Subjects treated with expanded non-genetically modified autologous Natural Killer cells (SNK01) show changes in CSF α-synuclein and in cognitive function

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Treatment of 10 mild, moderate or severe AD patients every 3 weeks for 3 months with autologous non genetically modified NK C ells (SNK01) resulted in no treatment related adverse events, **stable or improved ADCOMS scores in 90% of subjects** (despite 70% of subjects being treated at relatively low doses of SNK01), and a **decrease in CSF α-synuclein in 60% of subjects**

PURPOSE & OBJECTIVE

- In Alzheimer's Disease (AD), α-synuclein (α-syn) has been shown to be elevated in the cerebrospinal fluid (CSF).
- **Elevated levels of α-syn in the CSF have been linked to cognitive impairment in AD patients and correlate with worse cognitive performance** (Twohig and Nielson, 2019).
- The role of Natural Killer (NK) cells of the innate immune system in AD has largely been overlooked. In a murine model, depletion of NK cells augmented the accumulation of pathological α-syn.
- Human NK cells have been shown to efficiently internalize and degrade α-syn aggregates via the endosomal/ lysosomal pathway. This has been proposed as a protective mechanism of NK cells against α-synucleinopathy by removing harmful α-syn aggregates from the patient's brain.
- Our in vitro studies have shown that SNK01, an autologous non-genetically modified NK cell product, is able to effectively internalize and degrade α-syn aggregates.
- **SNK01 is a first-in-kind, autologous non-genetically modified NK cell product** with significantly increased cytotoxicity and over 90% activating receptor expression that can be consistently produced from any donor.
- **We hypothesize that SNK01 is safe, can cross the blood brain barrier (BBB) and can impact both cognition and protein aggregates in AD patients.**

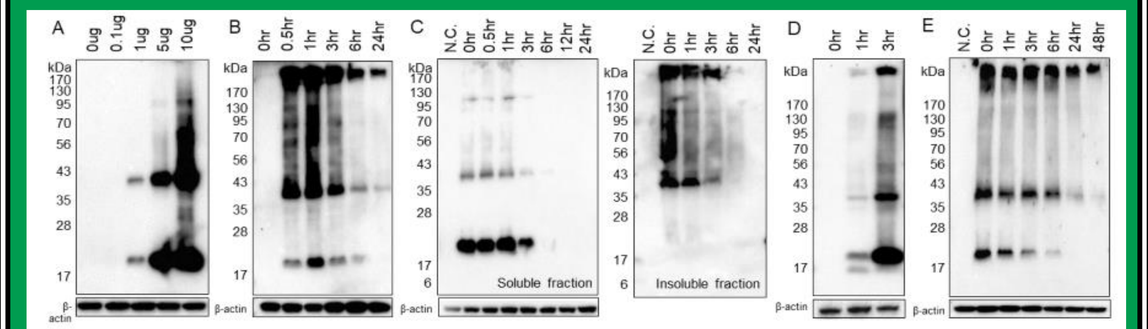
MATERIALS & METHODS

- To assess the in vitro internalization of α-syn aggregates in SNK01 cells, SNK01 cells were plated at 1 x 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 hour, or with 5 µg/ml α-syn aggregates for durations ranging from 0 to 24 hours. HMC3 cells (brain microglial cell line) were plated at 5 x 10^5 cells per well in a 6-well dish and allowed to settle for 24 hours. The next day, HMC3 cells were exposed to 5 µg/mL of α-syn aggregates for 1-3 hours, followed by three washes and harvested.
- In the Phase 1 dose escalation study (Study SNK01-MX04: Single Center, Open-Label, Phase 1 Study to Evaluate the Safety, Tolerability and Exploratory Efficacy of SNK01 in Subjects with Mild Cognitive Impairment (MCI) and Alzheimer's Disease (AD); NCT04678453), SNK01 was administered intravenously (IV) every three weeks for a total of 4 treatments using a 3+3 dose escalation design (1, 2 & 4 x 10^9 cells) in patients with either mild, moderate or severe AD (median MMSE of 14). Assessment of disease severity was based on the baseline CDR-SB score
- Cognitive assessments and CSF analysis of α-syn were performed at baseline and at one week and 12 weeks after the final dose (Weeks 11 and 22 respectively)
- Primary endpoint was safety and secondary endpoints included changes in cognitive assessments [composite score (ADCOMS)] and CSF levels of α-syn. The ADCOMS is a composite measure composed of clinically sensitive items from the ADAS-Cog, the MMSE, and the CDR-SB scales (Wang et al., 2016). A change in ADCOMS score of 0.1 can be considered a clinically meaningful change (Tahami Monfared et al., 2022)

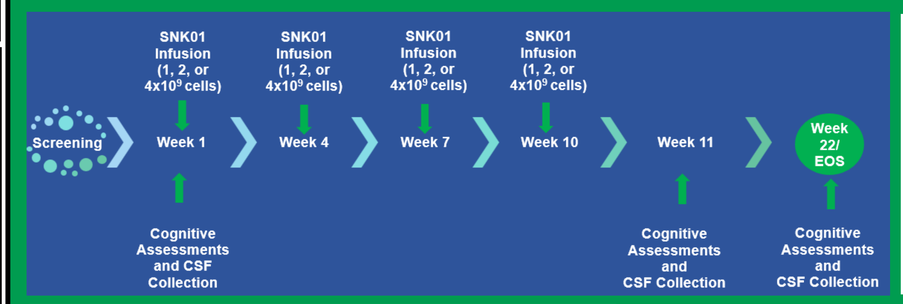
RESULTS

In vitro uptake and degradation of extracellular α-synuclein (α-syn) aggregates by SNK01.

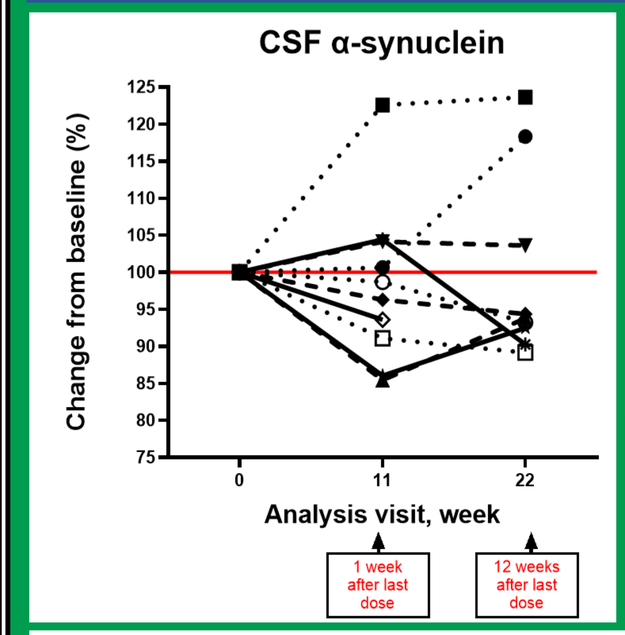
- (A-E) Intracellular levels of α-syn aggregates in SNK01 and HMC3 cells were analyzed by Western blot analysis.

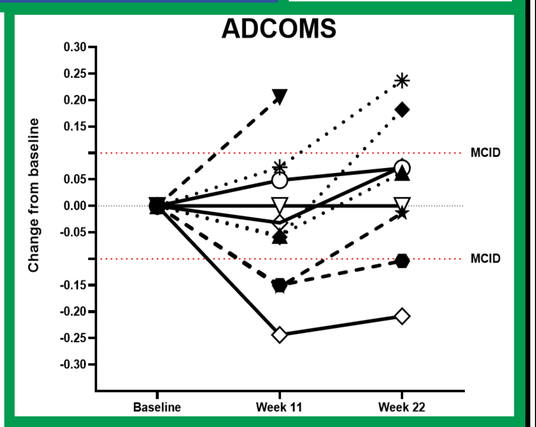


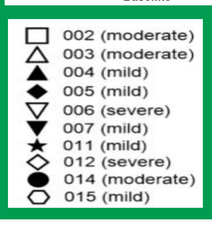
A: SNK01 internalized α-syn aggregates in a concentration dependant manner. B: α-syn aggregate internalization by SNK01 peaked at 1h then decreased. C: internalized α-syn was rapidly degraded by SNK01 (to undetectable levels within 6h of incubation). D: HMC3 cells (brain microglial cell line) internalized α-syn . E: HMC3 cells (brain microglial cell line) degraded α-syn .

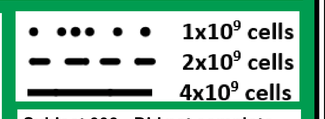


- Eleven subjects (5 males and 6 females) were enrolled, of which 10 were evaluable.
- The median age of the participants was 79 years (56 to 85 years).









Subject 006 – Did not complete W22 cognitive assessments
Subject 012 – Did not complete W22 biomarker assessments

- Despite 70% of subjects being treated at relatively low doses of SNK01, **90%** of all evaluable subjects had either **stable or improved (±0.1) composite ADCOMS scores** at week 11 (one-week after the final dose)
- **60%** of subjects (6/10) had a **decrease in CSF α-syn** compared to baseline values.

SUMMARY

- SNK01 is an autologous non genetically modified NK Cell product
- SNK01 has been shown in vitro to internalize and degrade α-synuclein
- **No treatment related adverse events were observed.**
- Despite 70% of subjects being treated at relatively low doses of SNK01
  - ➢ **90% of all evaluable subjects had either stable or improved (±0.1) composite ADCOMS scores at week 11 (one-week after the final dose)**
  - ➢ **60% of subjects (6/10) had a decrease in CSF α-syn compared to baseline values.**
  - ➢ At week 11, the decreases in α-syn corresponded to stable/decrease in ADCOMS in 5/6 subjects and where data was available, the α-syn levels continued below baseline through week 22. One additional subject had a decrease in CSF α-syn compared to baseline values at week 22

CONCLUSIONS

- SNK01 is well-tolerated by AD subjects with no dose-limiting toxicities observed at all doses tested
- SNK01 appears to stabilize or improve cognitive function in majority of the subjects, despite 70% of subjects being treated at relatively low doses
- SNK01 appears to reduce α-synuclein levels in CSF
- This data warrants further investigation in a larger Phase II trial with higher doses and a longer treatment duration

CONTACT

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