK cells migrate to the mouse brain in a CX3CL1-dependent manner, displaying early anti-inflammatory effects and heightened cytotoxicity against myelin proteolipid protein (PLP) autoreactive T cells [64]. Moreover, in vivo NK cell depletion in EAE leads to disease exacerbation, with increased autoreactive T cell proliferation and inflammation [65]. NK cells also can inhibit the activation of T helper 17 cell (Th17) signature transcription factors in pathogenic T cells by microglia, thereby decreasing neuroinflammation [25]. NK cells negatively regulate T cell responses by recognizing and killing activated CD4 + and CD8 + T cells in vitro, perforin-dependent mechanisms, and increased expression of DNAM1 and NKG2D ligands [5, 24, 36, 40, 45].

Reports on NK cell numbers in AD patients or the proper function of cells differ according to the research team. NK cell distribution in AD was described as presenting no changes compared to age-matched elderly healthy subjects [46], with increased activity [52] or a decrease in number, cytotoxic effects or activity [4, 43]. The diversity of NK cells from samples in patients with AD and healthy control subjects was assessed using web-based single-cell RNA sequencing or flow cytometry [43]. There were no differences in age or gender between groups. The researchers found both reduced cell number and cytotoxic activity of blood NK cells in AD. Although this study was performed using samples from a small sample size of 10 patients, changes in the transcriptomic profile of peripheral NK cells suggest their involvement in AD pathology [43]. This study opens the door to the possibility of restoring the number and function of NK cells in patients with AD.

We have shown in vitro that our expanded NK cells can kill activated T cells, secrete anti-inflammatory cytokines such as IL-10 and TGF-β, and internalize and degrade both Aβ and α-syn aggregates. To the best of our knowledge, this is the first report of NK cells' ability to internalize and degrade Aβ aggregates. SNK01 is a novel cell-based, patient-specific ex vivo expanded autologous NK cell immunotherapy that exhibits high CD56 expression, degranulation, cytokine secretion and cytotoxicity, with high expression of cell surface activating receptors such as NKG2D, NKp30, NKp44 and NKp46, which are expressed at high levels in both regulatory and cytotoxic NK cells [42].

In the present study, SNK01 was used to treat ten (evaluable) AD subjects (mild, moderate, and severe) for 10 weeks (once every three weeks for four doses). Doses ranged from 1 to 4×10^9 cells and no treatment-related adverse events were reported. As this was primarily a safety study, the primary endpoint was achieved. Preliminary efficacy data was also available for this study and suggested that treatment with autologous expanded NK cells may stabilize or improve cognitive function, improve protein aggregate levels, and reduce neuroinflammation.

Despite limitations such as a small sample size, short treatment duration, and lack of placebo control, the results suggest that NK cell treatment is having some effect in the CNS of AD subjects. Despite 70% of subjects being treated at relatively low doses of SNK01 (1 or 2×10^9 cells), 50–70% of all subjects in the trial had either stable or improved CDR-SB, ADAS-Cog and/or MMSE scores at one-week post-treatment (week 11). Using the composite ADCOMS score, 90% of subjects were stable or improved (30% improved, 60% stable) at week 11. The recent report on the phase 3 lecanemab trial [57] showed that all subjects (placebo or lecanemab-treated) had decreases in ADCOMS scores even at the 3-month time point, a time point analogous to our 10-week treatment duration. Although our population included moderate and severe AD, unlike the lecanemab mild cognitive impairment and mild AD population, our data seems to suggest that NK cell therapy may not only slow progression but may have at least a stabilizing effect. This will need to be elucidated in comparison to a placebo control in a follow-up efficacy study.

The present study demonstrated the ability of adoptive NK cell therapy to potentially have a beneficial effect on protein aggregate levels in the CNS of AD subjects:

at week 11 (one week after the last dose of SNK01), an increase in CSF Aβ42 and Aβ42/40 was seen in 40% and 50% of subjects, respectively (in AD, Aβ42 and Aβ42/40 are reduced in the CSF; [2]). For the other proteins, where a decrease in the CSF is seen to be beneficial, 50% of subjects (5/10) had a decrease in CSF pTau217, 70% (7/10) had decreased CSF pTau181 and 60% (6/10) had a decrease in CSF α-syn compared to baseline values after 4 doses of NK cell therapy. The decrease in pTau181 appears to be dose-dependent as all 3 subjects given the highest dose (4×10^9 cells) showed decreased levels after NK cell therapy. However, given that there was no placebo control, the within-subject differences cannot at this point be directly related to study drug – we can only observe the changes from baseline after treatment, which are suggestive of a potential action of the NK cell therapy.

This study also provided preliminary CSF biomarker evidence of the ability of NK cell therapy to reduce neuroinflammation in some AD subjects: 60% (6/10) had a decrease in GFAP and YKL-40 at week 11 (one week after the last dose). This effect also appeared to be dose-dependent as all 3 subjects given the highest dose showed decreases in GFAP and 2 of these showed decreases in YKL-40.

Interestingly, at week 22, 12 weeks after the last dose, some of the biomarkers remained changed compared to baseline, despite being off treatment for that period of time. This decrease was noted for CSF pTau217 for all subjects (4), for CSF pTau181 in 5/7 subjects, for α-syn in 5/6 subjects, for GFAP for all subjects (5), and for YKL-40 for 4/5 subjects. It is difficult to understand what lasting changes a 4-dose therapy could have resulted in; however, this finding is intriguing and begs further research.

Finally, when relating the changes in CSF biomarkers to the cognitive changes seen at week 11 (one week after the last dose), the decreases in pTau181 corresponded to stable/decreased CDR-SB scores in 5/8 subjects, for pTau217 in 2/5 subjects, for α-syn in 3/6 subjects for GFAP in 3/6 subjects and for YKL-40 in 4/6 subjects (data not shown). For ADCOMS scores, where 90% of subjects had stable or decreased scores at week 11, corresponding changes were seen for Aβ42/40 in 3/9 subjects, for Aβ42 and total Tau in 4/9 subjects, for pTau217, α-syn and GFAP in 5/9 subjects, for YKL-40 in 6/9 subjects and for pTau181 in 7/9 subjects (see Table 7). Given the different doses used in this study (with 7/10 of subjects receiving the lower doses), and a 10-week NK cell treatment, it will be interesting to see if biomarker changes will be reflected in cognitive changes when all subjects are provided higher doses for a longer period of time.

Limitations

The limitations of the present study include a small sample size, short treatment duration and lack of placebo control. Furthermore, although within-patient cognitive changes from baseline can be assessed based on the scores, it is unclear what the clinically relevant changes in biomarkers in this population are without having a placebo comparison group. Given the small size of the study and the lack of a placebo, the effects on both the cognitive assessments and the biomarkers will need to be confirmed in a larger study with longer treatment durations, at the highest (or higher) dose levels. A North American study has begun recruiting moderate AD subjects treated with SNK01 at a dose of 6×10^9 cells for a 12-month duration in comparison to placebo treatment (NCT 06189963).

Conclusions

The present study strove to elucidate the potential mechanism of action of NK cells in AD subjects and was able to demonstrate that adoptive NK cell therapy was well tolerated in these subjects. This study also suggested that NK cell therapy using SNK01 expanded autologous NK cells was able to reduce neuroinflammation and have a positive effect on brain protein aggregates (as suggested by CSF biomarker levels), which appeared to translate into a stabilization or even an improvement in cognitive function.

Given the role of the innate immune system in AD pathology, a reduction in neuroinflammation as a therapeutic strategy may complement the current available anti-Aβ antibodies. Since AD is a complex disease, it stands to reason that a multi-pronged approach to treatment which includes NK cell adoptive therapy, and potentially other anti-inflammatory therapeutic agents, may be able to stabilize or even improve this disease beyond the effect on slowing progression seen with the anti-Aβ antibodies. The additional ability of NK cell therapy to potentially cause degradation of protein aggregates suggests that this treatment on its own may have therapeutic and/or disease-modifying benefits.

Abbreviations

°C	Celsius
Aβ	Beta-amyloid
A-syn/ α-syn	Alpha-synuclein
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's Disease Assessment Scale – Cognitive Subscale
ADCOMS	Alzheimer's Disease Composite Score
ADNI	Alzheimer's Disease Neuroimaging Initiative
AE	Adverse event
CCL	Chemokine (C–C motif) ligand
CD	Cluster of differentiation
CDR-SB	Clinical Dementia Rating Scale – Sum of Boxes
CNS	Central nervous system
CRS	Cytokine release syndrome
CSF	Cerebrospinal fluid
DLT	Dose-limiting toxicity
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FDA	Food and Drug Administration (U.S.)
FDG	Fluorodeoxyglucose
GCP	Good Clinical Practice
GFAP	Glial fibrillary acidic protein
HIV	Human immunodeficiency virus

HMC3	Human microglia clone 3
HRP	Horse-radish peroxidase
IFN-γ	Interferon-gamma
IL	Interleukin
ITT	Intention-to-Treat
IU	International units
LPS	Lipopolysaccharide
MCI	Mild cognitive impairment
MCID	Minimal clinically important difference
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
MMSE	Mini-mental State Evaluation
MRI	Magnetic resonance imaging
MSD	Meso Scale Discovery
MTD	Maximum tolerated dose
N, n	Number
NfL	Neurofilament light
NFT	Neurofibrillary tangles
NK	Natural killer cells
PD	Parkinson's Disease
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PET	Positron emission tomography
PMA	Phorbol myristate acetate
RP2D	Recommended Phase 2 dose
RPMI 1640	Roswell Park Memorial Institute Medium 1640
SD	Standard deviation
SOC	System Organ Class
SRC	Safety review committee
TEAE	Treatment emergent adverse event
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
YKL-40	Chitinase 3-like I
REC	Research Ethics Committee
COFEPRIS	Federal Commission for the Protection against Sanitary Risk

Supplementary Information

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Supplementary Figure S1. Lysosomal Pathway Involvement in Intracellular Aβ Aggregate Degradation in SNK01 Cells: (A) Co-localization of Aβ aggregates with LAMP1 in SNK01 cells. SNK01 cells were treated with 5 μM Aβ for 3 hours and then stained with anti-CD56 (green), anti-Aβ (yellow), and anti-LAMP1 (red) antibodies to assess the co-localization of Aβ (yellow) and LAMP1, with CD56 as a surface marker of SNK01 cells. Aβ internalized and co-localized with LAMP1 in SNK01 cell is indicated by white arrows. (B-C) Western blot and densitometry analysis of Aβ aggregate degradation was performed on SNK01 cells treated with 5 μM Aβ aggregates, with or without the lysosomal pathway inhibitor chloroquine (CQ). SNK01 cells were treated with 5 μM Aβ aggregates for 1 hour, washed three times with PBS, and then incubated in fresh medium with or without 25 μM CQ for 6 and 24 hours. Intracellular levels of Aβ aggregates were subsequently analyzed by Western blot (B) and quantified using ImageJ (C). The measured values were normalized to the internal control β-actin levels and expressed as relative values with the 0-hour value, which was set to 1.

Supplementary Material 1. Gel images for Figures 1 and 2

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Authors' contributions
CHZG was the principal investigator, RMD and CAA were the sub-investigators and BIAG was the study coordinator at the site. PYS was the study medical monitor and was involved in the interpretation of the data. PYC was involved in the interpretation of the data. LH and SH were involved in biomarker analysis and interpretation of the data. KB was involved in the interpretation of the data and authoring the manuscript. MG, YR, SY, MK, and YMK were involved in the preclinical studies. All authors read and approved the final manuscript.

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Data availability
All data generated or analysed during this study for cognitive assessment scores are included in this published article. Additional datasets generated or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The protocol was reviewed by the institution's research board (Research Ethics Committee of Tijuana Angeles Hospital, registration number 02 CEI 003 20180322, approval received May 7, 2020). All patients signed informed consent prior to study enrolment.

Consent for publication
Not applicable.

Competing interests
Authors PYS, KB, PYC, LH, and SH are all current or past employees and shareholders of NKGen Biotech, Inc. which was the sponsor of this trial. Authors MG, YR, SY, MK, and YMK are all employees of NKMAX Co Ltd, a shareholder of NKGen Biotech, Inc.

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