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.

AMX0035

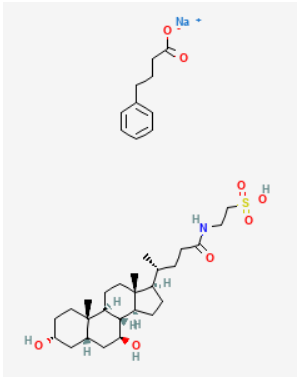
Evidence Summary

AMX0035 slows functional decline and prolongs survival in ALS patients. A longer, larger trial is needed to assess potential benefits for AD. AMX0035 has many interactions with drugs, diseases, and food.

Neuroprotective Benefit: The phase 2 study in AD was not designed to detect cognitive or functional effects. The phase 2 study in ALS reported slower rate of functional decline, prolonged survival, and 44% lower risk of death.

Aging and related health concerns: No studies with AMX0035 have been carried out for age-related diseases other than Alzheimer's disease and ALS. In ALS patients, AMX0035 treatment significantly prolonged survival compared to placebo.

Safety: The most common adverse events with AMX0035 are gastrointestinal in nature. AMX0035 may increase bile acid levels and fluid retention. In a minority of ALS patients, asymptomatic electrocardiographic changes have also been observed.

Availability: Rx for ALS; in development for Alzheimer's disease and Wolfram Syndrome	**Dose**: In ALS patients, 1 packet (3 g of sodium phenylbutyrate and 1 g of tauroursodeoxycholic acid) daily is taken for the first 3 weeks, followed by 1 packet twice daily (Relyvrio.com).	**Chemical formula:** $C_{26}H_{45}NO_6S$ **MW**: 187.19 (sodium phenylbutyrate; top); 499.7 (TUDCA; bottom)  Source: PubChem
Product/Company: Relyvrio™/ Amylyx Pharmaceuticals Inc.		
Half life: 45 minutes for sodium phenylbutyrate	**BBB**: penetrant	
Clinical trials: A phase 2 trial in AD enrolled 95 patients. A phase 2 trial in ALS enrolled 137 patients.	**Observational studies**: N/A	

What is it? AMX0035 (marketed as Relyvrio™) is a combination therapy of two active compounds, sodium phenylbutyrate and tauroursodeoxycholic acid (TUDCA; also known as taurursodiol, or TURSO). It is approved for the treatment of amyotrophic lateral sclerosis (ALS). This combination therapy is designed to reduce neuronal death through two mechanisms: sodium phenylbutyrate decreases endoplasmic reticulum (ER) stress (by upregulating the master chaperone regulator DJ-1) and TUDCA mitigates mitochondrial dysfunction (by incorporating into the mitochondrial membrane and increasing apoptotic threshold)(Paganoni et al., 2020; Jiang et al., 2022).

After the completion of the phase 2 study for ALS, the company developing AMX0035, Amylyx, submitted a New Drug Application to the US FDA (before completion of phase 3 trials). ALS patient advocacy groups also submitted over 50,000 signatures to the FDA, calling on the agency to approve AMX0035 for the treatment of ALS. In September 2022, the FDA convened an advisory committee meeting, at which members voted in favor of approving the drug (7-2). On September 29, 2022, the FDA approved AMX0035 (Relyvrio) for the treatment of ALS (FDA.gov). AMX0035 is also under clinical development for Alzheimer's disease and Wolfram syndrome, a rare genetic disorder that causes insulin-dependent diabetes mellitus and progressive optic atrophy (Amylyx pipeline).

For details on the individual components of this combination therapy, please see Cognitive Vitality Reports on sodium phenylbutyrate and tauroursodeoxycholic acid (TUDCA).

Neuroprotective Benefit: The phase 2 study in AD was not designed to detect cognitive or functional effects. The phase 2 study in ALS reported slower rate of functional decline, prolonged survival, and 44% lower risk of death.

Types of evidence:

- 1 phase 2 study in Alzheimer's disease patients
- 1 phase 2 study and an open-label extension study in ALS patients
- Numerous laboratory studies for sodium phenylbutyrate and TUDCA, individually

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:
None available.

Human research to suggest benefits to patients with dementia:
Results from the phase 2 double-blind, randomized, controlled trial of AMX0035 (PEGASUS trial; NCT03533257) in mild cognitive impairment or Alzheimer's disease were announced at the 14th Clinical Trials on Alzheimer's Disease (CTAD) conference held in November 2021 (BusinessWire; NeurologyLive). This study was co-funded by the ADDF and the Alzheimer's Association. The study met the primary end point of safety and tolerability (discussed below under "Safety" section). This phase 2 study was not designed to evaluate differences between placebo and treatment groups in efficacy outcomes, and there were no differences found in cognitive or functional measures, or in hippocampal volume. This clinical trial did have some baseline imbalances. A higher baseline level of cognitive impairment was present in the AMX0035 group compared with the placebo group (ADAS-Cog14, MoCA, and mild/moderate Alzheimer's disease composite scale; $p<0.01$ for all). The proportion of APOE4 carriers was also higher in the AMX0035 group (77.1%) compared with placebo (61.4%).

For exploratory outcomes, CSF and plasma biomarkers were evaluated (NCT03533257). When compared with placebo, significant reductions were seen in CSF total tau ($p=0.0005$) and p-tau181 ($p=0.0008$) in patients treated with AMX0035, as well as an increase in Aβ42/Aβ40 ratio ($p<0.05$), suggesting less accumulation of amyloid in the brain (NeurologyLive).

There was an increase in an oxidative stress marker, 8-OHdG, in the CSF of the AMX0035 group compared to the placebo group ($p<0.01$)(BusinessWire). This increase was unexpected and potentially a cause for concern, as studies have reported that CSF levels of 8-OHdG are elevated in several neurodegenerative diseases including Alzheimer's disease (Isobe et al., 2010) and Parkinson's disease (Gmitterova et al., 2009). It is not clear if AMX0035 treatment is responsible for the elevated 8-OHdG levels or if the level difference is related to the baseline imbalance between the AMX0035 and placebo groups.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:
For mechanisms of action of the individual components of AMX0035, please see Cognitive Vitality Reports on sodium phenylbutyrate and tauroursodeoxycholic acid (TUDCA).

ALS: AMX0035 has been tested in clinical trials for ALS patients. ALS is characterized by motor neuron degeneration that leads to progressive muscle weakness, with respiratory failure being the predominant cause of death several years after symptom onset.

A phase 2 double-blind, randomized controlled trial (the CENTAUR trial) enrolled 137 ALS patients who received AMX0035 (n=89) or placebo (n=48) for 24 weeks (Paganoni et al., 2020). Patients were within 18 months after symptom onset and had clinical evidence of both upper and lower motor neuron signs in at least 3 body regions. The AMX0035 dose was 3 g of sodium phenylbutyrate and 1 g of TUDCA per sachet, that was dissolved in room temperature water, and taken orally or through a feeding tube, once daily for the first 3 weeks and 2 sachets per day (1 in the morning, 1 in the evening) thereafter. Most participants were receiving riluzole or edaravone concurrently, with 28% of participants receiving both. A higher percentage of the placebo group (50%) were receiving edaravone than the AMX0035 group (25%). This trial was designed by and conducted through the Northeast Amyotrophic Lateral Sclerosis Consortium (NEALS; www.neals.org) in collaboration with Amylyx Pharmaceuticals Inc. Amylyx Pharmaceuticals Inc. provided AMX0035 and placebo and was involved in the trial design, data analysis, and manuscript development. Statistical analyses were performed by Pentara Corp, the Massachusetts General Hospital Biostatistics Center, and an independent consultant.

For the primary outcome, AMX0035 treatment significantly slowed the mean rate of change in the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised score (ALSFRS-R-score; 48-point scale), indicating slower functional decline than placebo over a 24-week period (-1.24 points per month with AMX0035; -1.66 points per month with placebo; difference of 0.42 points per month; 95% CI, 0.03-0.81; $p=0.03$)(Paganoni et al., 2020). In a sensitivity analysis, correcting for the use of edaravone gave a similar

result as the primary analysis, with an estimated between-group difference of 2.15 points in the ALSFRS-R score (95% CI, -0.05 to 4.35). A sensitivity analysis that corrected for the use of riluzole showed an estimated between-group difference of 2.34 points in the ALSFRS-R score (95% CI, 0.19 to 4.48). The ALSFRS-R-score consists of 4 subdomains and the change that was most prominent was the fine-motor subscale.

For secondary outcomes, there were no statistically significant differences between the AMX0035 and placebo groups, even though the direction of change was mostly in the same direction as the primary outcome. Secondary efficacy outcomes were assessed in the following hierarchical order: rate of decline in isometric muscle strength as measured by the Accurate Test of Limb Isometric Strength (ATLIS) device; the rate of decline in plasma levels of the phosphorylated axonal neurofilament H subunit (pNF-H; a potential biomarker of motor neuron degeneration); the rate of decline in the slow vital capacity (SVC); the time to death, tracheostomy, permanent assisted ventilation, or hospitalization; the pharmacokinetics of sodium phenylbutyrate and TUDCA; and 18-kD translocator protein (TSPO) uptake on the MR-PET scan (in a subgroup). The hierarchical analysis with the first secondary outcome (rate of decline in isometric muscle strength) failed to reach statistical significance, with the mean rate of change per month at -3.03% with AMX0035 treatment and -3.54% with placebo (difference, 0.51% per month; 95% CI, -0.12 to 1.14). Thus, subsequent secondary outcomes were analyzed without p-values.

The mean rate of change in the plasma pNF-H concentration was 3.58 pg/mL per month with AMX0035 and −2.34 pg/mL per month with placebo (difference, 5.92 pg/mL per month; 95% CI, −4.41 to 16.26). The mean rate of change in the SVC was −3.10% of the predicted normal value per month with AMX0035 and −4.03% of the predicted normal value per month with placebo (difference, 0.93% per month; 95% CI, −0.10 to 1.95). The cumulative hazard ratio for death, tracheostomy, or hospitalization in the AMX0035 group compared with the placebo group, was 0.53 (95% CI, 0.27 to 1.05). Death occurred in 5 participants (6%) who received AMX0035 and in 2 participants (4%) who received placebo. The most common cause of death was respiratory failure, accounting for 4 out of the 7 deaths, consistent with ALS pathology.

In an open-label extension study of the CENTAUR trial patients who were followed for up to 35 months post-randomization, patients who were originally randomized to AMX0035 treatment had a 44% lower risk of death than those randomized to placebo (HR=0.56; 95% CI, 0.34 to 0.92; p=0.023)(Paganoni et al., 2020). Vital status was obtained for 135 out of 137 participants originally randomized in the CENTAUR trial. Median overall survival was 25.0 months among participants originally randomized to AMX0035

and 18.5 months among those originally randomized to placebo. Thus, initiation of AMX0035 treatment at baseline resulted in a 6.5-month longer median survival compared with placebo.

The estimated probability of survival at 12 months among participants originally randomized to AMX0035 and placebo was 80.9% (95% CI, 71.1 to 87.7%) and 72.9% (95% CI, 58.0 to 83.3%), respectively. At 24 months, the estimates were 51.6% (95% CI, 38.9 to 62.9%) and 33.9% (95% CI, 19.4 to 49.1%), respectively. In patients originally randomized to AMX0035, the mean AMX0035 exposure duration was 10.6 months; and in the group originally randomized to placebo, the mean AMX0035 exposure duration was 4.7 months.

In an update to the open-label extension study of CENTAUR trial participants, after 6 months of AMX0035 treatment followed by an open-label extension of up to 30 months resulted in a 47% lower risk of any key event in those originally randomized to AMX0035 versus placebo (HR=0.53; 95% CI, 0.35 to 0.81; p=0.003)(Paganoni et al., 2022). Median event-free durations were 14.8 (6.5-29.1) and 10.0 (4.0-15.0) months, respectively; thus, 4.8 months longer in participants originally randomized to AMX0035. Risks of death or tracheostomy or permanent assistant ventilation (PAV) were 49% lower in those originally randomized to AMX0035 (HR=0.51; 95% CI, 0.32 to 0.84; p=0.007), with median tracheostomy/PAV-free survival durations of 25.8 (14.8–33.6) months and 18.5 months (11.7 months–not reached), respectively. First hospitalization was 44% lower in those originally randomized to AMX0035 (HR=0.56; 95% CI 0.34 to 0.95; p=0.03), with median hospitalization-free duration not reached (6.9 months to not reached) in those originally randomized to AMX0035 versus 14.1 months (4.2 months to not reached) in those originally randomized to placebo. Consistent with the earlier publication, early AMX0035 treatment prolonged tracheostomy/PAV-free survival and delayed first hospitalization in ALS patients. Riluzole and edaravone are two other FDA-approved therapies for ALS, but only riluzole has shown a survival benefit in randomized clinical trials (Miller et al., 2012). It is worth noting that AMX0035 treatment demonstrated a dual benefit on survival and function (Paganoni et al., 2022).

A Bayesian decision analysis of AMX0035 reported that the benefits of therapeutic effects seem to outweigh the risks of adverse effects in ALS patients (Xu t al., 2022).

The ongoing phase 3 trial (PHOENIX; NCT05021536) will further evaluate the safety and efficacy of AMX0035 over 48 weeks in a more heterogeneous, international population of 600 individuals with ALS.

In primary skin fibroblasts of sporadic ALS patients, exposure to AMX0035 changed more genes and metabolites than either phenylbutyrate or TUDCA individually (Fels et al., 2022). The gene expression

changes unique to AMX0035 affected nucleocytoplasmic transport, unfolded protein response (promoting cell survival under ER stress), mitochondrial oxidative phosphorylation, RNA metabolism, and innate immune activation (clearing misfolded proteins). Modulation of these pathways could underlie the neuroprotective benefits seen with AMX0035 in ALS patients. The weighted gene co-expression correlation analysis showed significant correlations between ALS gene expression modules and clinical parameters that were reversed by AMX0035 administration.

APOE4 interactions: Unknown.

Aging and related health concerns: No studies with AMX0035 have been carried out for age-related diseases other than Alzheimer's disease and ALS. In ALS patients, AMX0035 treatment significantly prolonged survival compared to placebo.

Types of evidence:

- 1 clinical trial in ALS patients
- 0 laboratory studies

AMX0035 has not been studied for age-related diseases other than the neurodegenerative diseases discussed under the "Neuroprotective Benefit" section above.

As discussed above, the open-label extension study in ALS patients who were followed up for a maximum of 35 months reported that AMX0035 provided survival benefits in ALS patients (Paganoni et al., 2020). Median overall survival was 25.0 months among participants originally randomized to AMX0035 and 18.5 months among those originally randomized to placebo (HR=0.56; 95% CI, 0.34 to 0.92; p=0.023).

Safety: The most common adverse events with AMX0035 are gastrointestinal in nature. AMX0035 may increase bile acid levels and fluid retention. In a minority of ALS patients, asymptomatic electrocardiographic changes have also been observed.

Types of evidence:

- 2 clinical trials, 1 in ALS and 1 in Alzheimer's disease
- Several clinical and laboratory studies for sodium phenylbutyrate and TUDCA, individually

AMX0035 may cause serious side effects, including changes in bile acid levels. If you already have problems with liver, bile ducts, or pancreas, AMX0035 may increase bile acid levels, causing worsening diarrhea (Drugs.com). AMX0035 also contains a high amount of salt, which may lead to fluid retention. People with heart failure, high blood pressure, or kidney problems may need to limit dietary intake of salt. The most common side effects with AMX0035 are diarrhea, abdominal pain, nausea, and upper respiratory tract infection. AMX0035 may make you dizzy or drowsy (WebMD.com).

In a phase 2 double-blind, randomized controlled trial (the CENTAUR trial) enrolling 137 ALS patients, adverse events with AMX0035 (n=89) were mainly gastrointestinal (Paganoni et al., 2020). In the modified intention-to-treat population, 69% of the subjects in the AMX0035 group and 77% of the subjects in the placebo group completed the trial regimen. Death occurred in 5 subjects (6%) who received AMX0035 and in 2 subjects (4%) who received placebo. The most common cause of death was respiratory failure (4 out of 7), a finding consistent with ALS pathology.

A total of 97% of the participants in the AMX0035 group and 96% in the placebo group had one or more adverse events during the trial (Paganoni et al., 2020). Events occurring at greater than 2% frequency in the AMX0035 group were primarily gastrointestinal (diarrhea, nausea, salivary hypersecretion, and abdominal discomfort). Gastrointestinal adverse events were reported more frequently in the AMX0035 group than in the placebo group during the first 3 weeks, with nausea, diarrhea, and abdominal pain accounting for most events; thereafter, these events were reported less frequently in the AMX0035 group than in the placebo group for the remainder of the trial. Mean changes in body weight from baseline to week 24 were similar in AMX0035 and placebo groups.

Digital electrocardiography showed asymptomatic electrocardiographic changes, including left anterior hemiblock, left bundle-branch block, and nonspecific T-wave changes in 7 participants in the AMX0035 group and 3 participants in the placebo group (Paganoni et al., 2020). Corrected QT intervals remained stable and did not differ significantly between the AMX0035 and placebo groups at any time point.

Serious adverse events were less frequent in the AMX0035 group than in the placebo group (12% vs. 19%)(Paganoni et al., 2020). The incidence of respiratory serious adverse events was 8% in the placebo group and 3% in the AMX0035 group. A total of 19% of the participants in the AMX0035 group prematurely discontinued the trial regimen owing to adverse events, as compared with 8% in the placebo group. The most common adverse events leading to discontinuation of the trial were diarrhea

(6% in the AMX0035 and 0% in the placebo) and respiratory failure (6% in the placebo group and 0% in the AMX0035 group).

In an open-label extension study of the CENTAUR trial ALS patients who were followed for up to 35 months post-randomization, the AMX0035 and placebo groups had similar rates of death-equivalent events (Paganoni et al., 2020). Six (6.7%) patients originally randomized to AMX0035 and 4 (8.3%) patients originally randomized to placebo experienced death-equivalent events.

In the phase 2 double-blind, randomized, controlled trial that tested AMX0035 (PEGASUS trial; NCT03533257) in mild cognitive impairment or Alzheimer's disease, the primary end point of safety and tolerability was met (NeurologyLive). Treatment with AMX0035 for 24 weeks was found to be associated with a higher incidence of gastrointestinal events when compared with placebo. In the AMX0035 treatment group, 36 patients (67%) reported treatment-emergent adverse events (TEAEs), compared with 26 patients (59%) in the placebo group. The greatest proportion of TEAEs in the treatment group were gastrointestinal events, primarily diarrhea, occurring in 20 (39%) patients, compared with 6 (14%) patients in the placebo group. In the AMX0035 treatment group, 10 out of 51 participants (19.6%) discontinued the study, and in the placebo group, 2 out of 44 participants (4.5%) discontinued.

Drug interactions: AMX0035 has 559 known drug interactions (Drugs.com). Examples of major drug interactions include aluminum-containing antacids, bile acid-binding resins (such as choestyramine, colestipol, and colesevelam), cyclosporine, probenecid, vorinostat, romidepsin, panobinostat, belinostat, and others (Drugs.com; WebMD.com).

AMX0035 has 4 disease interactions: liver disease, kidney disease, neurotoxicity, and biliary disorders (Drugs.com). The use of sodium phenylbutyrate may cause fluid retention in patients with congestive heart failure, renal dysfunction, or sodium retention with edema. Sodium phenylbutyrate is metabolized primarily by the liver and kidney; thus, caution should be exercised in patients with liver/kidney disease. Sodium phenylbutyrate is a prodrug and is metabolized to the active phenylacetate, which when administered intravenously, has been associated with exacerbation of preexisting neuropathy. Care should be exercised when sodium phenylbutyrate is used in patients at risk for neurotoxicity.

AMX0035 has 3 interactions with food/drinks: high-fat/calorie meals, nicotine, and caffeine (Drugs.com). Co-administration of sodium phenylbutyrate with a high-fat/high-calorie meal may reduce the rate and extent of absorption of sodium phenylbutyrate. Taurursodiol may alter the blood levels and effects of caffeine and nicotine.

In human liver cell culture, TUDCA and UDCA reduced cell death induced by alcohol when administered at the same time (Henzel et al., 2004). However, when TUDCA or UDCA was administered before ethanol insult, damage to liver cells was exacerbated. The mechanisms driving these opposing effects are unclear. It is also unknown whether these effects extend to humans.

Sources and dosing: AMX0035 (marketed as Relyvrio™, Amylyx Pharmaceuticals, Inc) was approved for the treatment of ALS in September 2022. In ALS patients, the recommended dosage for the first 3 weeks is 1 packet (3 g of sodium phenylbutyrate and 1 g of taurursodiol) daily (Relyvrio.com). After 3 weeks, the dosage increases to 1 packet twice daily. A packet is emptied into a cup of room temperature water (8 oz) and stirred well. The medication can be taken before a snack or meal. AMX0035 is also under clinical development by Amylyx Pharmaceuticals Inc. for the treatment of Alzheimer's disease and Wolfram syndrome (Amylyx pipeline).

Research underway: There are currently 4 ongoing clinical trials testing AMX0035. The randomized, double-blind, placebo-controlled phase 3 trial of AMX0035 for ALS is enrolling 600 participants (NCT05021536). They are testing AMX0035 (once daily for first 3 weeks, then twice daily thereafter) for 48 weeks and the primary outcomes are the ALS score (ALSFRS-R) slope change and survival at 48 weeks; the number of participants with adverse events, and the number of participants who remained on the study drug. This phase 3 trial is scheduled to be completed in March 2024. The open-label extension study of this phase 3 trial will evaluate the safety and tolerability of AMX0035 over 108 weeks (NCT05619783). This phase 3b study is scheduled to be completed in August 2026. A pharmacokinetic and pharmacodynamic study of AMX0035 is being carried out in 14 patients with ALS and this study is scheduled to be completed in October 2022 (NCT04987671). A phase 2 trial is testing the safety and efficacy of AMX0035 in 12 adult patients with Wolfram Syndrome (NCT05676034). It is an open-label trial and AMX0035 will be administered for 24 weeks (once daily for first 3 weeks and twice daily for the remainder of the study if tolerated by participant). This study is scheduled to be completed in February 2024.

Search terms:
Pubmed, Google: AMX0035, AMX-0035, Relyvrio

Websites visited for AMX0035, AMX-0035, Relyvrio:

- Clinicaltrials.gov
- NIH RePORTER (0)
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com
- WebMD.com
- PubChem
- DrugBank.ca (TUDCA; PB)
- Cafepharma
- Pharmapro.com (0)