Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

NSC001 (also known as AF267B, NGX267, and NI004)

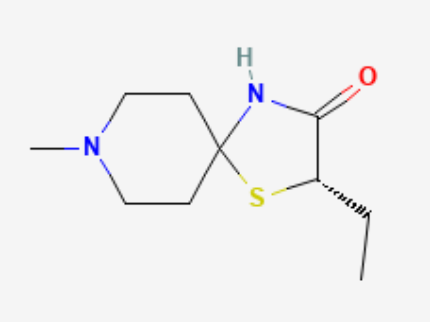
Evidence Summary

NSC001 reduced CSF p-tau181 but did not alter other CSF biomarkers in a phase 1b study of healthy elderly people. Early phase studies have reported that NSC001 is generally well tolerated.

Neuroprotective Benefit: In a phase 1b study in healthy elderly people, NSC001 treatment resulted in high brain uptake and significantly reduced CSF p-tau181, but did not alter CSF Aβ40, Aβ42, or NfL levels.

Aging and related health concerns: In a small phase 2 study in Sjogren's syndrome patients, NGX267 treatment increased salivary flow production.

Safety: Phase I and II studies have reported that NSC001 is well tolerated. Adverse events included headache, salivary hypersecretion, sweating, and GI issues. Most trials have tested single doses or short treatment durations. Long-term safety is not established.

Availability: in clinical development	**Dose**: Not established; in a phase 1b study of 65 healthy elderly volunteers, daily doses of 2.5, 5, 10, or 15 mg were tested.	**Chemical formula:** $C_{10}H_{18}N_2OS$ **MW**: 214.33  Source: PubChem
Half-life: 6-8 hours	**BBB**: penetrant	
Clinical trials: A phase 1b trial of NSC001 enrolled 65 healthy elderly volunteers.	**Observational studies**: N/A	

What is it?

NSC001 (also known as AF267B, NGX267, and NI004) is a rigid analog of acetylcholine and is a selective orthosteric M1 muscarinic acetylcholine receptor (mAChR) agonist. M1 muscarinic receptors play an important role in memory and learning and is abundantly expressed in the cortex and hippocampus (reviewed in Fisher, 2008, Giacobini et al., 2022). Degeneration of basal forebrain cholinergic neurons precedes Alzheimer's disease pathology. While nicotinic acetylcholine receptors are markedly lost in Alzheimer's disease, M1 receptors are not (Nordberg and Winblad, 1986). There are three main mechanisms through which M1 agonists may benefit Alzheimer's disease: 1) increase PKC activation, which in turn activates MAPK (ERK1/2), leading to restoration of cognitive function; 2) activate ADAM17 and inhibit BACE-1, promoting a non-amyloidogenic processing of APP and reducing levels of Aβ; and 3) inhibit GSK-3β, decreasing phosphorylation of tau (Fisher, 2008).

The first generation of muscarinic agonists (e.g., milameline, sabcomeline, talsaclidine, alvameline) have failed in clinical trials for Alzheimer's disease, due to adverse events, narrow safety margin, low bioavailability, extensive metabolism, low intrinsic activity, and/or lack of selectivity for the M1 muscarinic receptor (reviewed in Giacobini et al., 2022).

NSC001 is under development by NSC Therapeutics GmbH for the treatment of neurodegenerative diseases with cholinergic deficits, such as Alzheimer's disease and dementia with Lewy bodies (Alzforum,

5/10/2024). NGX267 was previously tested in a phase 2 trial for the treatment of dry mouth in Sjogren's syndrome, a chronic autoimmune condition that causes the immune system to attack the glands that produce saliva and tears (BioSpace, 12/2/2008).

Neuroprotective Benefit: In a phase 1b study in healthy elderly people, NSC001 treatment resulted in high brain uptake and significantly reduced CSF p-tau181, but did not alter CSF Aβ40, Aβ42, or NfL levels.

Types of evidence:

- A phase 1b trial in healthy elderly volunteers (not published in a peer-reviewed journal)
- 1 biomarker study in Alzheimer's patients of a different M1 selective agonist, AF102B
- Several laboratory studies
- Numerous review articles

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

In a phase 1b trial in 65 healthy elderly volunteers, NSC001 treatment (2.5, 5, 10, or 15 mg, daily) for 4 weeks had no significant effects on cognition or psychiatric symptoms, based on a presentation at the 2024 AD/PD conference (Alzforum, 5/10/2024). Cognitive effects were not expected given the subjects were cognitively healthy at baseline and the treatment duration was short. Brain uptake of NSC001 was high, with CSF concentrations reaching half that in plasma. NSC001 treatment did not result in significant changes in CSF Aβ40, Aβ42, or NfL, and resulted in a slight reduction in total tau. NSC001 treatment significantly reduced CSF p-tau181, which was attributed to GSK3β inhibition. NSC001 treatment altered circadian rhythms and normalized alpha waves on EEG. Detailed results of the phase 1b study have not been published in a peer-reviewed journal as of July 2024.

Human research to suggest benefits to patients with dementia:

No results from clinical trials testing NSC001 in dementia patients have been published in peer-reviewed journals.

In a biomarker study of 19 Alzheimer's patients, treatment with a different M1 (and M3) agonist, AF102B, for 4 weeks (Snow Brand Milk Products Company, Tokyo Japan; 20 mg, 3 times daily during

week 1; 40 mg, 3 times daily during week 2; 60 mg, 3 times daily during week 3; 80 mg, 3 times daily during week 4) resulted in a 22% decrease in CSF Aβ levels (total) in 14 patients, increased CSF Aβ in 3 patients, and no changes in 2 patients, with an overall decrease as a group being statistically significant (p=0.004; baseline=22.8 ± 7.5 vs after AF102B treatment=19.9 ± 7.9)(Nitsch et al., 2000). This reduction in CSF Aβ levels was not accompanied by an increase in APPs.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

In the 3xTg-AD mouse model of Alzheimer's disease, at 8 months of age, there is widespread intraneuronal Aβ accumulation throughout the cortex, hippocampus, and the amygdala and diffuse plaques in specific cortical regions and in the CA1/subiculum region. In 6-month old 3xTg-AD mice, AF267B (1 or 3 mg/kg/day, i.p.) for 10 weeks rescued spatial memory, measured by the Morris water maze, while significantly reducing Aβ42 and tau pathologies (HT7, AT8, and PHF-1 immunoreactivity) in the hippocampus and cortex (Caccamo et al., 2006). AF267B-treated 3xTg-AD mice reached learning criterion after 4 days, comparable to the performance of non-transgenic mice, while vehicle-treated 3xTg-AD mice required 6 days to reach criterion. In 3xTg-AD mice, AF267B treatment rescued the day-to-day memory retention deficit and memory impairments (measured by the latency to cross the platform, number of platform crosses, and time spent in the opposite quadrant) on the Morris water maze. AF267B treatment reduced Aβ pathologies through a decrease in brain BACE1 levels and the selective activation of ADAM17, shifting APP processing toward the nonamyloidogenic pathway. AF267B-mediated modulation of APP processing is mediated, in part, by increased activities of ERK1/2 and PKC. Reduction in tau pathology (e.g., tau phosphorylation) was mediated by decreased GSK3β activity. In contrast, administration of dicyclomine, an M1 antagonist, exacerbated Aβ and tau pathologies. AF267B treatment did not rescue amygdala-dependent contextual fear conditioning or alter Aβ and tau pathologies in the amygdala of 3xTg-AD mice. In non-transgenic mice, AF267B treatment did not influence cognitive function.

In mice with small hippocampi, which have spatial learning deficits, treatment with AF267B significantly attenuated the impairment in acquisition, memory, and reversal-learning (Fisher et al., 2002). AF267B treatment also improved escape latency in reversal learning in the Morris water maze test.

In rabbits, which have identical Aβ amino acid sequence to that of humans, treatment with AF267B (2 mg/kg/day, s.c.) for 5 days significantly decreased CSF Aβ42 by 40.8% and CSF Aβ40 by 45.5%, but levels of CSF-secreted APP (sum of sAPPα and sAPPβ) were not significantly altered (Beach et al., 2001). AF267B-treated rabbits had a non-significant trend for lower levels of total secreted β-APP.

In hypercholesterolemic rabbits, AF267B treatment decreased brain Aβ levels (reviewed in Fisher 2007, Fisher, 2008). AF267B treatment also decreased CSF Aβ42 levels and removed vascular Aβ42 deposition from the cortex in cholinotoxin-treated rabbits.

In PC12 cells stably transfected with M1 receptors (PC12M1 cells), starvation of these cells decreased cell viability, and this was further exacerbated by exposure to Aβ42 and Aβ25-35 (Fisher et al., 2002). AF267B administration significantly protected the cells from apoptosis induced by each insult and all of them combined. AF267B treatment had no effect on cell viability in PC12 cells (not transfected with M1 receptors).

APOE4 interactions: Unknown

Aging and related health concerns: In a small phase 2 study in Sjogren's syndrome patients, NGX267 treatment increased salivary flow production.

Types of evidence:

- A phase 2 trial in Sjogren's syndrome

Sjögren's syndrome is a chronic autoimmune disease that causes the immune system to attack the glands that produce saliva and tears, and this condition typically develops between the ages of 45 and 55. In a double-blind randomized placebo-controlled crossover study in 26 patients with xerostomia (dry mouth) associated with primary or secondary Sjogren's syndrome, a single dose of NGX267 (10, 15, and 20 mg) significantly increased salivary flow production (primary outcome) compared to placebo across a number of time points, including at 24 hours post-dosing (BioSpace, 12/2/2008). NGX267 treatment at the 15 mg and 20 mg doses also significantly improved the secondary outcome, the 8-item salivary flow questionnaire, with statistically significant differences compared to placebo in domains that rated speaking and swallowing, dryness of mouth, and level of thirst.

Safety: Phase I and II studies have reported that NSC001 is well tolerated. Adverse events included headache, salivary hypersecretion, sweating, and GI issues. Most trials have tested single doses or short treatment durations. Long-term safety is not established.

Types of evidence:

- A phase 2 trial in Sjogren's syndrome
- A phase 1b trial in healthy elderly volunteers
- A dose-escalation study in healthy volunteers
- Two other phase I studies
- Several laboratory studies

In a double-blind randomized placebo-controlled crossover phase 2 study in 26 patients with xerostomia (dry mouth) associated with primary or secondary Sjogren's syndrome, a single dose of NGX267 (10, 15, and 20 mg) was safe and well tolerated, with few reports of excessive sweating and gastrointestinal complaints (BioSpace, 12/2/2008). Adverse events were generally of mild to moderate severity and occurred with all doses and with placebo. The frequency of adverse events was dose-related, with the 20 mg group reporting the highest number of events.

In a phase 1b trial in 65 healthy elderly volunteers, NSC001 treatment (2.5, 5, 10, or 15 mg, daily) for 4 weeks resulted in adverse events that were mostly mild or moderate, based on a presentation at the 2024 AD/PD conference (Alzforum, 5/10/2024). Severe adverse events were mainly attributed to lumbar puncture and 24-hour CSF collection on day 1. There were no typical cholinergic side effects observed. The most common adverse event was headache, which was seen equally in the treatment and placebo groups.

In a dose-escalation study in 34 healthy male volunteers, 2 subjects each received single NGX267 doses of 1, 2.5, 5, 10, 15, 25, and 45 mg, 10 subjects received an NGX267 dose of 35 mg, and 10 subjects received placebo (Ivanova and Murphy, 2009). The study was designed to estimate the maximally tolerated dose and to gain clinical and pharmacokinetic data. The dose increase was terminated when the adverse event rate met predefined criteria after the NGX267 dose of 45 mg, and the 35 mg dose was repeated in the following two cohorts of 4 subjects. A single, oral 35 mg dose of NGX267 was estimated to be the maximally tolerated dose in normal male subjects. At this dose, 8 out of 10 subjects reported a total of 31 adverse events and 2 subjects reported no adverse events (European Patent: EP3523310A1). Treatment-emergent adverse events reported by more than one subject treated with this dose were

salivary hypersecretion (n=4), hyperhidrosis (excessive sweating; n=4), cold sweat (n=4), abdominal discomfort (n=2), and dysgeusia (distorted taste; n=2).

In a different phase I study that randomized healthy elderly subjects (age 65-80 years, men and women), 20 received NGX267 and 6 received placebo, and the maximum tolerated oral dose was determined to be 20 mg (European Patent: EP3523310A1).

In a double-blind, placebo-controlled, multiple-dose, sequential cohort study that randomized 60 healthy male volunteers (age 18-54 years), 48 received anhydrous NGX267 ("Compound A Form II") at 10, 20, 30, 35 mg once daily dose for 4 days and 12 received placebo (European Patent: EP3523310A1). Plasma concentrations of NGX267 and its active desmethyl metabolite increased dose-proportionally. The apparent elimination half-life of NGX267 was similar across doses, with mean half-life estimates ranging from 7.06 to 7.57 hours on Day 0 and 6.58 to 7.14 hours on Day 3.

In 6-month old 3xTg-AD mice, AF267B treatment (1 or 3 mg/kg/day, i.p.) for 10 weeks did not result in adverse changes in general health (Caccamo et al., 2006).

Drug interactions: Drug interactions of NSC001 have not been documented.

Sources and dosing:

NSC001 is under development by NSC Therapeutics GmbH for the treatment of neurodegenerative diseases with cholinergic deficits, such as Alzheimer's disease and dementia with Lewy bodies (Alzforum, 5/10/2024). Dosage has not been established. A phase 1b study in healthy elderly people tested NSC001 doses ranging from 2.5 mg to 15 mg, daily (Alzforum, 5/10/2024).

Research underway:

No clinical trials are currently ongoing or registered for NSC001, based on ClinicalTrials.gov or EUClinicalTrials.eu.

Search terms:

Pubmed, Google: NSC001, NGX267, AF267B

Websites visited for NSC001, NGX267, AF267B:

- Clinicaltrials.gov
- NIH RePORTER (0)
- Drugs.com (0)
- PubChem
- DrugBank.ca