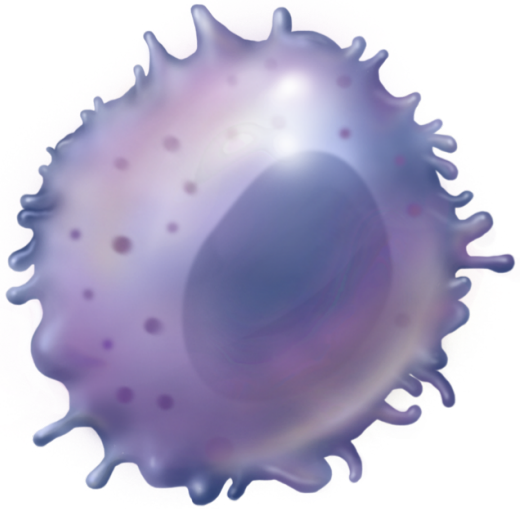
SNK01 for Neurodegenerative Disease

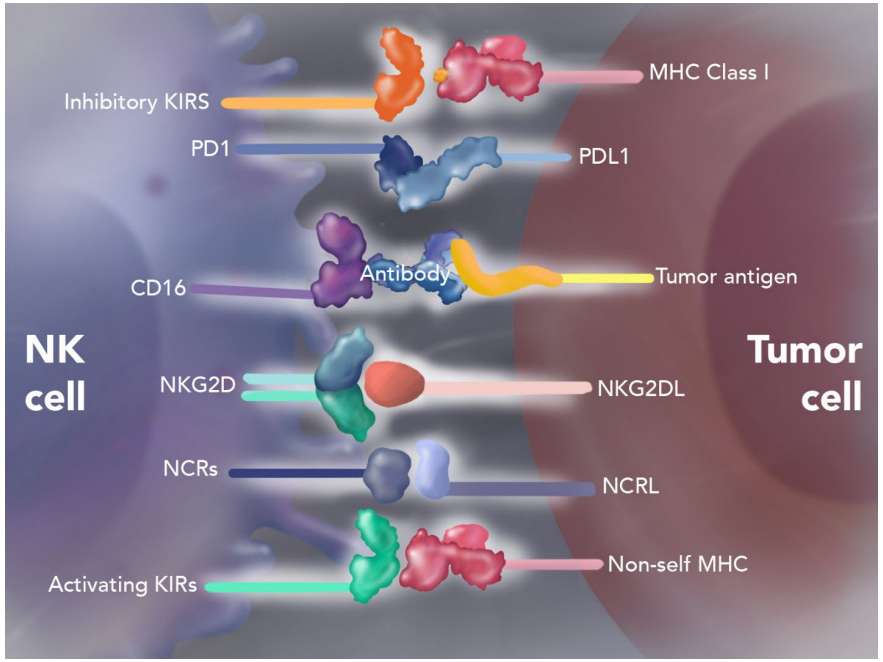
Paul Y. Song, MD

NKGEN BIOTECH

April 2024

Natural Killer Cells





Innate Lymphoid Cells:

5-20% of circulating lymphocytes (CD3- CD56+)

Can Distinguish Healthy Cells (self) From Dangerous Cells (non-self):

Ability to identify and eliminate “dangerous” cells

Mediate antibody-dependent cellular cytotoxicity (ADCC)

Immune regulatory capabilities, mediated by secreted cytokines

Natural Killers cells can get weaker with aging or stress.

Weak and/or deficient NK cells have been shown to be correlated with various disease conditions.

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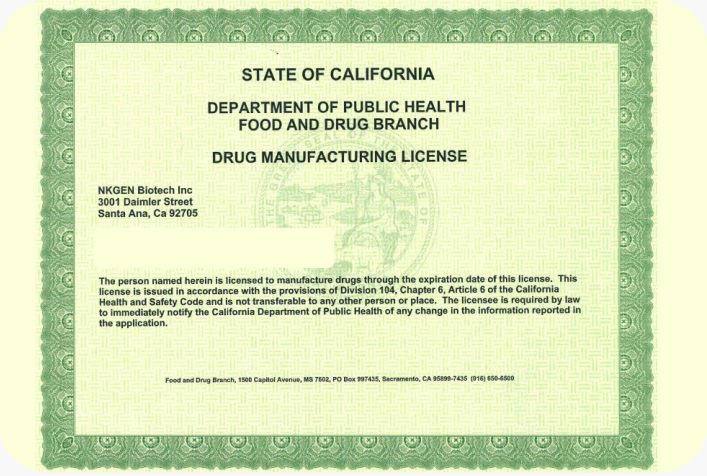
Liu, S., Galat, V., Galat4, Y. et al. NK cell-based cancer immunotherapy: from basic biology to clinical development. J Hematol Oncol 14, 7 (2021). https://doi.org/10.1186/s13045-020-01014-w

State-Of-The-Art GMP Manufacturing Facility

Licensed cell therapy manufacturing facility

- 25,000 sq ft facility (12,000 sq ft for GMP) completed in 2019
- CAP/CLIA Laboratory
- Facility owned and operated by NKGen Biotech


STATE OF CALIFORNIA

DEPARTMENT OF PUBLIC HEALTH
FOOD AND DRUG BRANCH

DRUG MANUFACTURING LICENSE

NKGEN Biotech Inc
3001 Daimler Street
Santa Ana, Ca 92705

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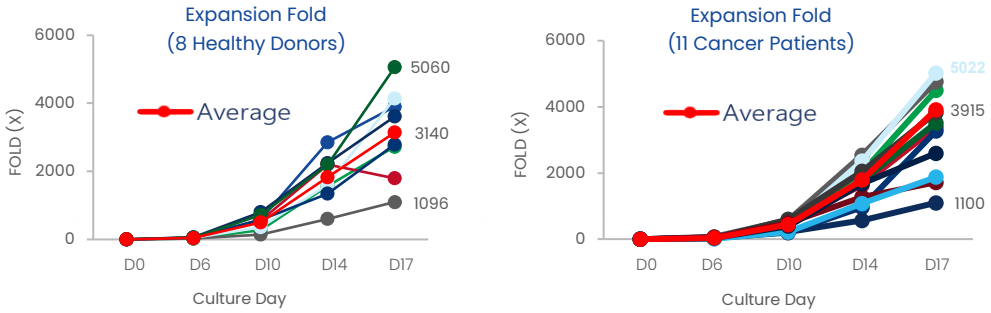
Food and Drug Branch, 1500 Capitol Avenue, MS 7602, PO Box 997435, Sacramento, CA 95899-7435 (916) 650-6500

NKGen's Manufacturing (CMC) Process results in Super NK cells

Superior Cell Expansion, Increased Cytotoxicity, and Increased Activating Receptor Expression

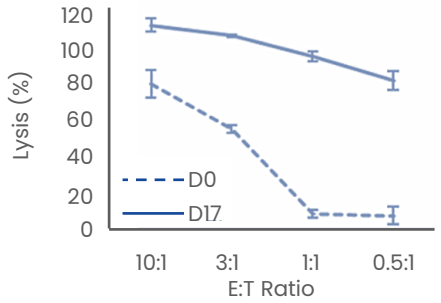
Expansion

NKGen can expand NK cells from any donor!



Cytotoxicity

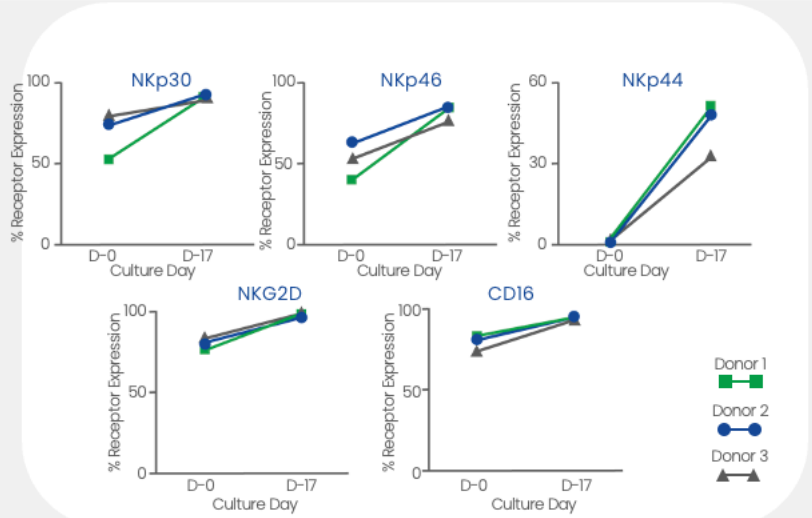
NKGen increases NK cell killing potential!



E:T Ratio = NK Cell : Cancer Cell Ratio
Target Cell = Myelogenous Leukemia

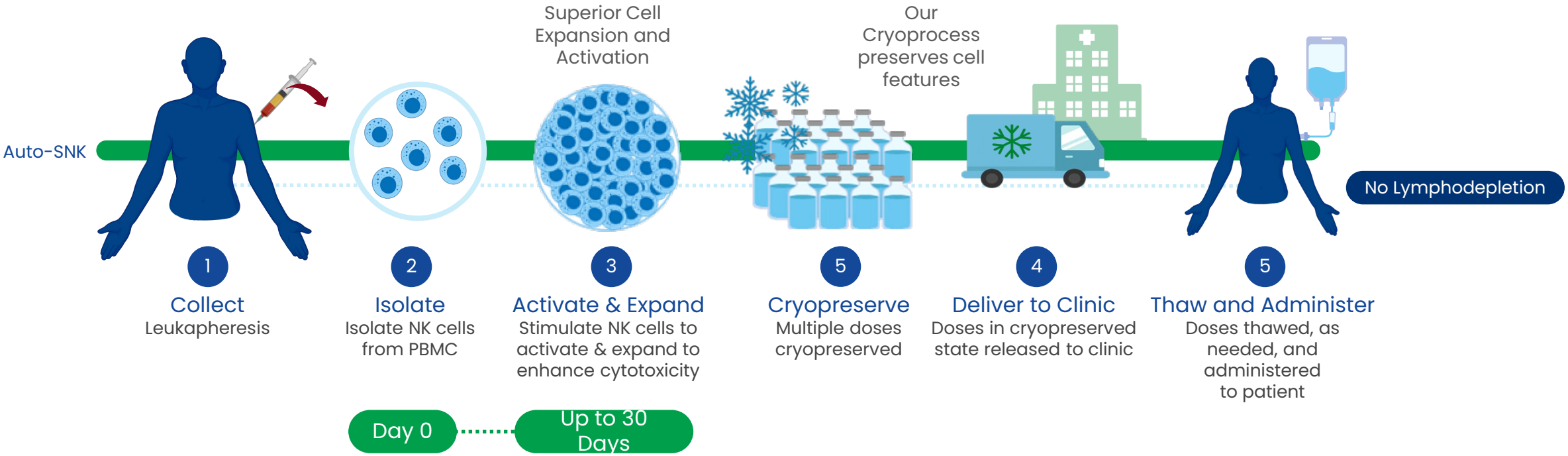
Receptor Expression Levels

NKGen increases receptor expression!



Autologous Next Gen Manufacturing

NKGen "Off-the-Shelf Autologous" SNK Cell Therapy



- Cryopreserved autologous manufacturing process takes ~ 30 days from NK cell isolation to cryopreserved product release
- **One-time production for all the doses; SNK cells are frozen and stored on site, ready for release**
- Ability to produce multiple doses (6 x 10^9 cells each) from single leukapheresis to fulfill 4–6 months of weekly treatments
- Multiple doses are produced at once; approx. 20 doses=1 batch release and cryopreserved
- Cryopreserved autologous process fully developed

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SNK01 – Mechanism of Action in Neurodegenerative Disease

Market Opportunity in Neurodegenerative Disease

NKGen's SNK01, not yet FDA approved, will seek to address a multi-billion/year market

Over 55 million people worldwide had **dementia in 2023**, with a new case diagnosed every 3 seconds; nearly 10 million new cases every year[1]

Alzheimer's and Parkinson's patients are estimated to account for **~46 million** of the 55 million people with dementia worldwide[2]

We are aware of no therapies currently on the market that **halt or reverse progression of AD or PD**

The annual global **cost** of dementia is **>$1.3 trillion USD**[1]

Global **Parkinson's and Alzheimer's** disease therapeutics **markets forecast to be ~$16.8 billion** by 2029[2]

Evidence of actual cognitive improvement in early trials and compassionate use AD and PD patients treated with SNK01[3]

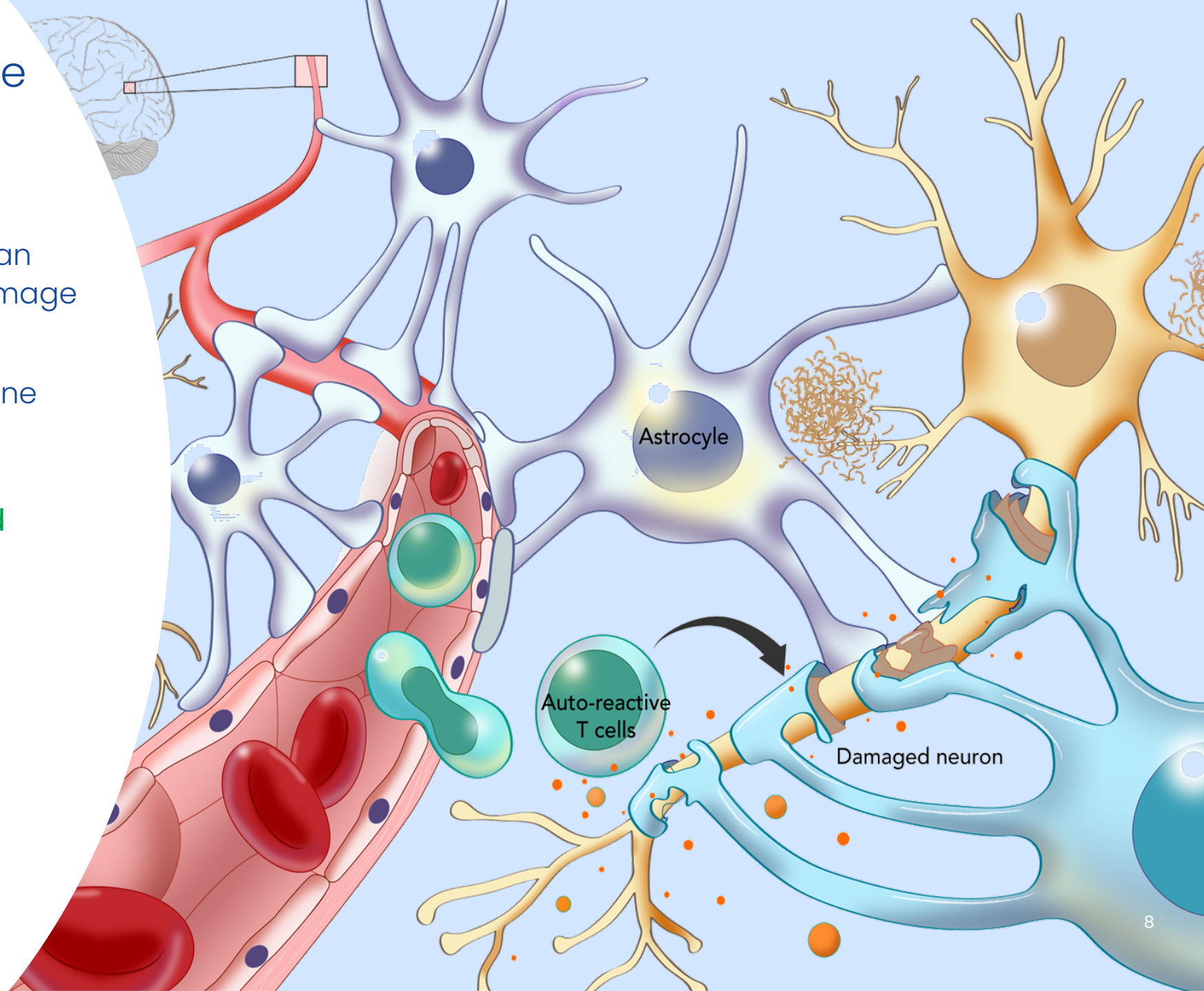
1. Dementia Statistics published by World Health Organization, Newsroom, Dementia Fact Sheet, on March 15, 2023.
2. "Global Alzheimer's Disease Market $6.3 Billion by 2029" and "Global Parkinson's Disease Therapeutics Market $10.5 Billion by 2029", February 22, 2023 by iHealthcareAnalyst, Inc.
3. Reports of patient experiences from the compassionate use case studies have not been verified or validated. Only controlled clinical trials can support benefit for patients. These compassionate case study results and early trials may not be predictive of clinical trial results and cannot be used to establish safety or efficacy for regulatory approval.

Autoreactive T cells Cause Neuroinflammation And Damage

Chronic protein deposition leads to an autoinflammatory cascade and damage

Removing proteins only addresses one aspect of the underlying pathology

Activation of **Autoreactive CD4+ and CD8+ T cells**[1-5] which migrate to the brain via **CXCR3**[6]



1. Lindestam Arlehamn - NATURE Communications (2020) 11:1875 1-11.
2. Stojić-Vukanić Z - Front Immunol (2020) 11: 566225.
3. Monsonego - J. Clin. Invest. (2003) 112:415–422.
4. Machhi - Journal of Neuroinflammation (2021) 18:272.
5. Heneka - Lancet Neurol. (2015) 14(4): 388–405.
6. Zhou - Current Neuropharmacology, (2019) 17:142-150

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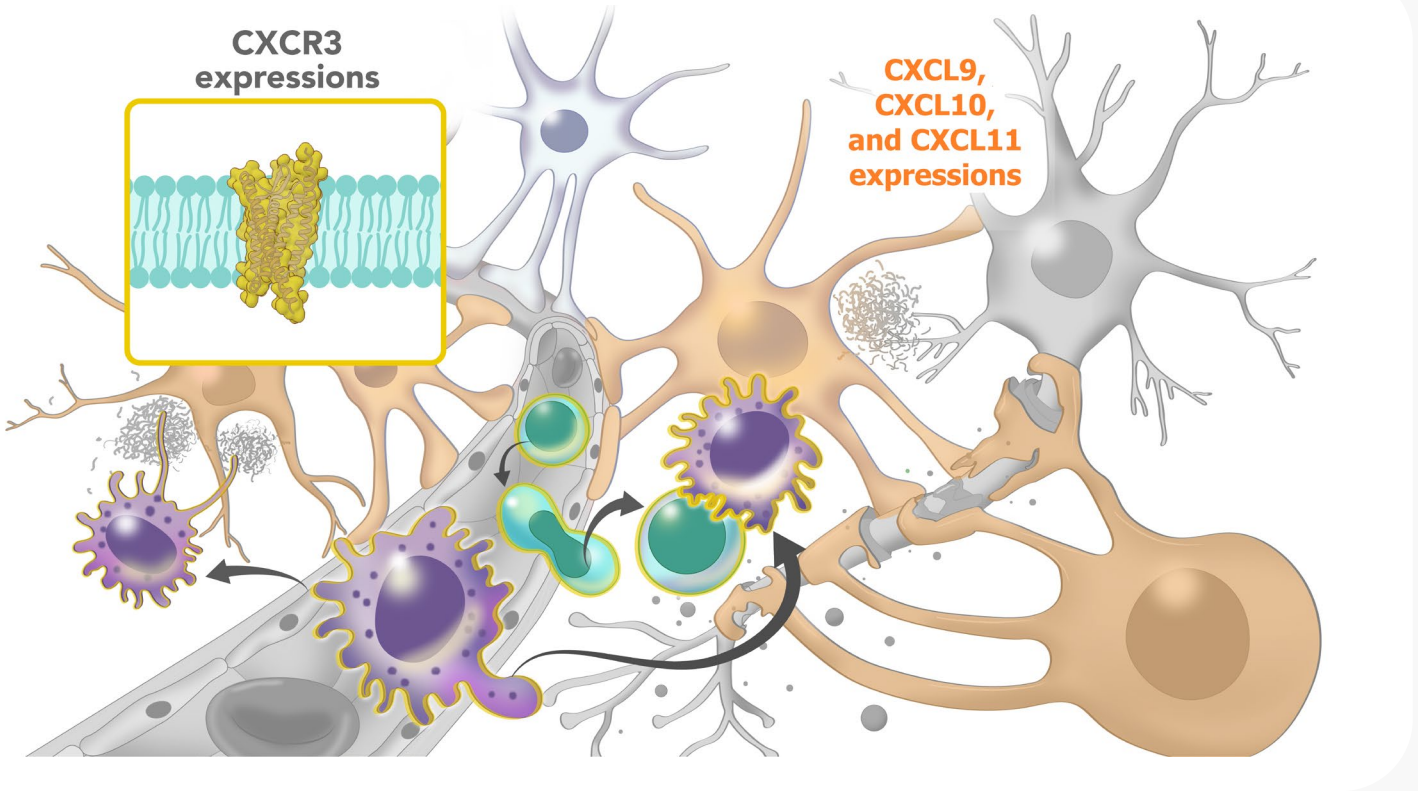
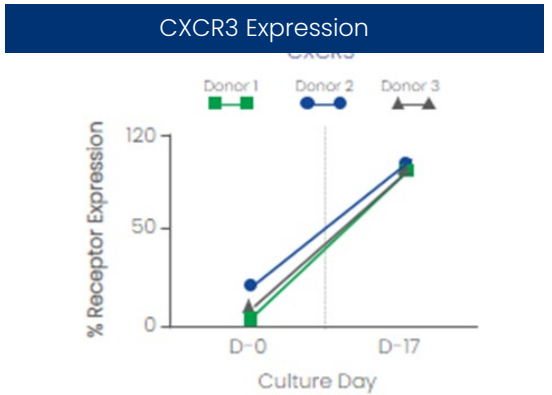
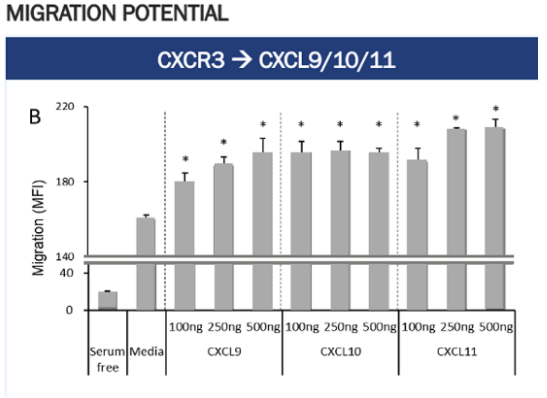
Autoreactive T cells And SNK01 Cross BBB (Blood Brain Barrier) Via CXCR3

SNK01 Has High CXCR3 Expression And Strong Migration Potential to cross the BBB







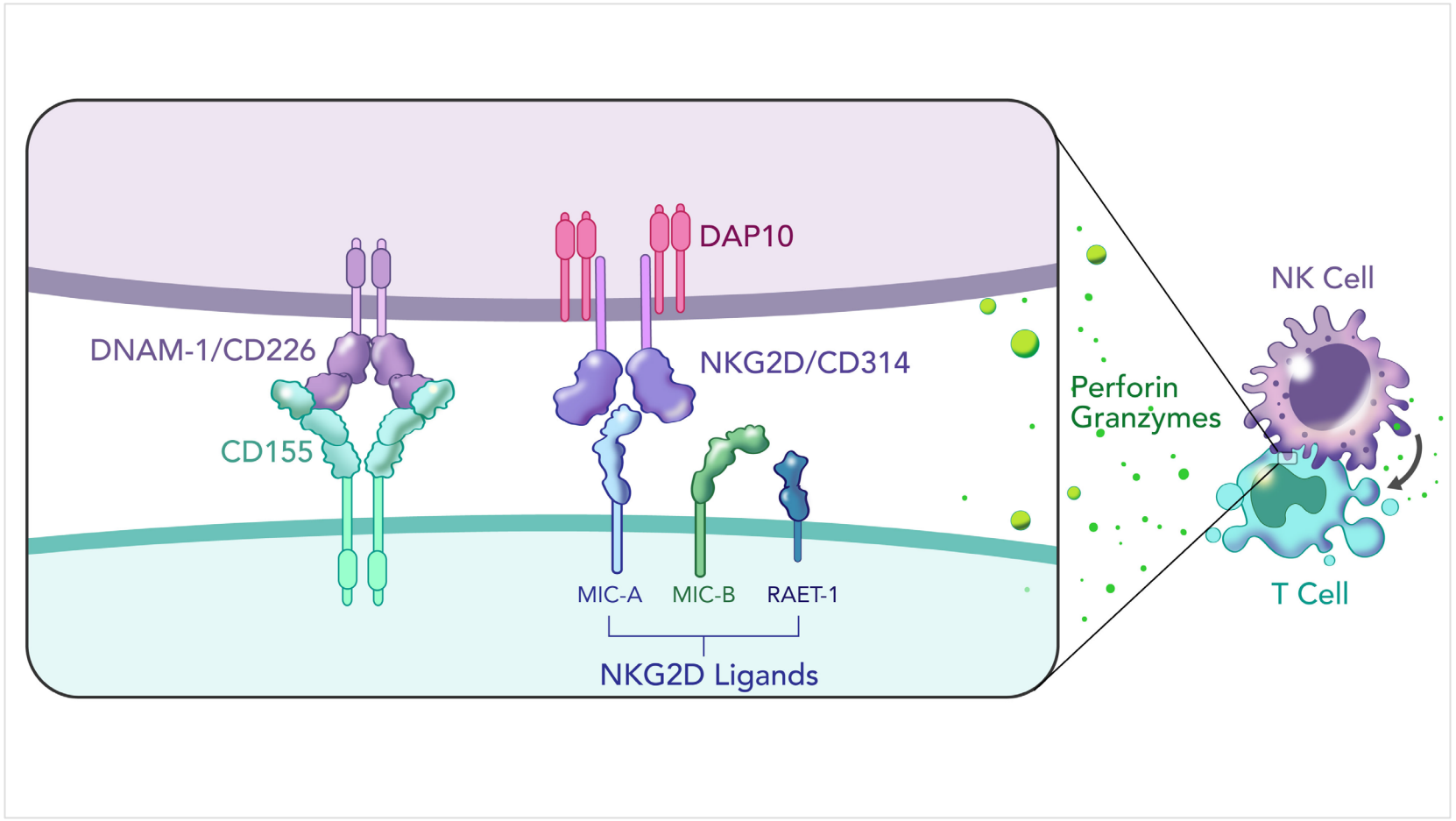
CXCR3+ T cells migrate to CXCL10 positive astrocytes that frequently are associated with amyloid deposits.[1]

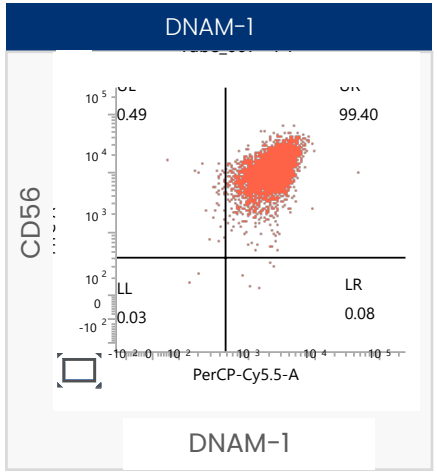
CXCR3 was highly expressed on a subpopulation of neurons and neuronal processes in the neocortex, hippocampus, striatum, cerebellum, and spinal cord.[1]

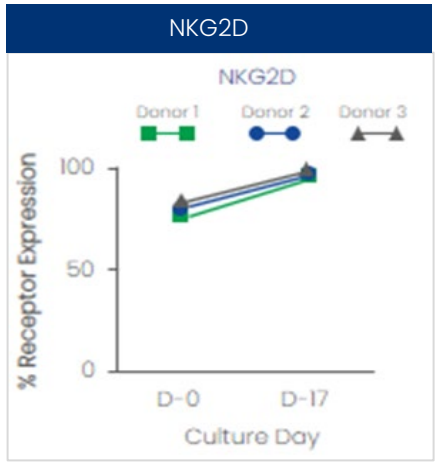
1. Xia - Neuroimmunol., 2000, 108(1-2), 227-235.

NK Cells Regulate Autoreactive T cells[1-4]

SNK01 Has Enhanced NKG2D and DNAM-1 Expression that helps identify and eliminate autoreactive T cells to reduce auto-immune inflammation.







1. Rabinovich - J Immunol (2003) 170 (7): 3572–3576.
2. Lu - Immunity. 2007 May ; 26(5): 593–604.
3. Nielsen - PLoS ONE 7(2): e31959.
4. Ardolino - Blood (2011) 117 (18): 4778–4786.

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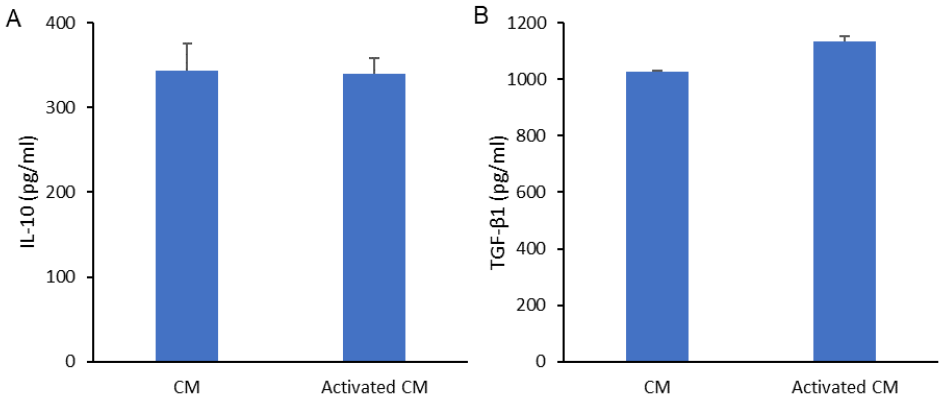
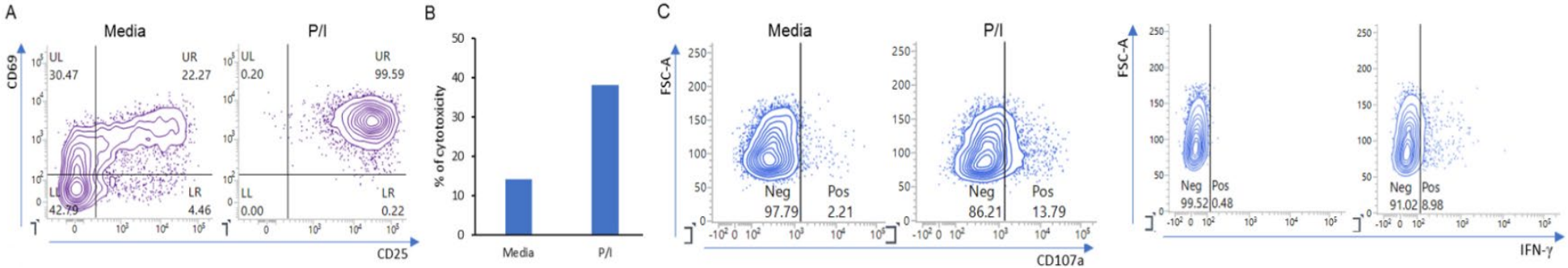
Level of immunosuppressive cytokines in conditioned media of SNK01 cells



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 hours 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).

Cytotoxic and degranulation activity and IFN-γ expression level of SNK01 cells when incubated with activated T cells



(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 hours. (C) Degranulation activity and cytokine 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 or IFN-γ in CD56+ population was assessed by flow cytometry after 5- or 3- hour incubation, respectively.

Weak or Deficient NK cells common in Active Autoimmune Disease

Table 1
Evidences of NK cell involvement in immune-mediated diseases as emerging from experimental models and human diseases.

Disease	Murine models (NK depletion)	Human diseases	
		Peripheral blood	Tissue site
Rheumatoid Arthritis	Protective/promoting	Reduced levels, impaired activity	Synovial membrane
Spondyloarthritis	Nd[a]	Impaired activity	Nd
Psoriasis	Nd	Reduced levels, impaired activity	Psoriatic skin lesion
Inflammatory bowel diseases	Protective	Normal levels, impaired activity	Colonic lamina propria
Diabetes	Protective	Reduced levels	Nd
Systemic lupus erythematosus	Protective	Reduced levels, impaired activity	Nd
Anti-phospholipid syndrome	Nd	Increased levels	Nd
Multiple sclerosis	Protective/promoting	Reduced levels, impaired activity	Nd
Myasthenia Gravis	Promoting	Reduced levels, impaired activity	Nd

[a] Nd: not determined.

Schleinitz - Immunology, 131, 451–458

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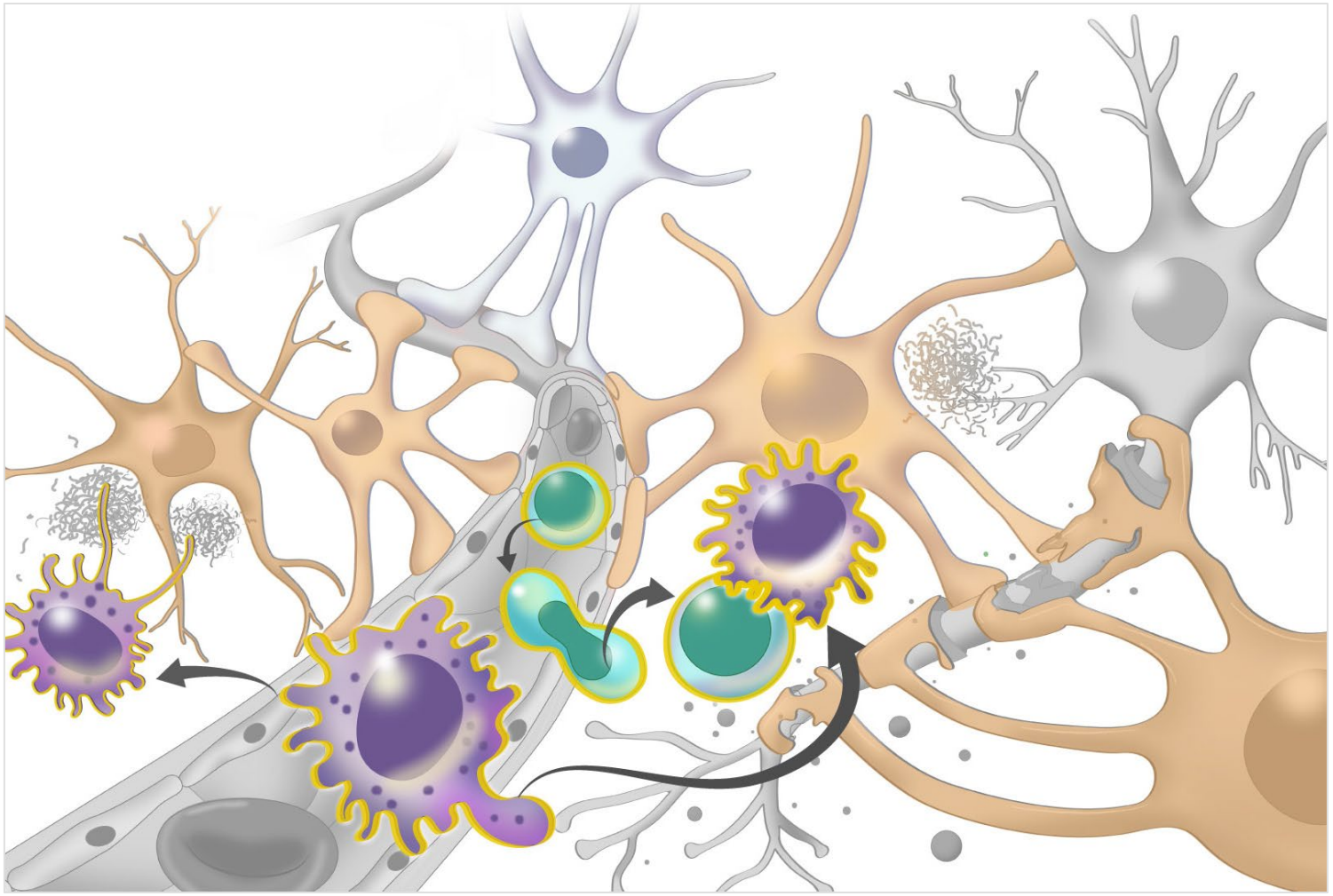
SNK01 Can Reduce Amyloid, p-Tau, Alpha-Synuclein, and Neuroinflammation



NK cells have been found to **reduce protein accumulation**[1,2]

NK cells have also been found to **identify and degenerate intact sensory axons** after nerve injury[3]

1. Earls - PNAS - January 2020 117 (3) 1762-1771.
2. Marsh et al. PNAS February 2016 -E1317.
3. Davies et al., 2019, Cell 176, 716–728.

NK Cells Play a Protective Role in Protein Deposition

NK cells clear α-synuclein and the depletion of NK cells exacerbates synuclein pathology in a mouse model of α-synucleinopathy

Rachael H. Earls[a,1], Kelly B. Menees[a,1], Jaegwon Chung[a,1], Claire-Anne Gutekunst[b], Hyun Joon Lee[c,d], Manuel G. Hazim[b], Balázs Rada[e], Levi B. Wood[f,g], and Jae-Kyung Lee[a,2]

[a]Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602; [b]Department of Neurosurgery, Emory University School of Medicine, Atlanta, GA 30322; [c]Department of Neurology, University of Mississippi Medical Center, Jackson, MS 39216; [d]Research Service, G.V. (Sonny) Montgomery VA Medical Center, Jackson, MS 39216; [e]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602; [f]Coulter Department of Biomedical Engineering, Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA 30332; and [g]Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332

Significance

Parkinson's disease, Lewy body dementia, and other synucleinopathies are characterized by the accumulation of abnormally aggregated α-synuclein (α-syn) protein, which is the principal component of Lewy bodies. Immunotherapeutic approaches aimed at halting the propagation of both central and peripheral extracellular α-syn aggregates may be a promising therapeutic avenue for synucleinopathies. Our study demonstrates that natural killer (NK) cells act as efficient scavengers of abnormal α-syn aggregates without becoming aberrantly activated. We demonstrate that systemic depletion of NK cells significantly exacerbates synuclein pathology. Overall, our results provide strong evidence for a protective role of NK cells in synuclein-related neurodegenerative diseases.

The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function

Samuel E. Marsh[a,b], Edsel M. Abud[a,b,1], Anita Lakatos[c,1], Alborz Karimzadeh[b,d], Stephen T. Yeung[c,2], Hayk Davtyan[e], Gianna M. Fote[a,b], Lydia Lau[c], Jason G. Weinger[d,3], Thomas E. Lane[b,c,d,4], Matthew A. Inlay[b,d], Wayne W. Poon[c], and Mathew Blurton-Jones[a,b,c,5]

[a]Department of Neurobiology and Behavior, University of California, Irvine, CA 92697; [b]Sue and Bill Gross Stem Cell Research Center, University of California, Irvine, CA 92697; [c]Institute for Memory Impairments and Neurological Disorders, University of California, Irvine, CA 92697; [d]Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697; and [e]Department of Molecular Immunology, Institute for Molecular Medicine, Huntington Beach, CA 92647

Significance

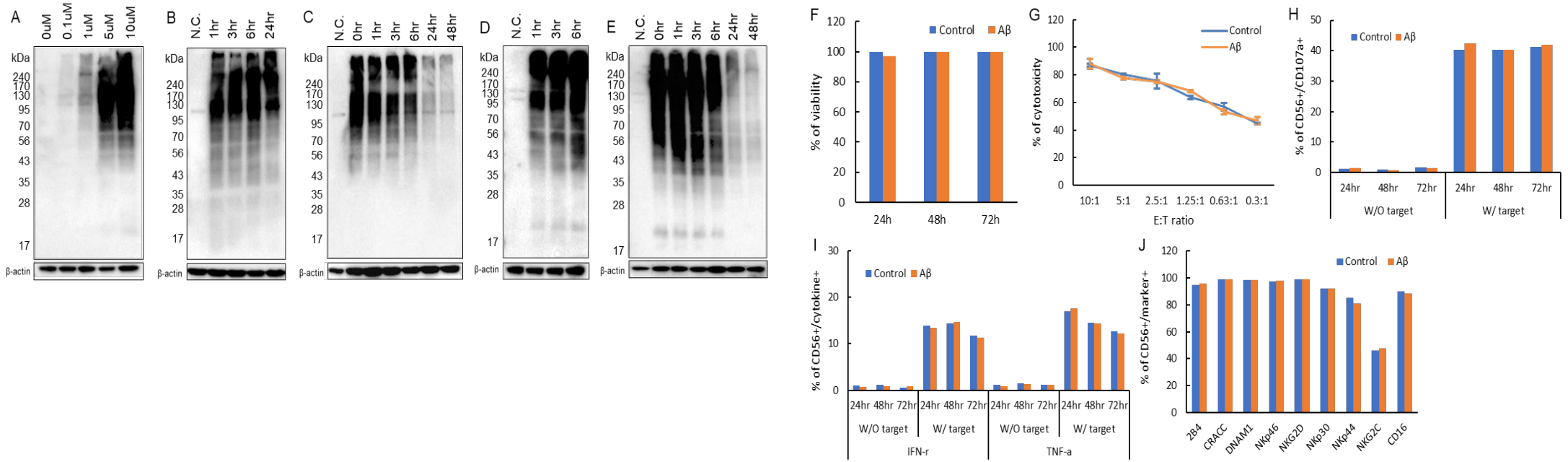
Neuroinflammation and activation of innate immunity are pathological hallmarks of Alzheimer's disease (AD). In contrast, very few studies have examined the impact of the adaptive immune system in AD pathogenesis. Here, we find that genetic ablation of peripheral immune cell populations significantly accelerates amyloid pathogenesis, worsens neuroinflammation, and alters microglial activation state. Critically, it appears that loss of IgG-producing B cells impairs microglial phagocytosis, thereby exacerbating amyloid deposition. Conversely, replacement of IgGs via direct injection or bone marrow transplantation reverses these effects and reduces Aβ pathology. Together, these results highlight the importance of the adaptive immune system and its interactions with microglia in the pathogenesis of AD.

Knockout/Depletion of NK cells in both models caused rapid acceleration of protein deposition

1. Earls RH, Menees KB, Chung J, et al. NK cells clear α-synuclein and the depletion of NK cells exacerbates synuclein pathology in a mouse model of α-synucleinopathy. Proc Natl Acad Sci U S A. 2020;117(3):1762-1771. doi:10.1073/pnas.1909110117
2. Marsh SE, Abud EM, Lakatos A, et al. The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function. Proc Natl Acad Sci U S A. 2016;113(9):E1316-E1325. doi:10.1073/pnas.1525466113

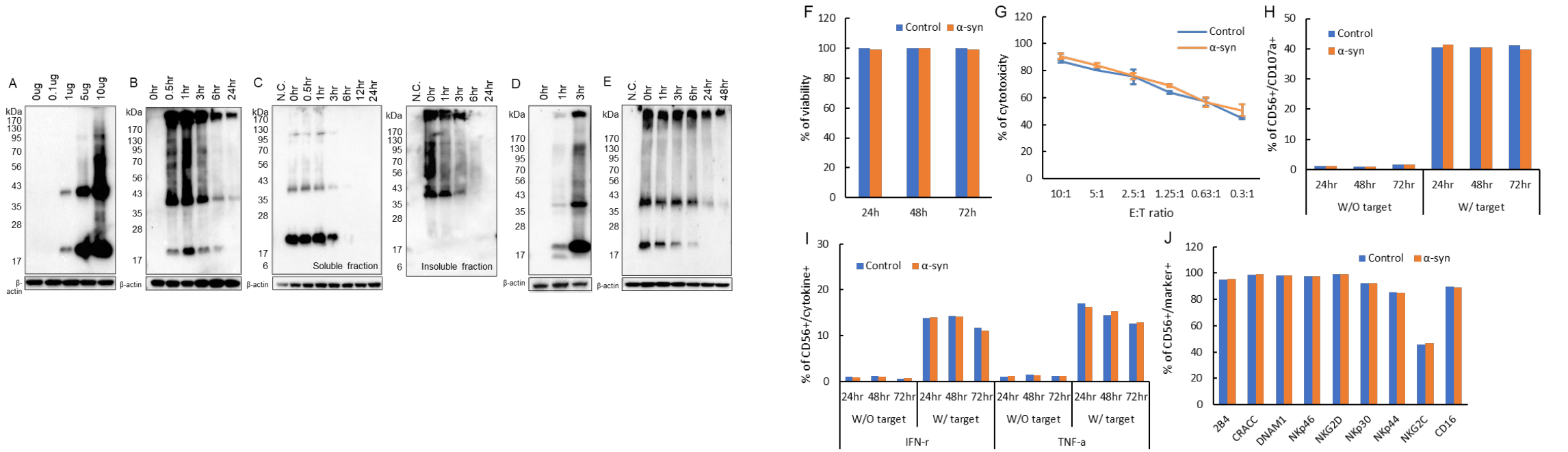
Functional Characteristics of SNK01 Cells for Alzheimer's Disease (AD)

Uptake and degradation of aggregated amyloid-β (Aβ) proteins



(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 hour and harvested. (B) SNK01 cells were treated with 5 μM of Aβ aggregates and harvested at 1, 3, 6, 9, and 24 hours after treatment. (C) SNK01 cells were treated with 5 μM Aβ aggregates for 1 hour and washed 3 times with PBS. Then SNK01 cells were incubated in fresh medium for up to 48 hours and harvested at 1, 3, 6, 24, and 48 hours after wash. (D) HMC3 cells were treated with 5 μM of Aβ aggregates for 1, 3, and 6 hours. N.C: negative control without treatment with Aβ aggregates. (E) HMC3 cells were treated with 5 μM Aβ aggregates for 1 hour and washed 3 times with PBS. Then cells were incubated further in fresh medium for up to 48 hours and harvested at 1, 3, 6, 24, and 48 hours after wash. (F-J) SNK01 cells were incubated with 5 μM of Aβ aggregates for 24, 48, and 72 hours and analyzed for viability (F), cytotoxicity at 72-hour after treatment (G), degranulation (H), intracellular expression of IFN-γ and TNF-α (I), and surface expression of various activating NK receptors at 72-hour after treatment (J).

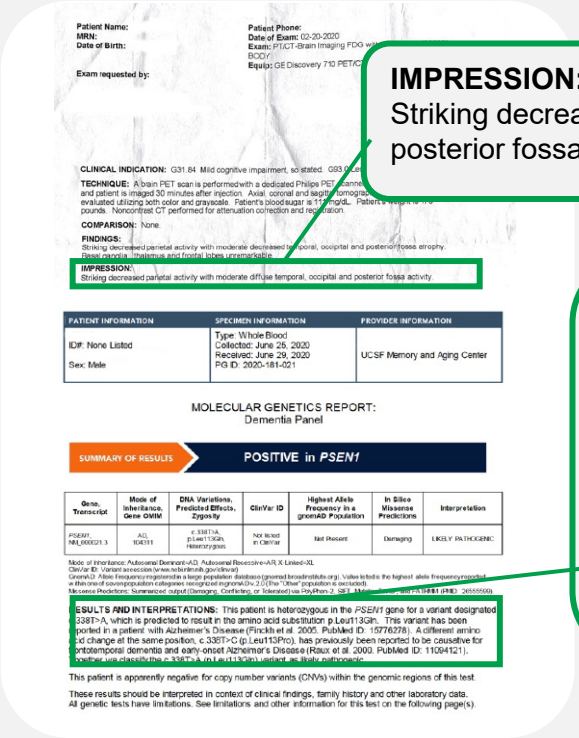
Uptake and degradation of aggregated α-synuclein proteins



(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 hour 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 hours after treatment. (C) SNK01 cells were treated with 5 μg/mL of α-syn aggregates for 1 hour and washed 3 times with PBS. Then SNK01 cells were incubated in fresh medium for up to 24 hours and harvested at 0.5, 1, 3, 6, 12, and 24 hours 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 hours. 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 hour and washed 3 times with PBS. Then cells were incubated further in fresh medium for up to 48 hours and harvested at 1, 3, 6, 24, and 48 hours after wash. (F-J) SNK01 cells were incubated with 5 μg/mL of α-syn aggregates for 24, 48, and 72 hours and analyzed for viability (F), cytotoxicity at 72-hour after treatment (G), degranulation (H), intracellular expression of IFN-γ and TNF-α (I), and surface expression of various activating NK receptors at 72-hour after treatment (J).

Case Studies

Alzheimer's Compassionate Case Study # 1
38 Y.O. With *PSEN1* Mutation And Advanced Alzheimer's Treated With SNK01



Patient Name:
MRN:
Date of Birth:

Patient Phone:
Date of Exam: 02-20-2020
Exam: PT/CT-Brain Imaging FDG
BODY
Equip: GE Discovery 710 PET/C

Exam requested by:

CLINICAL INDICATION: G31.84 Mild cognitive impairment, so stated. G93.1 C

TECHNIQUE: A brain PET scan is performed with a dedicated Philips PET scanner and patient is imaged 30 minutes after injection. Axial, coronal and sagittal tomograms evaluated utilizing both color and grayscale. Patient's blood sugar is 1 mg/dL. Patient weighs 1 pounds. Noncontrast CT performed for attenuation correction and registration.

COMPARISON: None.

FINDINGS:
Striking decreased parietal activity with moderate decreased temporal, occipital and posterior fossa atrophy.
Basal ganglia, thalamus and frontal lobes unremarkable.

IMPRESSION:
Striking decreased parietal activity with moderate diffuse temporal, occipital and posterior fossa activity.

PATIENT INFORMATION	SPECIMEN INFORMATION	PROVIDER INFORMATION
ID#: None Listed Sex: Male	Type: Whole Blood Collected: June 26, 2020 Received: June 29, 2020 PG ID: 2020-181-021	UCSF Memory and Aging Center

MOLECULAR GENETICS REPORT:
Dementia Panel

SUMMARY OF RESULTS — POSITIVE in *PSEN1*

Gene, Transcript	Mode of Inheritance, Gene OMIM	DNA Variations, Predicted Effects, Zygosity	ClinVar ID	Highest Allele Frequency in a gnomAD Population	In Silico Missense Predictions	Interpretation
PSEN1, NM_000021.3	AD, 104311	c.338T>A, p.Leu113Gln, Heterozygous	Not listed in ClinVar	Not Present	Damaging	LIKELY PATHOGENIC

Mode of Inheritance: Autosomal Dominant-AD, Autosomal Recessive-AR, X-Linked-XL
ClinVar ID: Variant accession (www.ncbi.nlm.nih.gov/clinvar)
gnomAD: Allele Frequency reported in a large population database (gnomad.broadinstitute.org). Value listed is the highest allele frequency reported in either one of seven population subgroups recognized in gnomAD v.2.0 (the "Other" population is excluded).
Missense Predictions: Summarized output (Damaging, Conflicting, or Tolerated) via PolyPhen-2, SIFT, MutationTaster, and PROVEAN (HGVS: 26555500)

RESULTS AND INTERPRETATIONS: This patient is heterozygous in the *PSEN1* gene for a variant designated c.338T>A, which is predicted to result in the amino acid substitution p.Leu113Gln. This variant has been reported in a patient with Alzheimer's Disease (Finckh et al. 2005. PubMed ID: 15776278). A different amino acid change at the same position, c.338T>C (p.Leu113Pro), has previously been reported to be causative for frontotemporal dementia and early-onset Alzheimer's Disease (Raux et al. 2000. PubMed ID: 11094121). Together we classify the c.338T>A (p.Leu113Gln) variant as likely pathogenic.

This patient is apparently negative for copy number variants (CNVs) within the genomic regions of this test.

These results should be interpreted in context of clinical findings, family history and other laboratory data. All genetic tests have limitations. See limitations and other information for this test on the following page(s).

IMPRESSION:
Striking decreased parietal activity with moderate diffuse temporal, occipital and posterior fossa activity.

RESULTS AND INTERPRETATIONS: This patient is heterozygous in the *PSEN1* gene for a variant designated c.338T>A, which is predicted to result in the amino acid substitution p.Leu113Gln. This variant has been reported in a patients with Alzheimer's Disease (Finckh et al. 2005. PubMed ID: 15776278). A different amino acid change at the same position, c.338T>C (p.Leu113Pro), has previously been reported to be causative for frontotemporal dementia and early-onset Alzheimer's Disease (Raux et al. 2000. PubMed ID: 11094121). Together we classify the c.338T>A (p.Leu113Gln) variant as likely pathogenic.

Patient was independently seen and evaluated by Dr. Ming Guo (at UCLA Medical Center) before initiating SNK01 treatment.

Alzheimer's Case Study #1 - Patient Baseline

Prior to SNK01 treatment, patient was unable to talk, feed himself, hold a pen, or get out of a wheelchair by himself

- Daniel is still declining and no longer able to (1) feed himself independently (caregiver helps him eat since he can't hold his spoon), (2) recognize when he has to urinate *(constantly goes to the bathroom with little success), (3) speak more than a few words,* (4) use his phone to call family or friends, (5) etc.

Baseline Report

Treatment #	Date	Observations
1	9/15/20	• Daniel is still declining and no longer able to (1) feed himself independently (caregiver helps him eat since he can't hold his spoon), (2) recognize when he has to urinate *(constantly goes to the bathroom with little success), (3) speak more than a few words,* (4) use his phone to call family or friends, (5) etc. • Week 2 of the first treatment I noticed that Daniel's facial expressions were brighter when he smiled.

After 3 Doses of SNK01 – Patient Able To Walk, Talk And Feed Himself

Post-treatment Report

10/13/20	• Daniel is still unable to sleep at night, continues to wander, moderately agitated and *seems depressed / anxious now. Daniel now communicates that he wants to go home* but is willing to stay at the adult care facility to continue receiving his treatments. He seems very aware of his surroundings which could be a possible explanation on why he is sad about his situation and living arrangement. • The caregivers noticed that Daniel is more independent now with eating and going to the bathroom. They also mentioned that he was engaged while watching the NBA finals, offered to buy them pizza and looked up "pizza" on his phone. They even saw him text "Hello" to his brother. • Daniel wasn't apprehensive during his third treatment. It looked like he knew this was a treatment to help him as opposed to the last treatment where he wasn't sure if this was good. • Daniel initiated a handshake with Dr. Bayer for the first time when we were saying goodbye after the treatment. He also saw a blue Porsche on the highway and said "nice car" on the drive back. • Daniel was able to eat noodles with chopsticks for the first time in many months. However, he used the chopsticks like a fork, but he was able to hold it firmly and go through the motions as if using chopsticks. • Daniel was able to shoot a basketball at the park, but he was still off balance. He wasn't able to make a shot in the basket but still managed to hit the backboard (in the general area of the basket).

10/13/20

After 5 Doses of SNK01 – Patient Was Able To Regain Ability To Walk And Run

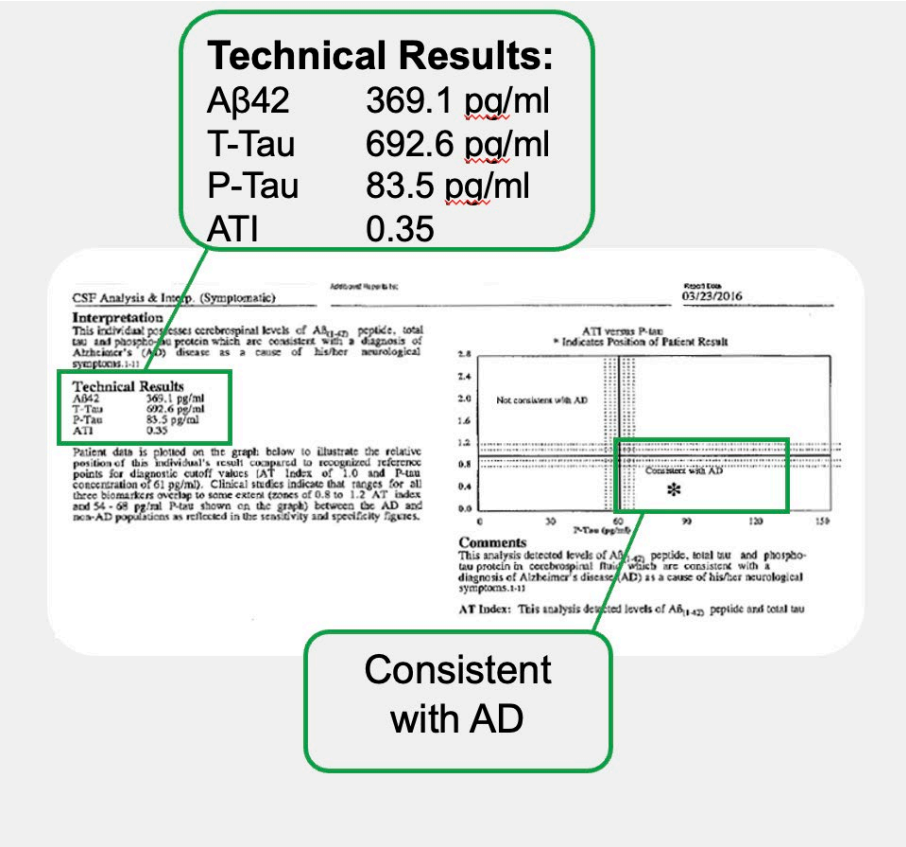
"Hi Dr. Song, it was really nice catching up. Here are some messages I have of Daniel's Board and Care reporting falls due to balance issues, videos of Daniel having difficulty walking and wearing a helmet as the Board and Care required this after many falls, and then videos of **Daniel running after 2 months of additional treatments. At this point, the Board and Care no longer required Daniel to wear a helmet. Hope these help!"**

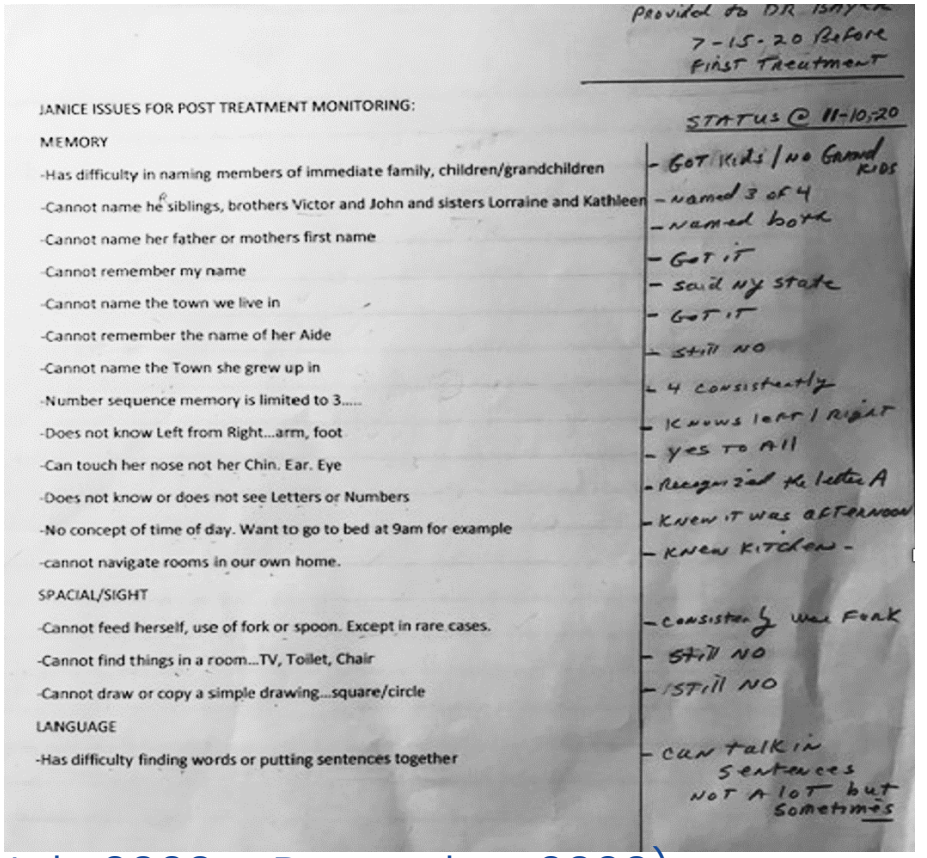
- Daniel's brother

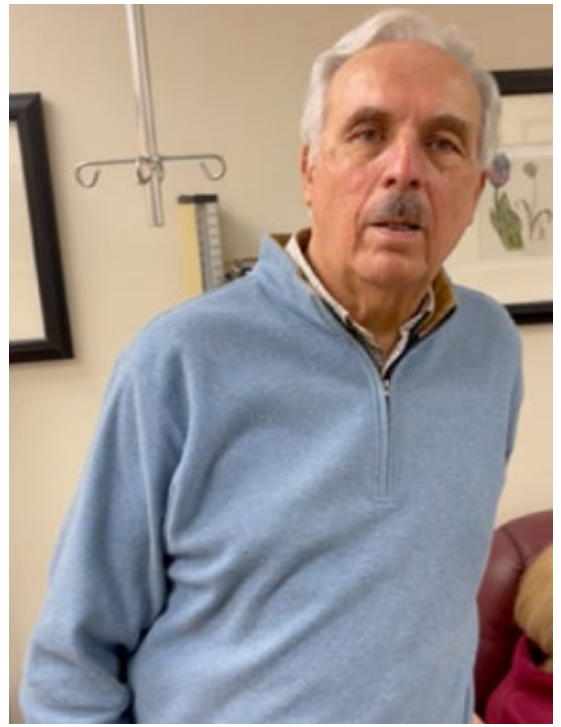
1/5/21

5/19/21

Alzheimer's Compassionate Use Case Study # 2
70 Y.O With Advanced Alzheimer's Treated With SNK01







Clinical response **after six treatments** (July 2020 – December 2020).

Patient had **noticeable improvement** in her overall cognitive function.

But treatment was stopped for 2 years due to Covid.

After A Two-Year Hiatus, Treatment Resumed and Patient Showed Immediate Response

Neurological Associates of Long Island, P.C.
3d ·

Dr. Vincent DeOrchis and Dr Paul Y Song of NKGen Biotech provided the first ever infusion of autologous enhanced NK cells as part of a clinical trial for Advanced Alzheimer's Type Dementia. #dementiatreatment

"By the way. Everyone sees a different Janice recently. Energy. Eye contact. General interaction. Can't wait for you to see.

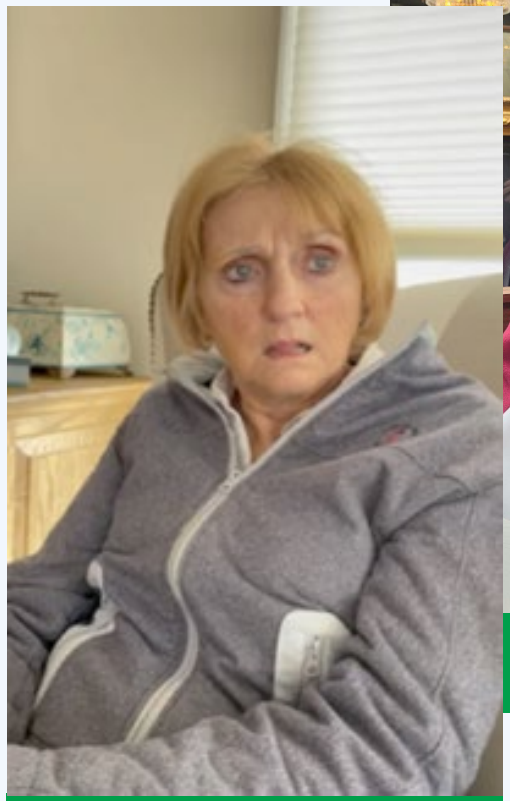
This morning I said each day of the week and she repeated each day after I said it. Sounds small but big from where we were for sure."

- *Janice's husband*

"For your listening pleasure. Sunday 2-19-23. Not sure if this is meaningful, but thought you would appreciate it.

No way does she even attempt this before 1-27-23 first treatment."

- *Janice's husband*



Week 3

Week 5

Patient was granted single Compassionate Use IND approval by USFDA

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Patient Showed Continued Improvement After 16 Weeks – Patient Now Able To Walk, Talk, And Feed Herself

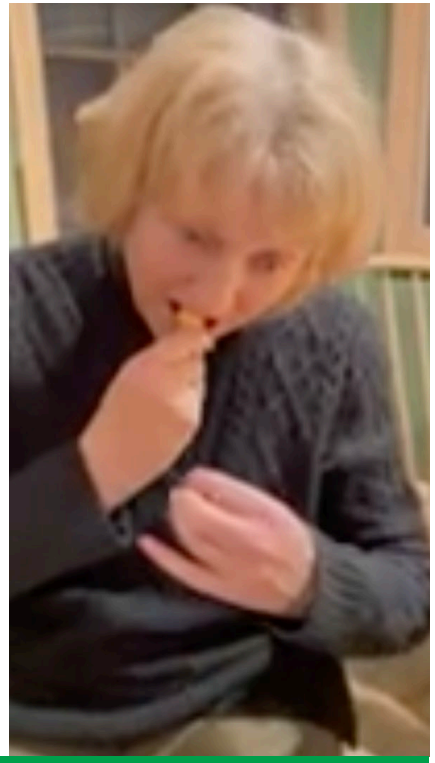
"This evening, I went from our family room to kitchen to clean things up before heading upstairs. To my surprise **Janice got up and walked thru two rooms and arrived to my surprise in the kitchen.**

The video I just sent you was her taking a bread flat and **feeding herself** with it.

Do not want to overstate this happening but thought you both would like to see it

Small step forward."

- *Janice's husband*



Week 9
Patient feeding herself which she has not done in years

Week 15
Walking without assistance

Week 21
Improved memory

12/22/23
Improved MMSE, more verbal with increased overall energy

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THESE REPORTS OF PATIENT EXPERIENCES AND IMAGES HAVE NOT BEEN VALIDATED. ONLY CONTROLLED CLINICAL TRIALS CAN SUPPORT BENEFIT FOR PATIENTS. THESE MAY NOT BE PREDICTIVE OF CLINICAL TRIAL RESULTS.

Patient Continues to Show Improvement…

Actively engaged in conversation

Able to get up out of a chair

Can effectively navigate surroundings

March and April 2024

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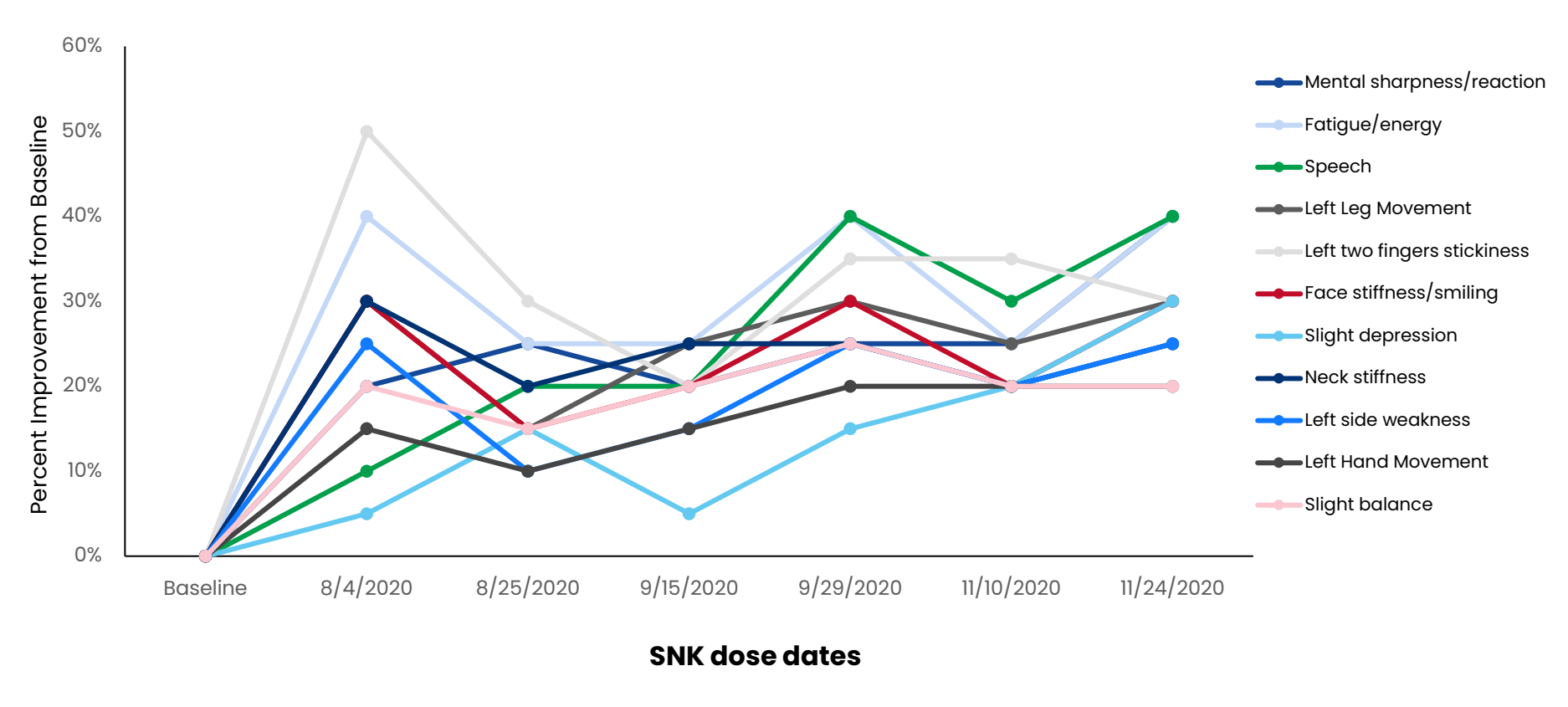
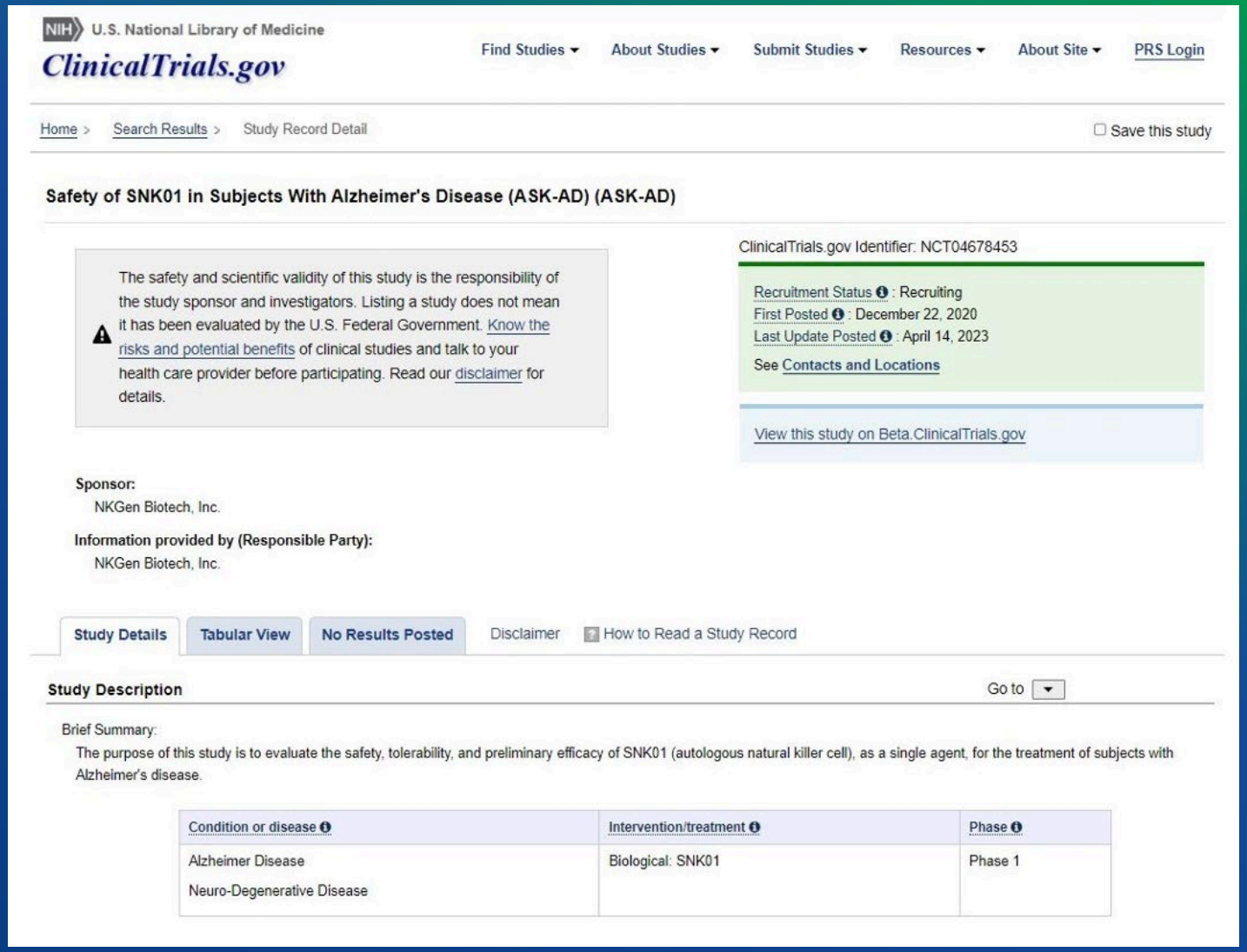
THESE REPORTS OF PATIENT EXPERIENCES AND IMAGES HAVE NOT BEEN VALIDATED. ONLY CONTROLLED CLINICAL TRIALS CAN SUPPORT BENEFIT FOR PATIENTS. THESE MAY NOT BE PREDICTIVE OF CLINICAL TRIAL RESULTS.

Parkinson's Compassionate Use Case Study # 1 : Qualitative Evaluation Of 47 Y.O. After Treatment with SNK01

Patient was independently seen and evaluated by Dr. Ming Guo (at UCLA Medical Center)



Phase I Data
MX04



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Home > Search Results > Study Record Detail

Save this study

Safety of SNK01 in Subjects With Alzheimer's Disease (ASK-AD) (ASK-AD)

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT04678453

Recruitment Status : Recruiting
First Posted : December 22, 2020
Last Update Posted : April 14, 2023
See Contacts and Locations

View this study on Beta.ClinicalTrials.gov

Sponsor:
NKGen Biotech, Inc.

Information provided by (Responsible Party):
NKGen Biotech, Inc.

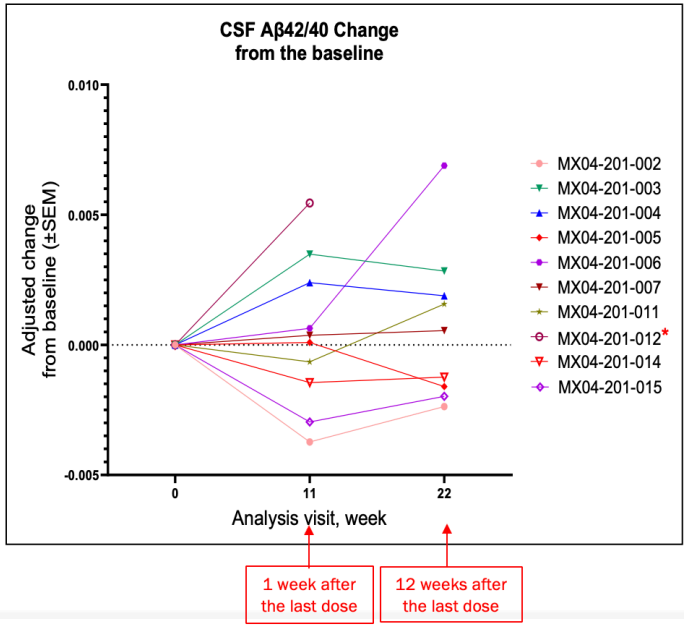
Study Details | Tabular View | No Results Posted | Disclaimer | How to Read a Study Record

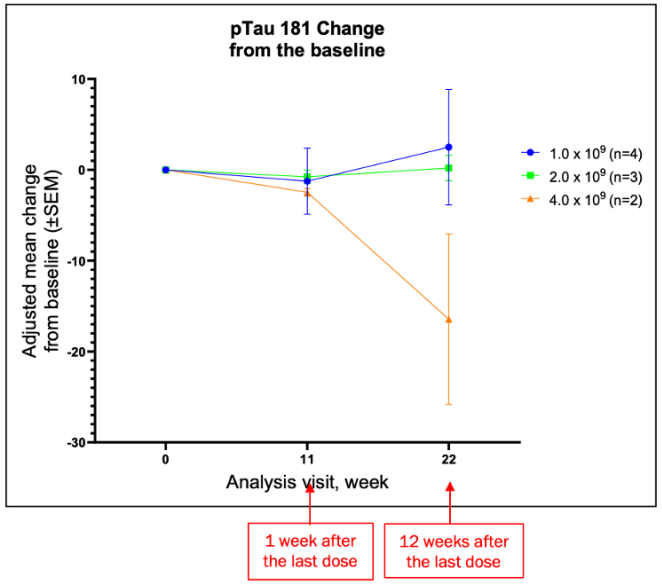
Study Description Go to

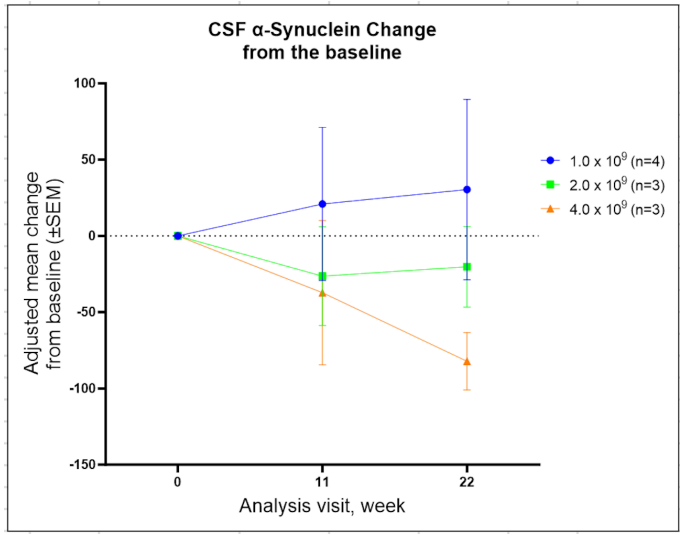
Brief Summary:
The purpose of this study is to evaluate the safety, tolerability, and preliminary efficacy of SNK01 (autologous natural killer cell), as a single agent, for the treatment of subjects with Alzheimer's disease.

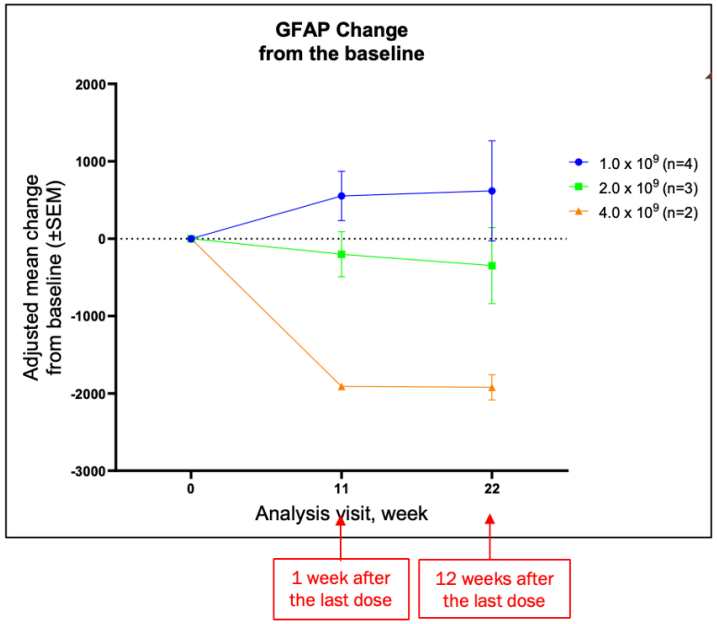
Condition or disease	Intervention/treatment	Phase
Alzheimer Disease Neuro-Degenerative Disease	Biological: SNK01	Phase 1

Data From MX04 Phase I Trial









No observed SAEs or dose limiting toxicity

Dose Response Observed for Several Biomarkers in CSF

Improvements seen in AB42/40, p-Tau, and alpha-synuclein protein levels

Reductions seen in neuroinflammation

YKL-40, NF-L were reduced or stable in 50-60% of patients but not in a dose dependent manner

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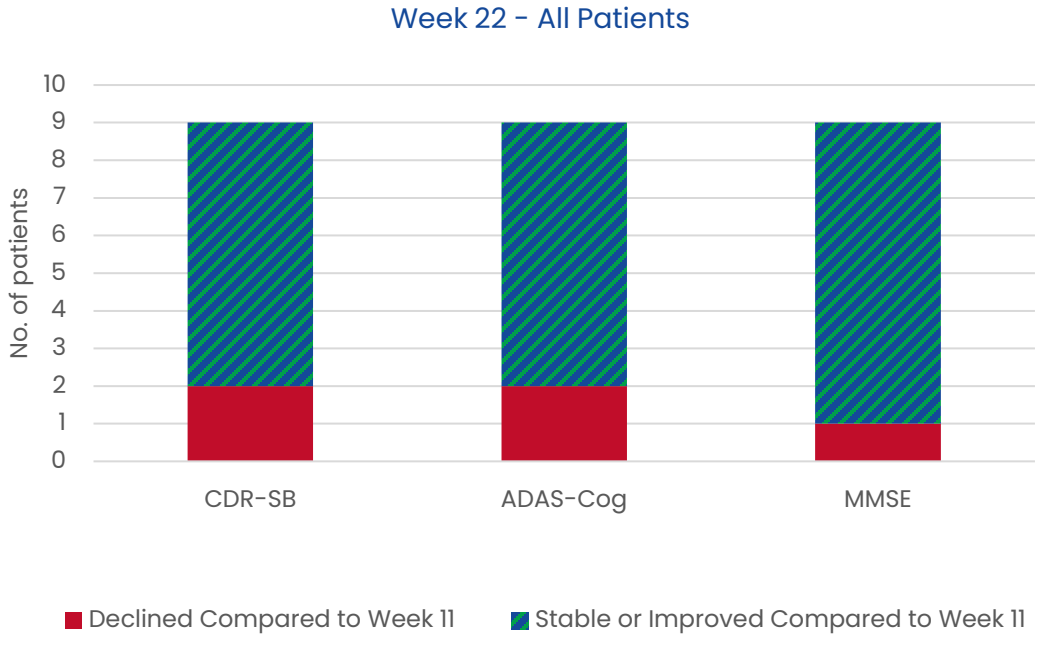
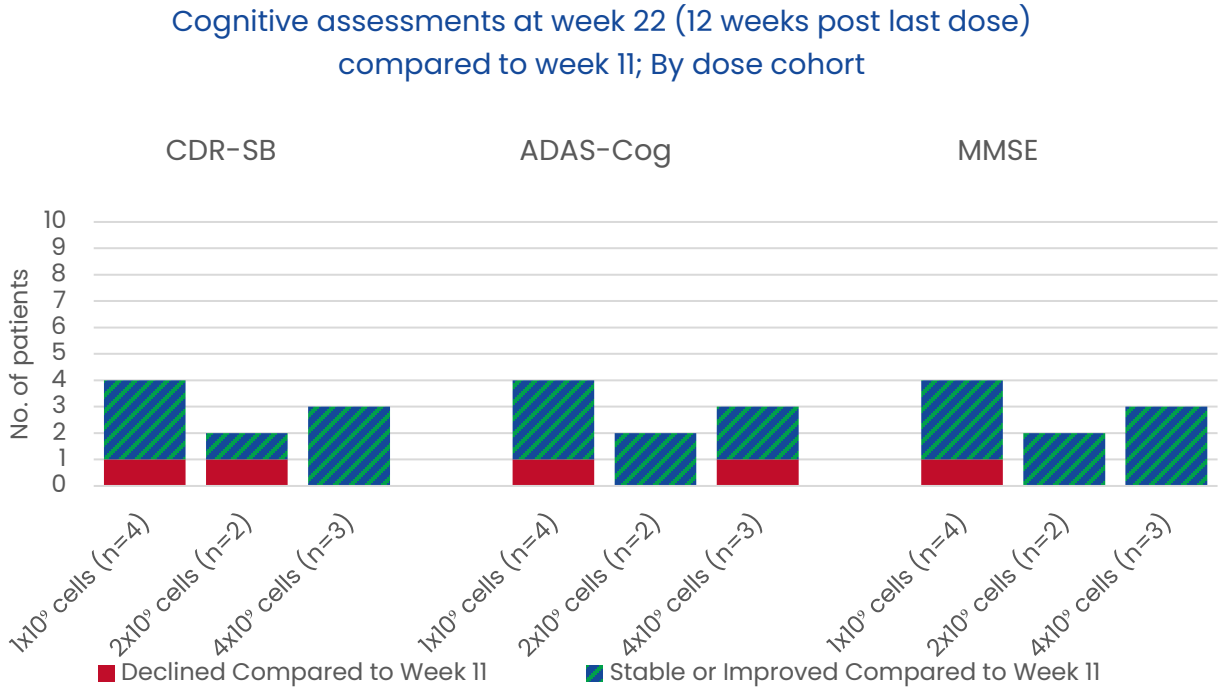
Changes In Cognitive Function Post SNK01 Treatment
Data From MX04 Phase I Trial





Despite 2/3 of patients being treated with low sub-optimal dosing, majority of patients showed improved or stable cognitive function at 6 months.

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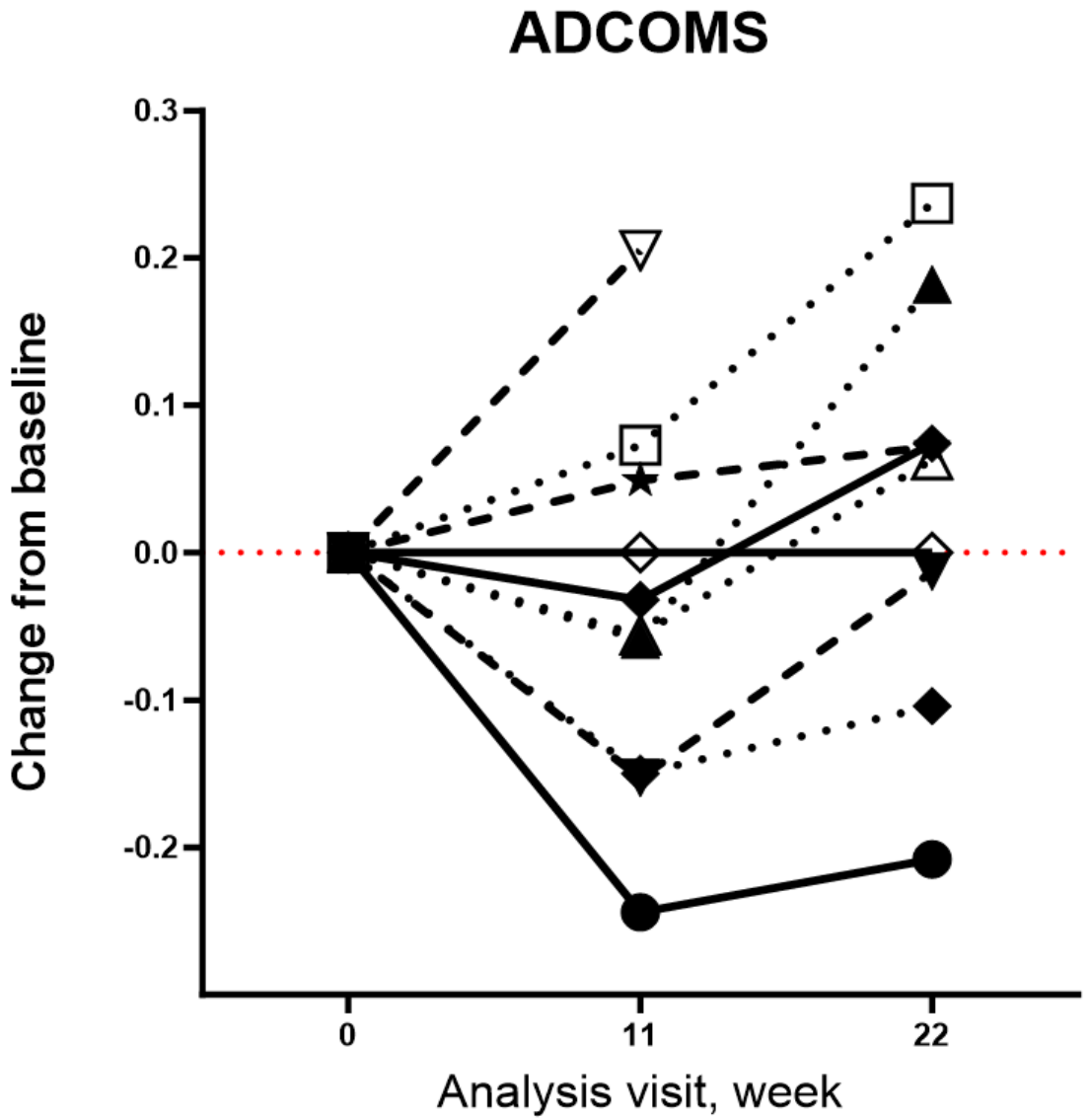
Results

***MCIDs used to determine stable or improved cognition: ADCOMS -0.1**

Dotted lines are subjects in Cohort 1 (1x10⁹ cells), Dashed lines are subjects in Cohort 2 (2x10⁹ cells) Solid lines are subjects in Cohort 3 (4x10⁹ cells)



90% had either stable or improved ADCOMS scores, at one-week post-treatment (week 11).

78% had either stable or improved ADCOMS scores, at 3 months post-treatment (week 22).

Phase I Clinical Trial Patient #15
85 Y.O. With Alzheimer's Disease Treated With SNK01

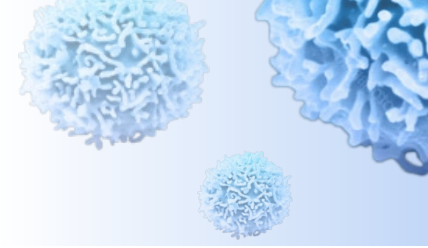
Patient MMSE Score Increased From 14 to 22

Subject 015. His walk was unsteady, and he couldn't dance prior to treatment. He was much more coordinated, could now dance, and was mentally sharper after treatment. See attached.

•Email message from June 12, 2023
Blanca Acosta, MD, Study Coordinator for MX04, Mexico

SNK01-MX04 Clinical trial, **NCT04678453**

ClinicalTrials.gov



RECRUITING

Clinical Study of SNK01 in Participants With Moderate Alzheimer's Disease

ClinicalTrials.gov ID NCT06189963

Sponsor NKGen Biotech, Inc.

Information provided by NKGen Biotech, Inc. (Responsible Party)

Last Update Posted 2024-01-08

Study Overview

Brief Summary

The goal of this clinical trial is to test SNK01 in participants with moderate Alzheimer's Disease. The main questions it aims to answer are:

1. Is SNK01 safe and tolerable when administered every 3 weeks for up to 1 year as an intravenous infusion
2. Can SNK01 administration improve cognitive assessment scores and biomarkers

Official Title

A Phase I/IIa, Study to Evaluate the Safety, Tolerability and Exploratory Efficacy of SNK01 in Participants With Moderate Alzheimer's Disease

Conditions

Moderate Alzheimer Disease

Study Start (Actual)

2023-11-21

Primary Completion (Estimated)

2024-12

Study Completion (Estimated)

2025-06

Enrollment (Estimated)

36

Study Type

Interventional

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Phase 1/2a SNK01 in Moderate Alzheimer's Clinical Trial Patient

" She has been doing great. No side effects… She is improving a little bit at a time…" *Patient's husband*

1-3. Product Testing of Cryopreserved SNK01 Products in Clinical Trial (from 3 patients in AD trial)

Test		Method	Specifications		Batch 1	Batch 2	Batch 3
Appearance		Macrographic Observation	Light-yellow cell suspension without cell aggregation or clumping		Pass	Pass	Pass
Sterility		BacT/Alert	No microorganism detected		No microorganism detected		
		Gram Staining[1)]	No microorganism detected		No microorganism detected		
		USP <71>[1)]	No microorganism detected		No microorganism detected		
Mycoplasma		MycoSEQ™ qPCR Assay Method	No mycoplasma contamination		No microorganism detected		
Identity		Flow Cytometry	$CD3^-CD56^+$	> 80%	99.8%	99.9%	99.4%
Purity		Flow Cytometry	CD3	< 5.0%	0.0%	0.0%	0.0%
			CD14	< 5.0%	0.0%	0.3%	0.0%
			CD20	< 5.0%	0.0%	0.1%	0.0%
Endotoxin		USP <85> Bacterial Endotoxin Test	≤ 0.5 EU/mL		<0.050 EU/mL	<0.050 EU/mL	<0.050 EU/mL
Potency	Cytotoxicity Assay	Calcein AM	Cytotoxicity against K562: ≥ 50% (E:T ratio of 10:1)		97%	93%	88%
	Cell Surface Marker Expression[2)]	Flow Cytometry	NKG2D	Report Result	98.8%	99.4%	98.8%
			CXCR3	Report Result	97.8%	95.0%	94.7%
			DNAM-1	Report Result	100.0%	99.9%	98.6%
			NKp46	Report Result	82.2%	73.7%	70.5%
Cell Viability		Trypan Blue Staining Method (EP <2.7.29>)	≥ 70%		97.0%	98%	96%
Total Cell Number		Trypan Blue Staining Method (EP <2.7.29>)	1.6 - 2.4 × 10^7 cells/mL		$1.9x10^7$ cells/mL, $2.9x10^9$ cell/bag	$2.1x10^7$ cells/mL, $3.2x10^9$ cell/bag	$1.9x10^7$ cells/mL, $2.9x10^9$ cell/bag

[1)] Additional confirmatory tests to assess sterility of the cryopreserved SNK01 final drug product.
[2)] Results are reported for information only. Specification will be determined based on the accumulated data during Phase I and II clinical trials.

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Article

https://doi.org/10.1038/s43587-023-00565-0

Plasma proteomic profiles predict future dementia in healthy adults

Received: 9 June 2023

Accepted: 22 December 2023

Published online: 12 February 2024

Check for updates

Yu Guo[1,4], Jia You[1,2,4], Yi Zhang[1,4], Wei-Shi Liu[1], Yu-Yuan Huang[1], Ya-Ru Zhang[1], Wei Zhang[2], Qiang Dong[1], Jian-Feng Feng[2,3] ✉, Wei Cheng[1,2,3] ✉ & Jin-Tai Yu[1] ✉

The advent of proteomics offers an unprecedented opportunity to predict dementia onset. We examined this in data from 52,645 adults without dementia in the UK Biobank, with 1,417 incident cases and a follow-up time of 14.1 years. Of 1,463 plasma proteins, GFAP, NEFL, GDF15 and LTBP2 consistently associated most with incident all-cause dementia (ACD), Alzheimer's disease (AD) and vascular dementia (VaD), and ranked high in protein importance ordering. Combining GFAP (or GDF15) with demographics produced desirable predictions for ACD (area under the curve (AUC) = 0.891) and AD (AUC = 0.872) (or VaD (AUC = 0.912)). This was also true when predicting over 10-year ACD, AD and VaD. Individuals with higher GFAP levels were 2.32 times more likely to develop dementia. Notably, GFAP and LTBP2 were highly specific for dementia prediction. GFAP and NEFL began to change at least 10 years before dementia diagnosis. Our findings strongly highlight GFAP as an optimal biomarker for dementia prediction, even more than 10 years before the diagnosis, with implications for screening people at high risk for dementia and for early intervention.

Fibrillary Acid Protein (GFAP)

Neurofilament-light (NfL)

Growth/Differentiation Factor-15 (GDF-15)

Latent Transforming Growth Factor beta binding protein 2 (ltbp2)

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Dementia Risk Panel – MX04

SNK01 can reduce all biomarkers associated with increased risk of Dementia suggesting possibility to be used as a preventative treatment.

CSF Analysis		GFAP - cfb		NFL - cfb		GDF-15- cfb		LTBP2 - cfb	
Subject	AD Stage	Week 11	Week 22	Week 11	Week 22	Week 11	Week 22	Week 11	Week 22
MX04-201-002	Moderate	↑	↑	↑	↑	↑	↑	↑	↑
MX04-201-003	Moderate	↑	↑	↑	↑	↓	↓	↑	↓
MX04-201-004	Mild	↑	↓	↑	↑	↑	↑	↑	↑
MX04-201-005	Mild	↓	↓	↓	↓	↑	↓	↑	↓
MX04-201-006	Severe	↓	↓	↑	↓	↑	↑	↓	↓
MX04-201-007	Mild	↑	↑	↑	↑	↓	↑	↑	↓
MX04-201-011	Mild	↓	↓	↓	↓	↓	↓	↓	↑
MX04-201-012	Severe	↓		↑		↑		↑	
MX04-201-014	Moderate	↓	↓	↑	↑	↑	↓	↑	↓
MX04-201-015	Mild	↓	↓	↓	↑	↓	↓	↓	↑

Plasma Analysis		GFAP - cfb		NFL - cfb		GDF-15- cfb		LTBP2 - cfb	
Subject	AD Stage	Week 11	Week 22	Week 11	Week 22	Week 11	Week 22	Week 11	Week 22
MX04-201-002	Moderate	↑	↑	↑	↑	↓	↓	↓	↓
MX04-201-003	Moderate	↓	↓	↑	↑	↓	↑	↑	↓
MX04-201-004	Mild	↑	↓	↑	↑	↑	↑	↑	↑
MX04-201-005	Mild	↑	↑	↑	↑	↑	↑	↓	↑
MX04-201-006	Severe	↑	↑	↓	↑	↑	↑	↓	↓
MX04-201-007	Mild	↓	↓	↓	↓	↓	↓	↑	↑
MX04-201-011	Mild	↓	↓	↑	↑	↓	↓	↑	↑
MX04-201-012	Severe	↑		↑		↓		↓	
MX04-201-014	Moderate	↑	↑	↑	↑	↓	↑	↑	↑
MX04-201-015	Mild	↓	↓	↓	↓	↑	↑	↑	↑

cfb=change from baseline

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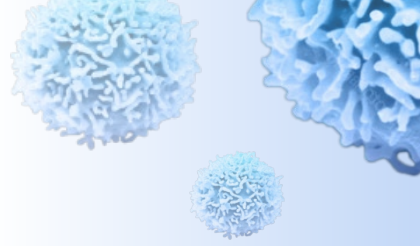
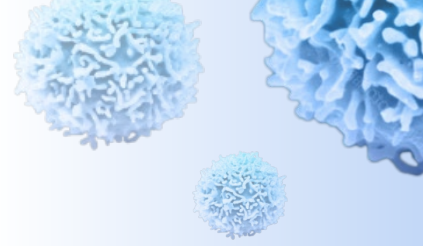
References For Neurodegenerative Disease



- Zhou, Y-Q, et al. (2019). The role of CXCR3 in Neurological Diseases. Curr Neuropharmacol, 17(2): 142-150.
- Marsh, S.E., et al. (2016). The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function. Proc Natl Acad Sci U S A,113(9):E1316-25.
- Alves, S., et al. (2017). Interleukin-2 improves amyloid pathology, synaptic failure and memory in Alzheimer's disease mice. Brain, 140(3):826-842.
- Mate, I., et al. (2015). Function and Redox State of Peritoneal leukocytes as preclinical and prodromic markers in a longitudinal study of triple-transgenic mice for Alzheimer's disease. J Alzheimers Dis, 43(1):213-26.
- Davies, A.J., et al. (2019). Natural Killer cells degenerate intact sensory afferents following nerve injury. Cell, 176(4), 716-728.
- Hoxha, E., et al. (2018). The emerging role of altered cerebellar synaptic processing in Alzheimer's disease. Front Aging Neurosci, 10:396.
- Desikan, R.S., et al. (2010). Selective disruption of the cerebral neocortex in Alzheimer's disease. PLoS One, 5(9):e12853.
- Rao, Y.L., et al. (2022). Hippocampus and its involvement in Alzheimer's disease: a review. 3 Biotech, 12(2):55.
- Qin, Q., et al. (2021). Prominent striatum amyloid retention in early-onset familial Alzheimer's disease with PSEN1 mutations: a pilot PET/MR study. Front Aging Neurosci, 13:732159.

References Neurodegenerative Disease (Cont.)



- Earls, R.H., et al., (2020). NK cells clear alpha-synuclein and the depletion of NK cells exacerbates synuclein pathology in a mouse model of alpha-synucleinopathy. Proc Natl Acad Sci U S A, 117(3):1762-1771.
- Rabinovich, B.A., et al. (2003). Activated but not resting, T cells can be recognized and killed by syngeneic NK cells. J Immunol, 170(7):3572-6.
- Betourney, Charlene. "'Natural Killer' cells could halt Parkinson's progression." UGA Today, March 12, 2020
- La Jolla Institute for Immunology. "New Therapies could stop T cells from attacking brain cells in Parkinson's disease." SciTech Daily, March 21, 2022.
- Lindestam Arlehamn, C.S., et al. (2020). Alpha-Synuclein-specific T cell reactivity is associated with preclinical and early Parkinson's disease. Nat Commun, (11)1:1875.
- Earls, R.H. and Lee, J-K., (2020). The role of natural killer cells in Parkinson's disease. Experimental & Molecular Medicine, 52:1517-1525.