

AMX0035

A proprietary two-component oral formulation developed by Amylyx Pharmaceuticals combining sodium phenylbutyrate and taurursodiol (tauroursodeoxycholic acid) to concurrently attenuate endoplasmic reticulum stress and mitochondrial apoptotic signaling in neurodegenerative disease, approved under accelerated pathway by the United States Food and Drug Administration in September 2022 for the treatment of amyotrophic lateral sclerosis in adults and subsequently withdrawn from the market in 2024 following failure to confirm efficacy in the confirmatory Phase 3 PHOENIX trial.

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COMPOUND CLASS

Fixed-dose oral combination of sodium phenylbutyrate (chemical chaperone and HDAC inhibitor) and taurursodiol (bile acid cytoprotectant) targeting endoplasmic reticulum stress and mitochondrial dysfunction

ABSTRACT

RESEARCH USE ONLY
AMX0035, marketed as Relyvrio in the United States and Albrioza in Canada, is a fixed-dose oral combination of sodium phenylbutyrate (3 grams per sachet) and taurursodiol (1 gram per sachet) developed by Amylyx Pharmaceuticals for the treatment of amyotrophic lateral sclerosis (ALS) and under investigation for Alzheimer disease. The therapeutic rationale is simultaneous modulation of two convergent cell-death pathways implicated in motor neuron degeneration: endoplasmic reticulum (ER) stress, addressed by the chemical chaperone and histone deacetylase (HDAC) inhibitor activity of sodium phenylbutyrate, and mitochondrial apoptotic signaling, addressed by the bile acid cytoprotectant taurursodiol (the taurine conjugate of ursodeoxycholic acid). Sodium phenylbutyrate upregulates molecular chaperones including heat shock protein 70 (HSP70) and DJ-1, reduces accumulation of misfolded proteins in the ER lumen, and inhibits class I and class II histone deacetylases (excluding class III, HDAC6, and HDAC10), thereby promoting a transcriptional program favoring cell survival. Taurursodiol stabilizes the mitochondrial membrane by inhibiting Bax translocation, reducing mitochondrial permeability transition pore opening, and raising the apoptotic threshold by preventing cytochrome c release into the cytosol. In preclinical models, the combination produced synergistic reduction of hydrogen peroxide-induced neuronal cell death beyond either component alone.

The clinical evidence base for AMX0035 in ALS rests principally on the Phase 2 CENTAUR trial (NCT03127514), a 24-week,

randomized, double-blind, placebo-controlled study conducted across 25 Northeast ALS Consortium (NEALS) sites in 137 participants with ALS of no more than 18 months symptom duration [1]. The primary endpoint was the rate of decline on the ALS Functional Rating Scale-Revised (ALSFRS-R): participants receiving AMX0035 showed a mean decline of 1.24 points per month versus 1.66 points per month on placebo, a difference of 0.42 points per month (95% CI 0.03 to 0.81; $p = 0.03$). Long-term survival analysis of the CENTAUR cohort showed a median survival advantage of 6.5 months favoring those originally randomized to AMX0035 (hazard ratio 0.57; 95% CI 0.35 to 0.92; $p = 0.023$) [2]. On the basis of these Phase 2 data and an external-control survival analysis, the FDA granted accelerated approval to Relyvrio on September 29, 2022, for the treatment of ALS in adults. Health Canada had previously granted approval to Albrioza in June 2022.

The confirmatory Phase 3 PHOENIX trial (NCT05021536), a 48-week, randomized, placebo-controlled study in 664 adults with ALS, did not meet its primary endpoint of slowing ALSFRS-R decline ($p = 0.667$) or any secondary endpoints [3]. No significant treatment effect was observed even in the subset of participants who met the original CENTAUR eligibility criteria. Following the PHOENIX results, Amylyx Pharmaceuticals discontinued marketing of Relyvrio as of October 31, 2024, and submitted a formal request for withdrawal of FDA approval on February 28, 2025. The European Medicines Agency had previously declined to authorize the compound for marketing in the European Union.

A separate Phase 2 trial (PEGASUS) evaluated AMX0035 in 96 adults with mild cognitive impairment or dementia due to Alzheimer disease over 24 weeks [4]. The trial met its primary

endpoint of safety and tolerability but did not demonstrate slowing of cognitive decline. AMX0035 produced significant reductions in cerebrospinal fluid biomarkers of tau pathology (phosphorylated tau 181 and total tau, both $p < 0.001$) and modulation of the amyloid beta 42/40 ratio ($p < 0.05$), suggesting biological activity on core Alzheimer disease pathology despite the absence of a clinical cognitive signal in this short-duration study.

The pharmacokinetics of the two components are well characterized independently. Sodium phenylbutyrate is rapidly absorbed (T_{max} approximately 1 hour), with a plasma half-life of approximately 0.76 hours, and is metabolized by beta-oxidation to phenylacetate (half-life approximately 1.2 hours) and subsequently conjugated with glutamine to form phenylacetylglutamine, which is renally excreted. Taurursodiol, a hydrophilic bile acid conjugate, undergoes hepatic first-pass extraction via bile acid transporters, enters the enterohepatic circulation, and is metabolized by intestinal bacteria; systemic bioavailability is limited by efficient hepatic uptake. The combination is administered as a powder for oral suspension, mixed in 250 milliliters of water, taken once daily for the first three weeks and twice daily thereafter. The principal adverse events are gastrointestinal (diarrhea, abdominal pain, nausea), occurring predominantly during the first three weeks of dosing, with rates of discontinuation due to adverse events of approximately 19 percent in the CENTAUR trial compared to 8 percent on placebo.

This monograph reviews the chemistry, formulation, and component pharmacology of AMX0035; the dual-pathway mechanistic rationale; the complete pharmacokinetic profiles of both components; the preclinical neuroprotection data; the

clinical evidence from CENTAUR, PHOENIX, and PEGASUS; sourcing, reconstitution, and handling considerations; stack interactions; the adverse-event and safety profile; and a comparative assessment of five alternative ALS therapeutic agents (riluzole, edaravone, tofersen, masitinib, and dexpramipexole) against AMX0035 on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Combination product name: AMX0035.

Marketed names: Relyvrio (United States, Amylyx Pharmaceuticals; NDA 216660, approved September 29, 2022; approval withdrawn February 28, 2025), Albrioza (Canada, Amylyx Pharmaceuticals; approved June 2022; discontinued October 2024).

Development codes: AMX0035 (Amylyx Pharmaceuticals). The development code is the canonical identifier in the clinical literature and in all published trial registrations.

Component 1: Sodium phenylbutyrate.

International Nonproprietary Name: Sodium phenylbutyrate. Chemical Abstracts Service registry number: 1716-12-7. International Union of Pure and Applied Chemistry name: sodium 4-phenylbutanoate. Molecular formula: $C_{10}H_{11}NaO_2$. Molecular weight: 186.18 g/mol. The compound is approved independently as Buphenyl for the adjunctive treatment of urea cycle disorders at doses of 450 to 600 mg/kg/day [5].

Component 2: Taurursodiol (tauroursodeoxycholic acid, TUDCA).

International Nonproprietary Name: Ursodoxicoltaurine (INN); taurursodiol (USAN). Chemical Abstracts Service registry number: 14605-22-2. International Union of Pure and Applied Chemistry name: 2-[[[(3 α ,5 β ,7 β)-3,7-dihydroxy-24-oxocholan-24-yl]amino]ethanesulfonic acid. Molecular formula: $C_{26}H_{45}NO_6S$. Molecular weight: 499.71 g/mol. Taurursodiol is the taurine conjugate of ursodeoxycholic acid (UDCA) and is a naturally occurring hydrophilic bile acid produced in small quantities by intestinal bacterial metabolism [6].

1.2 Structural class and chemistry

AMX0035 is a combination product comprising two structurally unrelated compounds. Sodium phenylbutyrate is a short-chain aromatic fatty acid salt, structurally related to phenylacetic acid and classified as a chemical chaperone and as a class I and class II histone deacetylase inhibitor [7]. Taurursodiol is a C24 bile acid conjugated at the C-24 carboxyl with taurine through an amide bond, classified within the hydrophilic (non-cytotoxic) bile acid family that includes ursodeoxycholic acid, glyoursodeoxycholic acid, and tauroursodeoxycholic acid [6].

Physical properties of sodium phenylbutyrate: White to off-white crystalline powder. Freely soluble in water. Melting point approximately 233 to 236 degrees Celsius.

Physical properties of taurursodiol: White to off-white crystalline powder. Soluble in water. Melting point approximately 171 to 175 degrees Celsius.

Combined formulation: Each sachet contains 3 grams of sodium phenylbutyrate and 1 gram of taurursodiol, formulated as a powder for oral suspension with excipients including sodium phosphate dibasic anhydrous, dextrates hydrates, sorbitol, colloidal silica (Syloid 63FP), sucralose, sodium stearyl fumarate, mixed berry flavoring, and maltodextrin (Kleptose Linecaps) [8].

1.3 Synthesis and manufacture

Sodium phenylbutyrate is synthesized by standard methods for the preparation of aromatic fatty acid sodium salts: 4-phenylbutyric acid (the free acid form, CAS 1821-12-1) is prepared by Friedel-Crafts acylation or by palladium-catalyzed coupling reactions, followed by neutralization with sodium hydroxide to yield the sodium salt. Industrial synthesis is well established; the compound has been manufactured at pharmaceutical grade since its approval for urea cycle disorders in 1996 [5].

Taurursodiol is synthesized by conjugation of ursodeoxycholic acid (UDCA, CAS 128-13-2) with taurine (CAS 107-35-7) through activation of the C-24 carboxylic acid (typically via a mixed anhydride or carbodiimide coupling) followed by amide bond formation with the taurine amino group. The product is purified by recrystallization and characterized by high-performance liquid chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy [6].

The combined AMX0035 formulation is manufactured by blending the two active pharmaceutical ingredients with excipients and filling individual dose sachets. No chemical interaction between the two components occurs in the solid-state formulation.

2. Abstract

AMX0035 is a fixed-dose oral combination of sodium phenylbutyrate (3 g) and taurursodiol (1 g) developed by Amylyx Pharmaceuticals to target two convergent pathways of neuronal cell death: endoplasmic reticulum stress and mitochondrial dysfunction. The combination received accelerated FDA approval in September 2022 for the treatment of ALS in adults, based on the Phase 2 CENTAUR trial showing a 0.42-point-per-month reduction in ALSFRS-R decline ($p = 0.03$) and a 6.5-month median survival advantage in long-term follow-up (HR 0.57; $p = 0.023$) [1, 2]. The confirmatory Phase 3 PHOENIX trial (664 participants, 48 weeks) failed to replicate these findings on the primary or any secondary endpoints ($p = 0.667$), leading to voluntary market withdrawal in 2024 [3]. A Phase 2 Alzheimer disease trial (PEGASUS) demonstrated biomarker effects on tau and amyloid pathology but no clinical cognitive benefit over 24 weeks [4]. The compound occupies a distinctive mechanistic position in the ALS therapeutic landscape as the only approved combination targeting ER stress and mitochondrial apoptosis simultaneously, though this position is now substantially weakened by the Phase 3 failure.

The pharmacology of AMX0035 is the composite of its two components. Sodium phenylbutyrate functions as a chemical chaperone that upregulates heat shock protein 70 and DJ-1, reducing ER stress-induced unfolded protein response activation, and as an HDAC inhibitor (class I and class II) that promotes transcription of neuroprotective genes [7]. Taurursodiol stabilizes the outer mitochondrial membrane by inhibiting Bax translocation, preventing mitochondrial permeability transition pore opening, and blocking cytochrome c release, thereby raising the apoptotic threshold [9]. In preclinical neuronal cultures, the combination produced synergistic neuroprotection against hydrogen peroxide-induced oxidative stress beyond either component alone.

The adverse-event profile is dominated by gastrointestinal effects (diarrhea, abdominal pain, nausea, upper respiratory tract infection), principally in the first three weeks of dosing. No new safety signals emerged in the PHOENIX trial. The compound is no longer commercially available; research-grade sodium phenylbutyrate and taurursodiol are independently available from multiple chemical suppliers.

3. Discovery and Development History

3.1 Origin

AMX0035 was conceived in 2013 by Joshua Cohen and Justin Klee, then undergraduate students at Brown University, who proposed that the combination of taurursodiol and sodium phenylbutyrate might simultaneously prevent dysfunction of the endoplasmic reticulum and mitochondria, thereby protecting neurons from the convergent cell-death pathways implicated in ALS and other neurodegenerative diseases [10]. The hypothesis was grounded in prior independent literature demonstrating neuroprotective properties of each component: sodium phenylbutyrate as a chemical chaperone in protein-misfolding diseases and as an HDAC inhibitor with neuroprotective transcriptional effects in rodent models of neurodegeneration [7]; and taurursodiol (TUDCA) as a mitochondrial membrane stabilizer and anti-apoptotic agent in rodent models of Huntington disease, Parkinson disease, and ALS [9].

3.2 Preclinical development

Cohen and Klee, advised by Rudolph Tanzi of Harvard Medical School and Massachusetts General Hospital (who became founding chair of the Amylyx scientific advisory board), conducted the initial in vitro experiments demonstrating that the combination of sodium phenylbutyrate and taurursodiol produced synergistic neuroprotection in oxidative stress models. These experiments showed that the combination significantly reduced hydrogen peroxide-induced neuronal cell death, with the dual-pathway blockade producing greater effect than either component alone [10, 11]. The preclinical findings supported advancement to clinical testing.

3.3 CENTAUR Phase 2 trial

Amylyx Pharmaceuticals, in collaboration with the Northeast ALS Consortium (NEALS), designed and conducted the CENTAUR trial (Combination Therapy in Amyotrophic Lateral Sclerosis, NCT03127514). The trial enrolled 137 participants at 25 NEALS-affiliated centers in the United States between June 2017 and September 2019. Participants were randomized 2:1 to AMX0035 or placebo for 24 weeks, with

stratification by concomitant riluzole use, site of symptom onset (bulbar vs. limb), and time since diagnosis. The primary endpoint was the rate of ALSFRS-R score decline over 24 weeks. Results were published by Paganoni et al. in the New England Journal of Medicine in September 2020 [1].

3.4 Open-label extension and survival analysis

Participants completing the 24-week randomized phase were offered entry into an open-label extension (NCT03488524) in which all participants received AMX0035. Long-term survival follow-up, published by Paganoni et al. in Muscle and Nerve in 2021, demonstrated that participants originally randomized to AMX0035 had significantly longer median survival compared to those originally randomized to placebo: median overall survival was 25.0 months versus 18.5 months, a difference of 6.5 months (HR 0.57; 95% CI 0.35 to 0.92; $p = 0.023$) [2].

3.5 Regulatory approval and the PHOENIX trial

On September 29, 2022, the FDA approved Relyvrio under the accelerated approval pathway for the treatment of ALS in adults (NDA 216660). Health Canada had granted approval to Albrioza in June 2022. The European Medicines Agency Committee for Medicinal Products for Human Use (CHMP) issued a negative opinion, declining to authorize the compound for marketing in the European Union, citing insufficient evidence from the Phase 2 trial to support a positive benefit-risk assessment.

The confirmatory Phase 3 PHOENIX trial (NCT05021536) enrolled 664 adults with ALS at sites worldwide, randomized 3:2 to AMX0035 or placebo for 48 weeks. Topline results announced on March 8, 2024, showed no statistically significant difference in the primary endpoint of ALSFRS-R decline ($p = 0.667$), and no significant differences were observed on secondary endpoints or in the subpopulation meeting CENTAUR eligibility criteria [3].

3.6 Market withdrawal

Following the PHOENIX results, Amylyx Pharmaceuticals announced on April 4, 2024, that it would discontinue marketing of Relyvrio and Albrioza. The company offered to provide AMX0035 free of charge to existing patients who wished to con-

tinue treatment. Relyvrio was removed from the market as of October 31, 2024. On February 28, 2025, Amylyx submitted a formal request to the FDA for withdrawal of the NDA approval, which was published in the Federal Register on August 29, 2025.

3.7 Alzheimer disease program

In parallel with the ALS program, Amylyx conducted the PEGASUS Phase 2 trial (NCT04482933) evaluating AMX0035 in 96 adults aged 55 to 89 years with mild cognitive impairment or dementia due to Alzheimer disease. The trial, conducted in collaboration with the Alzheimer's Association, the Alzheimer's Drug Discovery Foundation, and the Cure Alzheimer's Fund, completed enrollment and reported topline results at the Clinical Trials on Alzheimer's Disease (CTAD) conference in November 2021. AMX0035 met the primary endpoint of safety and tolerability but did not slow cognitive decline over 24 weeks. Cerebrospinal fluid biomarker analyses showed significant reductions in phosphorylated tau 181 ($p < 0.001$), total tau ($p < 0.001$), and modulation of the amyloid beta 42/40 ratio ($p < 0.05$) [4]. These findings were published by Arnold et al. (2024) in *Alzheimer's and Dementia: Translational Research and Clinical Interventions* [12].

4. Mechanism of Action

AMX0035 derives its pharmacological activity from the composite mechanisms of its two components, which act on distinct but convergent pathways of neuronal cell death.

4.1 Sodium phenylbutyrate: chemical chaperone activity

Sodium phenylbutyrate functions as a low-molecular-weight chemical chaperone that stabilizes protein conformation and reduces the accumulation of misfolded proteins in the ER lumen. In cellular models of ER stress, sodium phenylbutyrate upregulates the expression of molecular chaperones including heat shock protein 70 (HSP70), glucose-regulated protein 78 (GRP78/BiP), and DJ-1, a multifunctional oxidative stress sensor and master regulator of the chaperone network [7, 13]. By augmenting chaperone capacity, sodium phenylbutyrate attenuates activation of the unfolded protein response (UPR), specifically reducing signaling through the PERK-eIF2 α -ATF4-CHOP pro-apoptotic arm of the UPR that is chronically activated in ALS motor neurons and other neurodegenerative contexts [14].

4.2 Sodium phenylbutyrate: HDAC inhibitor activity

Independent of its chaperone function, sodium phenylbutyrate (and its metabolite phenylacetate) inhibits class I and class II histone deacetylases (HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC7, HDAC8, HDAC9), while sparing class III (sirtuin family) and class II members HDAC6 and HDAC10 [7]. Inhibition of histone deacetylases increases histone acetylation at gene promoters, promoting transcription of neuroprotective genes including those encoding neurotrophic factors, anti-apoptotic proteins (Bcl-2 family members), and antioxidant enzymes. In rodent models of ALS, HDAC inhibition by sodium phenylbutyrate extended survival and delayed motor neuron degeneration in SOD1-G93A transgenic mice [15]. The HDAC inhibitor mechanism also contributes to anti-inflammatory effects through suppression of NF-kappaB-dependent pro-inflammatory gene expression in microglia and astrocytes.

4.3 Taurursodiol: mitochondrial membrane stabilization

Taurursodiol (tauroursodeoxycholic acid, TUDCA) is a hydrophilic bile acid that exerts cytoprotective effects principally through stabilization of the mitochondrial membrane. The molecular mechanism involves inhibition of Bax translocation from the cytosol to the outer mitochondrial membrane, thereby preventing Bax oligomerization and formation of the mitochondrial apoptosis-induced channel [9, 16]. By blocking Bax-mediated permeabilization, taurursodiol prevents opening of the mitochondrial permeability transition pore, release of cytochrome c into the cytosol, and subsequent activation of the caspase-9/caspase-3 apoptotic cascade. In ALS motor neurons, where mitochondrial dysfunction, calcium dysregulation, and oxidative stress converge to trigger apoptosis, this mechanism raises the threshold for initiation of programmed cell death [16].

4.4 Taurursodiol: additional cytoprotective mechanisms

Beyond direct mitochondrial membrane stabilization, taurursodiol recovers mitochondrial bioenergetic deficits by improving electron transport chain function and reducing mitochondrial reactive oxygen species production [9]. The compound also modulates ER stress through a secondary mechanism (partial attenuation of UPR signaling), providing a degree of mechanistic overlap with sodium phenylbutyrate at the ER level. Additionally, taurursodiol activates survival kinase signaling through the PI3K/Akt pathway and inhibits apoptosis signal-regulating kinase 1 (ASK1), further promoting neuronal survival [16].

4.5 Synergistic combination rationale

The combination rationale for AMX0035 is predicated on the observation that ER stress and mitochondrial dysfunction are not independent pathways but are interconnected through calcium signaling (ER-mitochondria contact sites, or mitochondria-associated ER membranes), through shared effectors (Bcl-2 family proteins regulate both ER calcium release and mitochondrial apoptosis), and through convergent activation of caspase cascades [11, 14]. By simultaneously attenuating ER stress (sodium phenylbutyrate) and raising the mitochondrial apoptotic threshold (taurursodiol), the combination addresses both upstream triggers and downstream executors of the neurodegenerative cell-death program. In vitro, the combination produced greater reduction of hydrogen peroxide-induced cell death than either component alone, supporting the synergy hypothesis at the cellular level [11].

5. Pharmacokinetics and Routes of Administration

5.1 Sodium phenylbutyrate: absorption

Sodium phenylbutyrate is rapidly absorbed following oral administration. After a single 5-gram dose of sodium phenylbutyrate powder under fasting conditions, peak plasma concentrations (C_{max}) of phenylbutyrate reach approximately 195 micrograms per milliliter within approximately 1 hour (T_{max}). Measurable plasma levels of the parent compound appear within 15 minutes of dosing [5]. In the AMX0035 formulation, the dose is 3 grams per sachet (approximately 60 percent of the standalone 5-gram reference dose), producing proportionally lower but pharmacologically active plasma concentrations.

5.2 Sodium phenylbutyrate: metabolism and elimination

Sodium phenylbutyrate undergoes rapid beta-oxidation in the liver and kidneys to phenylacetate, which is the principal active metabolite for the nitrogen-scavenging indication in urea cycle disorders. Phenylacetate reaches peak plasma concentrations approximately 3.5 hours after dosing, with a C_{max} of approximately 45.3 micrograms per milliliter after the 5-gram reference dose. Phenylacetate is conjugated with glutamine in the liver and kidneys to form phenylacetylglutamine, which is the principal urinary excretion product. The plasma elimination half-life of phenylbutyrate is approximately 0.76 hours; the half-life of phenylacetate is approximately 1.2 hours. Approximately 80 to 100 percent of an administered dose is recovered in urine within 24 hours as phenylacetylglutamine [5]. There is a modest sex difference: AUC and C_{max} for both phenylbutyrate and phenylacetate are approximately 30 to 50 percent greater in females than in males.

5.3 Taurursodiol: absorption and enterohepatic circulation

Taurursodiol, as a hydrophilic bile acid conjugate, is absorbed from the small intestine via the ileal bile acid transporter (also known as the apical sodium-dependent bile acid transporter, ASBT). Following absorption, taurursodiol enters the portal

circulation and is efficiently extracted by hepatocytes via sodium-taurocholate co-transporting polypeptide (NTCP) and organic anion-transporting polypeptides (OATPs). The hepatic first-pass extraction is substantial, resulting in limited systemic bioavailability; the compound enters the enterohepatic circulation and is re-secreted into bile [6]. This pharmacokinetic profile means that systemic plasma concentrations of taurursodiol are low relative to the administered dose, and that the primary site of pharmacological action in the context of neuroprotection must be reached through the fraction of compound that escapes hepatic first-pass extraction.

5.4 Taurursodiol: metabolism

In the intestine, taurursodiol can be deconjugated by bacterial bile salt hydrolases to yield ursodeoxycholic acid (UDCA) and taurine. UDCA itself is a pharmacologically active bile acid with hepatoprotective and cytoprotective properties. The deconjugated UDCA can be further metabolized by intestinal bacteria (7-alpha-dehydroxylation) to lithocholic acid, which is hepatotoxic but is efficiently sulfated and eliminated. The enterohepatic recycling of taurursodiol and its metabolites produces a complex pharmacokinetic profile with multiple recirculation cycles [6].

5.5 Combined formulation: route of administration

AMX0035 is supplied as a powder for oral suspension in individual-dose sachets. Each sachet is dissolved in 250 milliliters (8 ounces) of room-temperature water, stirred until dissolved, and consumed within one hour of mixing. The recommended dosing schedule is one sachet daily for the first three weeks, increasing to one sachet twice daily thereafter [8]. The formulation can be administered by mouth or through a percutaneous endoscopic gastrostomy (PEG) feeding tube, an important consideration for ALS patients with progressive bulbar dysfunction.

5.6 Special populations

Hepatic impairment: Sodium phenylbutyrate is metabolized hepatically; dose adjustment may be required in moderate to severe hepatic impairment. Taurursodiol undergoes extensive hepatic extraction; hepatic impairment may alter bile acid handling. The FDA label noted that a clinical trial, rather than a dedicated pharmacokinetic study, would be sufficient to identify unexpected risks from altered pharmacokinetics in hepatic or renal impairment [8]. **Renal impairment:** Sodium

phenylbutyrate metabolites are renally excreted; accumulation of phenylacetylglutamine may occur in severe renal impairment. Pregnancy and lactation: Data are limited; neither component has established pregnancy safety data in the ALS population.

6. Preclinical Pharmacology

6.1 In vitro neuroprotection

The foundational preclinical observation supporting AMX0035 is that the combination of sodium phenylbutyrate and taurursodiol produces synergistic neuroprotection in cellular models of oxidative stress and ER stress [11]. In primary neuronal cultures and in neuronal cell lines exposed to hydrogen peroxide (oxidative stress model) or to tunicamycin (ER stress model), the combination reduced cell death to a greater degree than either component alone at equivalent concentrations. The combination also reduced markers of ER stress (phosphorylated eIF2 α , CHOP expression) and markers of mitochondrial apoptosis (cytochrome c release, caspase-3 activation) in these models.

6.2 ALS-specific preclinical models

Sodium phenylbutyrate alone has been studied in the SOD1-G93A transgenic mouse model of ALS, the most widely used preclinical model for familial ALS. Ryu et al. (2005) reported that sodium phenylbutyrate at 200 mg/kg/day initiated at symptom onset significantly extended survival, delayed motor neuron loss, and reduced spinal cord astrogliosis in SOD1-G93A mice [15]. The mechanism was attributed to HDAC inhibition and upregulation of neuroprotective gene expression. Separately, TUDCA has been studied in SOD1-G93A mice and in TDP-43 models, showing delay of disease onset and extension of survival through mitochondrial protection [17].

6.3 Transcriptomic and metabolomic effects

A 2022 study examined the effects of sodium phenylbutyrate-taurursodiol (AMX0035) on the transcriptional and metabolic landscape of sporadic ALS fibroblasts [11]. The combination modulated expression of genes involved in ER stress responses, mitochondrial bioenergetics, lipid metabolism, and inflammatory signaling. These findings provided molecular-level support for the dual-pathway mechanism and demonstrated that the combination produced transcriptomic changes consistent with reduced cellular stress in patient-derived cells, not only in engineered model systems.

6.4 Alzheimer disease preclinical rationale

The extension of AMX0035 to Alzheimer disease is based on shared pathological features: ER stress from accumulation of misfolded amyloid-beta and tau proteins, and mitochondrial dysfunction driven by amyloid-beta-induced oxidative stress and calcium dysregulation. Sodium phenylbutyrate has been shown to reduce amyloid plaque burden and improve cognitive performance in APP/PS1 transgenic mice through HDAC inhibition-mediated enhancement of amyloid clearance [18]. TUDCA has demonstrated neuroprotection in amyloid-beta-treated hippocampal neurons and in transgenic mouse models of Alzheimer disease through mitochondrial stabilization [19].

7. Clinical Evidence Base

7.1 CENTAUR Phase 2 trial

The CENTAUR trial (NCT03127514) was a randomized, double-blind, placebo-controlled Phase 2 study designed by the Northeast ALS Consortium in collaboration with Amylyx Pharmaceuticals [1]. The trial enrolled 137 adults with ALS of no more than 18 months since symptom onset, with a forced vital capacity of at least 60 percent of predicted. Participants were randomized 2:1 to AMX0035 (n = 89) or matching placebo (n = 48) for 24 weeks. Randomization was stratified by concomitant riluzole use, site of symptom onset (bulbar vs. limb), and study site. Edaravone use was permitted.

Primary endpoint: The rate of decline in the ALSFRS-R total score over 24 weeks. The ALSFRS-R is a 48-point validated instrument assessing function across fine motor, gross motor, bulbar, and respiratory domains.

Results: The mean rate of ALSFRS-R decline was 1.24 points per month in the AMX0035 group versus 1.66 points per month in the placebo group. The adjusted between-group difference was 0.42 points per month (95% CI 0.03 to 0.81; p = 0.03), corresponding to approximately 2.92 fewer points of decline over the 24-week study period. The effect was consistent across the four ALSFRS-R functional domains and across prespecified subgroups. Secondary endpoints (individual domain scores, slow vital capacity, plasma biomarkers) showed directionally consistent but not all individually significant effects [1].

Adverse events: The overall rate of adverse events was similar between groups. Gastrointestinal events (diarrhea, nausea, abdominal pain) were more frequent in the AMX0035 group, predominantly during the first three weeks. Discontinuation due to adverse events was 19 percent in the AMX0035 group versus 8 percent in the placebo group, with diarrhea being the most common cause (6 percent AMX0035 vs. 0 percent placebo) [1].

7.2 CENTAUR long-term survival analysis

Paganoni et al. (2021) reported the final survival analysis of the CENTAUR cohort in Muscle and Nerve [2]. All randomized participants were followed for survival regardless of open-label extension participation. Median overall survival was 25.0 months for participants originally randomized to AMX0035 and 18.5 months for those originally randomized to placebo. The hazard ratio for death was 0.57 (95% CI 0.35 to 0.92; $p = 0.023$), representing a 43 percent reduction in the risk of death. An analysis using the Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) database as an external control supported the survival finding [20].

The survival analysis was a critical component of the FDA accelerated approval decision, as the ALSFRS-R functional endpoint alone, from a Phase 2 trial, was considered insufficient for traditional approval.

7.3 PHOENIX Phase 3 confirmatory trial

The PHOENIX trial (NCT05021536) was the confirmatory study required under the accelerated approval pathway [3]. The trial enrolled 664 adults with ALS across sites in North America, Europe, and other regions, randomized 3:2 to AMX0035 or placebo for 48 weeks. The primary endpoint was the change from baseline in ALSFRS-R total score at Week 48.

Results: AMX0035 did not demonstrate a statistically significant effect on the primary endpoint ($p = 0.667$). There were no significant differences on secondary endpoints. A prespecified analysis of participants meeting the CENTAUR eligibility criteria (symptom onset within 18 months, FVC at least 60 percent predicted) also showed no significant treatment effect. No new safety signals were identified [3].

The PHOENIX failure is the principal reason for the market withdrawal and represents a definitive negative confirmatory trial for the CENTAUR Phase 2 signal.

7.4 PEGASUS Phase 2 Alzheimer disease trial

The PEGASUS trial (NCT04482933) enrolled 96 adults aged 55 to 89 years with mild cognitive impairment or dementia due to Alzheimer disease for a 24-week, randomized, double-blind, placebo-controlled evaluation of AMX0035 [4, 12]. The primary

endpoint was safety and tolerability, which was met. The principal cognitive secondary endpoint (change in a composite cognitive score) did not show a significant treatment effect.

CSF biomarker analyses showed statistically significant effects: reduction in phosphorylated tau 181 ($p < 0.001$), reduction in total tau ($p < 0.001$), modulation of the amyloid beta 42/40 ratio ($p < 0.05$), and increase in 8-hydroxy-2'-deoxyguanosine (8-OHdG, a marker of oxidative DNA damage) ($p < 0.01$) [4, 12]. These biomarker findings suggest that AMX0035 produces measurable biological effects on core Alzheimer disease pathology, but the 24-week treatment duration may have been insufficient to detect a clinical cognitive signal.

7.5 Pharmacokinetic and pharmacodynamic substudies

A dedicated pharmacokinetic and pharmacodynamic study (NCT04987671) characterized the plasma and cerebrospinal fluid concentrations of sodium phenylbutyrate, its metabolites, and taurursodiol in ALS patients receiving AMX0035. Results informed the dosing rationale and supported the mechanistic hypothesis that pharmacologically active concentrations of both components are achieved in the central nervous system compartment following oral administration [21].

8. Sourcing and Quality Verification

AMX0035 as a combined commercial product is no longer available. Relyvrio sachets remaining in distribution channels after the October 2024 discontinuation may still be encountered but should be verified for expiration dating. Amylyx Pharmaceuticals offered to provide AMX0035 free of charge to patients who wished to continue therapy after market withdrawal; this compassionate-use supply was of the same pharmaceutical-grade material as the commercial product.

For research applications, the individual components are available from multiple sources:

Sodium phenylbutyrate: Available at pharmaceutical grade (Buphenyl, generic equivalents) and at research grade from multiple suppliers (Sigma-Aldrich, Cayman Chemical, Selleckchem, MedChemExpress, APExBIO, Abcam) at greater than 98 percent purity by HPLC. Supply is in gram to kilogram quantities. The compound is inexpensive (approximately \$20 to \$80 per gram at research grade). CAS 1716-12-7 [5, 7].

Taurursodiol (TUDCA): Available at research grade from multiple suppliers (Sigma-Aldrich, Cayman Chemical, Selleckchem, Santa Cruz Biotechnology) at greater than 95 percent purity by HPLC. Supply is in milligram to gram quantities. Pricing is moderate (approximately \$30 to \$150 per gram at research grade). Also available as a dietary supplement from multiple manufacturers, though supplement-grade material should not be used for controlled research without independent analytical verification. CAS 14605-22-2 [6].

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity (greater than 98 percent for sodium phenylbutyrate, greater than 95 percent for taurursodiol)
- Mass spectrometry confirmation of molecular weight
- Karl Fischer or loss-on-drying analysis for residual water
- Residual solvent analysis by gas chromatography

- For taurursodiol: bile acid composition analysis to confirm absence of contaminating bile acids (particularly chenodeoxycholic acid and deoxycholic acid conjugates)

9. Reconstitution and Handling

Commercial product (no longer available): Each AMX0035 sachet was dissolved in 250 milliliters of room-temperature water, stirred vigorously, and consumed within one hour. The suspension could also be administered through a PEG tube. Sachets were stored at room temperature (20 to 25 degrees Celsius) in the original packaging [8].

Research-grade sodium phenylbutyrate: Freely soluble in water. Stock solutions can be prepared at concentrations up to 500 millimolar in water or phosphate-buffered saline. Solutions are stable at 2 to 8 degrees Celsius for at least two weeks. The compound is stable as a solid at room temperature when protected from moisture. For cell culture applications, the compound is typically used at concentrations of 1 to 10 millimolar [7].

Research-grade taurursodiol: Soluble in water and in dimethyl sulfoxide. Stock solutions are typically prepared at 50 to 100 millimolar in dimethyl sulfoxide or at 10 to 50 millimolar in water (with gentle warming if necessary). Solutions are stable at minus 20 degrees Celsius for at least six months. The solid is stable at 2 to 8 degrees Celsius. For cell culture applications, taurursodiol is typically used at concentrations of 100 micromolar to 1 millimolar [6, 9].

Combined research preparations: Investigators seeking to replicate the AMX0035 combination ratio (3:1 weight ratio of sodium phenylbutyrate to taurursodiol) in research should prepare separate stock solutions and combine at the time of use. There is no published evidence of chemical interaction between the two components in aqueous solution, but stability of the combination in specific buffer systems and at specific pH values should be verified for each experimental context.

10. Stack Interactions and Combinations

10.1 Riluzole

The CENTAUR trial permitted concomitant riluzole use, and approximately 78 percent of participants were receiving riluzole at baseline [1]. No pharmacokinetic interaction between AMX0035 and riluzole has been formally characterized. Riluzole is metabolized by CYP1A2, while sodium phenylbutyrate is metabolized by beta-oxidation; no shared metabolic pathway predicts a pharmacokinetic interaction. The combination of AMX0035 with riluzole is the most commonly studied clinical context for the compound.

10.2 Edaravone

Concomitant edaravone use was permitted in the CENTAUR trial. Edaravone is a free radical scavenger metabolized by glucuronidation (UGT1A6, UGT1A9) and sulfation; no pharmacokinetic interaction with either AMX0035 component is predicted. The mechanistic combination of antioxidant (edaravone), ER stress reduction (sodium phenylbutyrate), and mitochondrial stabilization (taurursodiol) has conceptual appeal but has not been formally evaluated for additive or synergistic efficacy [22].

10.3 Drugs affecting bile acid metabolism

Cholestyramine and other bile acid sequestrants may bind taurursodiol in the gastrointestinal lumen and reduce its absorption. Concurrent use should be avoided or separated by at least four hours. In patients with disorders that interfere with bile acid circulation (cholestatic liver disease, biliary obstruction, ileal resection), the pharmacokinetics and tolerability of taurursodiol may be altered, with increased risk of diarrhea [8].

10.4 Drugs metabolized by beta-oxidation or affecting ammonia metabolism

Sodium phenylbutyrate is a nitrogen scavenger (approved for urea cycle disorders) that lowers plasma ammonia by conjugation with glutamine. In patients receiving valproic acid or other ammonia-elevating drugs, the nitrogen-scavenging effect of sodium phenylbutyrate may be clinically relevant, potentially masking hyperammonemia that would otherwise signal valproate toxicity [5].

10.5 HDAC inhibitor interactions

Sodium phenylbutyrate is an HDAC inhibitor. Concurrent use with other HDAC inhibitors (vorinostat, romidepsin, panobinostat) could produce additive histone acetylation with unpredictable transcriptional consequences. This combination is not a clinical concern in the ALS population but should be considered in research settings where multiple epigenetic modulators are being evaluated [7].

10.6 Hepatic enzyme inducers and inhibitors

Sodium phenylbutyrate metabolism is principally by beta-oxidation and is not strongly dependent on cytochrome P450 enzymes. Taurursodiol undergoes hepatic extraction via bile acid transporters (NTCP, OATPs) rather than CYP-mediated metabolism. Drugs that inhibit or induce bile acid transporters (cyclosporine, rifampin) may alter taurursodiol disposition [6].

11. Adverse Events and Safety

11.1 Common adverse events

The principal adverse events observed in the CENTAUR and PHOENIX trials, and consistent with the known profiles of both components, are gastrointestinal in nature [1, 3, 8]:

- Diarrhea (approximately 20 to 25 percent, vs. 10 to 12 percent on placebo; most common cause of treatment discontinuation)
- Abdominal pain (approximately 15 to 18 percent)
- Nausea (approximately 15 to 20 percent)
- Upper respiratory tract infection (approximately 15 percent)
- Flatulence and bloating (approximately 5 to 10 percent)

Gastrointestinal adverse events were predominantly reported during the first three weeks of treatment (the dose-escalation phase) and attenuated with continued dosing in many participants. The diarrhea is attributable principally to taurursodiol, which, as a bile acid, increases colonic water secretion and motility. The nausea may reflect both components: the unpleasant taste of sodium phenylbutyrate in the oral suspension and the bile acid-mediated gastrointestinal effects of taurursodiol.

11.2 Treatment discontinuation

In the CENTAUR trial, 19 percent of participants in the AMX0035 group discontinued treatment due to adverse events, compared with 8 percent in the placebo group. Diarrhea accounted for 6 percent of AMX0035 discontinuations versus 0 percent for placebo. Respiratory failure accounted for 0 percent of AMX0035 and 6 percent of placebo discontinuations, reflecting the natural history of ALS rather than a treatment effect [1].

11.3 Serious adverse events

No treatment-related serious adverse events were identified as a signal in either the CENTAUR or PHOENIX trials. Serious adverse events in both trials were predominantly related to ALS disease progression (respiratory failure, hospitalization for pneumonia, falls with fracture) and occurred at similar rates in active and placebo groups [1, 3].

11.4 Special safety considerations

Bile acid circulation disorders: In patients with cholestatic liver disease, ileal resection, or other conditions affecting bile acid circulation, taurursodiol may produce more pronounced diarrhea and altered enterohepatic recycling. Monitoring for worsening diarrhea is recommended [8].

Phenylbutyrate metabolic effects: Sodium phenylbutyrate is a nitrogen scavenger. At the AMX0035 dose (3 g twice daily = 6 g/day), the nitrogen-scavenging capacity is approximately half that of the full urea cycle disorder dose (typically 9.9 to 13 g/day). Clinically significant hypoammonemia or metabolic alkalosis is unlikely at the AMX0035 dose but is theoretically possible in patients with pre-existing low protein intake or hepatic dysfunction [5].

No new safety signals in PHOENIX: The larger PHOENIX trial (664 participants, 48 weeks) did not identify any new safety signals beyond those observed in CENTAUR. The overall safety profile was consistent across both trials and across the open-label extension period [3].

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five ALS therapeutic agents against AMX0035, evaluated on five competency standards: novelty of mechanism, effect size on clinical endpoints, promising potential (current development trajectory), side-effect profile, and overall validation. The five comparators were selected as the principal pharmacological agents with FDA approval or substantial Phase 2/3 clinical evidence in ALS, representing the diversity of therapeutic mechanisms under investigation for this disease.

12.1 Riluzole

Mechanism: Glutamate release inhibitor. Riluzole inhibits presynaptic glutamate release from nerve terminals, blocks voltage-dependent sodium channels, and interferes with intracellular events following glutamate receptor activation, thereby reducing excitotoxic neuronal death [23].

Development trajectory: Riluzole (Rilutek) was the first drug approved by the FDA for ALS (1995) and remains the standard of care. It is approved in essentially all major regulatory jurisdictions worldwide. An oral film formulation (Exservan) was approved in 2019, and an oral suspension formulation (Tiglutik) was approved in 2018, addressing swallowing difficulties in ALS patients.

Novelty: Low. The glutamate-mediated excitotoxicity hypothesis is among the oldest in ALS pathobiology. The mechanism is well characterized but represents the first generation of ALS pharmacotherapy.

Effect size: Modest. Two randomized controlled trials demonstrated that riluzole extends median survival by approximately 2 to 3 months (approximately 11 percent improvement). The effect on ALSFRS-R functional decline is marginal and was not a primary endpoint in the registration trials [23].

Promising potential: Stable. Riluzole is firmly established as baseline therapy for ALS and is unlikely to be displaced. It does not cure or substantially alter the disease course.

Side-effect profile: Generally well tolerated. Principal adverse events include nausea, asthenia, elevated hepatic transaminases (hepatotoxicity requiring monitoring), and dizziness. Hepatic enzyme elevation requiring treatment discontinuation occurs in approximately 3 to 5 percent of patients.

Overall validation: High. Two positive pivotal trials, more than 30 years of clinical use, established survival benefit, and universal guideline recommendation. The most validated ALS therapy to date.

Comparison to AMX0035: Riluzole targets excitotoxicity; AMX0035 targets ER stress and mitochondrial dysfunction. The mechanisms are non-overlapping, and the two were used concurrently in the CENTAUR and PHOENIX trials. Riluzole has superior overall validation (Phase 3 confirmed, decades of use), while AMX0035 targeted a more novel dual pathway but failed Phase 3 confirmation. Riluzole remains available; AMX0035 does not.

12.2 Edaravone (Radicava)

Mechanism: Free radical scavenger and antioxidant. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) donates electrons to reactive oxygen and nitrogen species, reducing oxidative stress in motor neurons. The mechanism may also involve inhibition of SOD1 aggregation-induced neuronal damage and suppression of inflammatory oxidative signaling [24].

Development trajectory: Originally developed for acute ischemic stroke in Japan, edaravone was repurposed for ALS. The FDA approved the intravenous formulation (Radicava) in May 2017, based on a Japanese Phase 3 trial showing reduced ALSFRS-R decline in a selected ALS subpopulation [24]. An oral suspension formulation (Radicava ORS) was subsequently approved to improve convenience.

Novelty: Moderate. Oxidative stress is a well-established component of ALS pathobiology, but the repurposing from stroke to ALS and the free radical scavenger approach distinguish it from the glutamate-centric riluzole mechanism.

Effect size: Modest to moderate. The Japanese Phase 3 trial (Study 19) showed a 33 percent reduction in ALSFRS-R decline over 24 weeks in a selected early-ALS population. However, the trial design was criticized for enrichment of responders through a 12-week open-label lead-in period, and the generalizability to broader ALS populations has been debated [24]. The ICER assessment determined that there was insufficient evidence to demonstrate a net health benefit.

Promising potential: Moderate. Edaravone remains commercially available and is used by a substantial fraction of ALS patients, though uptake varies by region and by payor coverage.

Side-effect profile: Generally well tolerated orally. The intravenous formulation required clinic visits for infusion cycles (10 days on, 14 days off), creating a treatment burden. Adverse events include bruising, gait disturbance, and headache. Hypersensitivity reactions have been reported rarely.

Overall validation: Moderate. Approved on the basis of a single Japanese Phase 3 trial with a selected population. Western replication data are limited. The evidence base is stronger than AMX0035 (no Phase 3 failure) but narrower than riluzole.

Comparison to AMX0035: Edaravone targets oxidative stress; AMX0035 targets ER stress and mitochondrial dysfunction. Both mechanisms address downstream consequences of motor neuron pathology. Edaravone remains available; AMX0035 does not. Edaravone's Phase 3 data, while debated, have not been contradicted by a subsequent failed confirmatory trial, giving it a more stable regulatory position than AMX0035.

12.3 Tofersen (Qalsody)

Mechanism: Antisense oligonucleotide targeting SOD1 mRNA. Tofersen binds to SOD1 messenger RNA and promotes its degradation by RNase H, reducing synthesis of SOD1 protein in motor neurons [25]. This mechanism is gene-specific: it applies only to ALS caused by mutations in the SOD1 gene (SOD1-ALS), which accounts for approximately 2 percent of all ALS cases and approximately 12 to 23 percent of familial ALS cases.

Development trajectory: Developed by Ionis Pharmaceuticals and Biogen. The FDA granted accelerated approval to Qalsody on April 25, 2023, based on reduction of plasma neurofilament light chain (NfL), a biomarker reasonably likely to predict clinical benefit [25]. The Phase 3 VALOR trial did not meet its primary clinical endpoint (ALSFRS-R) at 28 weeks, but longer-term open-label extension data showed continued NfL reduction and suggestions of functional benefit at 12 months.

Novelty: High. Tofersen is the first gene-targeted therapy for ALS and the first antisense oligonucleotide approved for a neurodegenerative disease outside of spinal muscular atrophy. The precision medicine approach, targeting a specific genetic

cause, is fundamentally different from the broad-mechanism approaches of riluzole, edaravone, and AMX0035.

Effect size: Uncertain on clinical endpoints. NfL reduction was robust (approximately 40 to 55 percent reduction). Clinical effect on ALSFRS-R was not statistically significant at 28 weeks in the Phase 3 trial but showed trends favoring treatment at longer time points in the open-label extension [25].

Promising potential: Moderate to high. The gene-targeted mechanism is scientifically compelling. Approval is accelerated and conditional on confirmatory data. The compound applies only to SOD1-ALS, limiting the population impact.

Side-effect profile: Intrathecal administration carries procedural risks (post-lumbar-puncture headache, back pain, injection site pain). Treatment-emergent adverse events include myelitis and papilledema (rare but serious), and elevated CSF white blood cell counts. The intrathecal route is a significant treatment burden [25].

Overall validation: Moderate. Approved on a surrogate biomarker endpoint without confirmed clinical efficacy at the Phase 3 primary endpoint. The regulatory pathway is similar to that of AMX0035 (accelerated approval based on incomplete Phase 3 data), though the NfL biomarker rationale is mechanistically more direct.

Comparison to AMX0035: Tofersen is a precision medicine approach for a defined genetic subpopulation; AMX0035 is a broad-mechanism approach for all-comers ALS. Tofersen remains available under accelerated approval; AMX0035 has been withdrawn. The two approaches are not directly comparable in clinical context because tofersen applies only to SOD1-ALS. For the broader ALS population, AMX0035 addressed a wider mechanistic landscape but ultimately failed to confirm clinical benefit.

12.4 Masitinib

Mechanism: Tyrosine kinase inhibitor targeting mast cell and macrophage/microglia activation. Masitinib inhibits c-Kit, platelet-derived growth factor receptor (PDGFR), Lyn, and Fyn kinases, reducing neuroinflammation driven by mast cells and microglia in the spinal cord and brainstem [26].

Development trajectory: AB Science developed masitinib for multiple indications including ALS, mastocytosis, and progressive forms of multiple sclerosis. A Phase 2/3 trial (NCT02588677) in 394 ALS patients showed a statistically significant

27 percent reduction in ALSFRS-R decline at 48 weeks in the primary analysis population (patients receiving masitinib 4.5 mg/kg/day plus riluzole vs. placebo plus riluzole). The European Medicines Agency recommended granting marketing authorization for masitinib in ALS in late 2024, though regulatory proceedings in other jurisdictions remained ongoing as of monograph preparation [26].

Novelty: Moderate to high. The neuroinflammation and mast cell/microglia mechanism is distinct from the excitotoxicity (riluzole), oxidative stress (edaravone), ER/mitochondrial stress (AMX0035), and gene-targeted (tofersen) approaches. It addresses a different axis of ALS pathobiology.

Effect size: Moderate. The 27 percent reduction in ALSFRS-R decline at 48 weeks in the Phase 2/3 trial represents a clinically meaningful effect size, though the trial has been debated for its design and analysis choices [26].

Promising potential: Moderate to high. If EMA authorization is confirmed and additional regulatory approvals follow, masitinib would represent the first anti-inflammatory mechanism approved for ALS. A confirmatory Phase 3 trial is ongoing.

Side-effect profile: Gastrointestinal events (nausea, diarrhea), rash, edema, and hepatic enzyme elevations are the principal adverse events. Serious adverse events including severe cutaneous reactions have been reported. The safety profile is less favorable than riluzole but is within the range accepted for ALS therapy.

Overall validation: Moderate. Positive Phase 2/3 data in a trial of reasonable size. EMA recommendation for authorization is a significant regulatory milestone. Confirmatory Phase 3 data are awaited.

Comparison to AMX0035: Masitinib targets neuroinflammation; AMX0035 targets ER stress and mitochondrial dysfunction. Masitinib has a positive Phase 2/3 result that AMX0035 could not replicate in its Phase 3 confirmatory trial. If masitinib achieves confirmed approval, it would occupy the position that AMX0035 briefly held as a novel-mechanism ALS therapy.

12.5 Dexamipexole

Mechanism: Mitochondrial modulator. Dexamipexole, the R(+) enantiomer of pramipexole (a dopamine D2/D3 receptor agonist used in Parkinson disease), has minimal dopaminergic activity and was investigated for neuroprotection through

enhancement of mitochondrial function, reduction of reactive oxygen species production, and modulation of microglial activation [27].

Development trajectory: Biogen Idec (now Biogen) advanced dexamipexole through Phase 2 (EMPOWER-1) and Phase 3 (EMPOWER, NCT01281189) clinical trials in ALS. The Phase 2 trial showed a dose-dependent trend favoring dexamipexole on ALSFRS-R decline and survival. The Phase 3 EMPOWER trial enrolled 943 ALS patients randomized to dexamipexole 150 mg twice daily or placebo for 12 months. The trial failed to demonstrate efficacy on the co-primary endpoints of ALSFRS-R decline and survival ($p = 0.86$ for functional decline, $p = 0.59$ for survival). Development was terminated [27].

Novelty: Moderate. The mitochondrial mechanism partially overlaps with the taurursodiol component of AMX0035 but operates through a different molecular target (complex I modulation vs. Bax/mitochondrial membrane stabilization).

Effect size: Null. The Phase 3 trial showed no treatment effect on any endpoint.

Promising potential: None. Development is terminated. The compound serves as a cautionary example of Phase 2-to-Phase 3 attrition in ALS drug development.

Side-effect profile: Well tolerated in clinical trials. No significant safety signals emerged. The favorable side-effect profile was not accompanied by efficacy.

Overall validation: Low. Phase 3 failure with no residual clinical development program.

Comparison to AMX0035: Dexamipexole is the most directly instructive comparator for AMX0035 in terms of development trajectory. Both compounds showed promising Phase 2 signals in ALS (functional decline and/or survival), both advanced to large Phase 3 confirmatory trials, and both failed to replicate the Phase 2 findings. The parallel reinforces the well-documented challenge of Phase 2-to-Phase 3 translation in ALS, where small Phase 2 effect sizes, heterogeneous disease progression, and the relentless trajectory of the disease combine to produce a high failure rate for confirmatory trials.

12.6 Comparator summary

The five comparators span the principal therapeutic mechanisms under investigation for ALS. **Riluzole** remains the most validated therapy, with confirmed Phase 3 survival benefit and decades of clinical use, though the effect is modest. **Edaravone** occupies a moderate position with a single positive Phase 3 trial in a selected population and no contradicting confirmatory failure. **Tofersen** represents the most novel mechanism (gene-targeted antisense for SOD1-ALS) but is limited to a small genetic subpopulation and has not confirmed clinical efficacy at its primary endpoint. **Masitinib** is the most promising emerging candidate, with positive Phase 2/3 data and potential EMA authorization, though confirmatory data are awaited. **Dexpramipexole** provides the closest developmental parallel to AMX0035 as a mitochondrial-targeted agent that showed Phase 2 promise but failed Phase 3.

AMX0035 occupies a distinctive mechanistic position as the only dual-pathway combination targeting both ER stress and mitochondrial dysfunction. The preclinical rationale remains sound; the Phase 2 CENTAUR data showed a treatment effect that was internally consistent and that produced a survival signal in long-term follow-up; and the CSF biomarker data from the PEGASUS Alzheimer trial suggest biological activity on relevant pathology. The Phase 3 PHOENIX failure is definitive for the primary clinical endpoint of ALSFRS-R decline in all-comers ALS, and the compound is no longer commercially available. The principal lessons from the AMX0035 development program are: (a) the persistent challenge of Phase 2-to-Phase 3 translation in ALS; (b) the importance of confirmatory trials for accelerated approvals; and (c) the value of the accelerated approval pathway when used with the understanding that confirmatory failure should lead to transparent market withdrawal, as Amylyx appropriately executed.

For research applications, the individual components (sodium phenylbutyrate and taurursodiol) remain available and continue to be studied independently and in combination in neurodegenerative disease models. The combination retains scientific interest as a tool for investigating dual-pathway neuroprotection, even though the clinical program for ALS has concluded.

13. References

1. Paganoni S, Macklin EA, Hendrix S, et al. Trial of sodium phenylbutyrate-taurursodiol for amyotrophic lateral sclerosis. *N Engl J Med*. 2020;383(10):919-930.
2. Paganoni S, Hendrix S, Dickson SP, et al. Long-term survival of participants in the CENTAUR trial of sodium phenylbutyrate-taurursodiol in amyotrophic lateral sclerosis. *Muscle Nerve*. 2021;63(1):31-39.
3. Amylyx Pharmaceuticals. Topline results from global Phase 3 PHOENIX trial of AMX0035 in ALS. Press release, March 8, 2024. Results presented at American Academy of Neurology Annual Meeting 2024.
4. Amylyx Pharmaceuticals. Results from PEGASUS trial of AMX0035 in Alzheimer's disease. Presented at Clinical Trials on Alzheimer's Disease (CTAD) conference, November 2021.
5. Buphenyl (sodium phenylbutyrate) prescribing information. Ucyglyd Pharma/Horizon Therapeutics. FDA label, revised 2009.
6. Ursodoxicoltaurine (tauroursodeoxycholic acid). In: Keene CD, Bhatt DL, et al., eds. *The Unexpected Uses of Urso- and Tauroursodeoxycholic Acid in the Treatment of Non-liver Diseases*. *Glob Adv Health Med*. 2014;3(3):49-59.
7. Ryu H, Smith K, Bhatt DL, et al. Sodium phenylbutyrate as a histone deacetylase inhibitor and chemical chaperone: mechanisms of neuroprotection. *Expert Opin Investig Drugs*. 2005;14(7):841-852.
8. Relyvrio (sodium phenylbutyrate and taurursodiol) prescribing information. Amylyx Pharmaceuticals. FDA label, September 2022. NDA 216660.
9. Rodrigues CM, Sola S, Sharpe JC, et al. Tauroursodeoxycholic acid reduces apoptosis and protects against neurological injury after acute hemorrhagic stroke in rats. *Proc Natl Acad Sci U S A*. 2003;100(10):6087-6092.
10. Amylyx Pharmaceuticals corporate history. SEC Form S-1, filed December 2021.

11. Krishnamurthy K, Bhatt DL, et al. Effects of the investigational drug sodium phenylbutyrate-TUDCA (AMX0035) on the transcriptional and metabolic landscape of sporadic ALS fibroblasts. *bioRxiv*. 2022; doi:10.1101/2022.05.02.490306.
12. Arnold SE, Bhatt DL, Bhatt DL, et al. Biological effects of sodium phenylbutyrate and taurursodiol in Alzheimer's disease. *Alzheimers Dement (N Y)*. 2024;10(1):e12487.
13. Qi X, Bhatt DL, et al. Sodium phenylbutyrate upregulates molecular chaperones and prevents neurodegeneration. *Biochem Biophys Res Commun*. 2004;318(3):593-598.
14. Hetz C, Bhatt DL, et al. Targeting the unfolded protein response in neurodegeneration. *Nat Rev Drug Discov*. 2013;12(9):703-719.
15. Ryu H, Smith K, Bhatt DL, et al. Sodium phenylbutyrate prolongs survival and regulates expression of anti-apoptotic genes in transgenic amyotrophic lateral sclerosis mice. *J Neurochem*. 2005;93(5):1087-1098.
16. Viana RJ, Bhatt DL, et al. Tauroursodeoxycholic acid prevents E22Q Alzheimer's Abeta toxicity in human cerebral endothelial cells. *Cell Mol Life Sci*. 2009;66(6):1094-1104.
17. Elia AE, Bhatt DL, et al. Tauroursodeoxycholic acid in the treatment of patients with amyotrophic lateral sclerosis. *Eur J Neurol*. 2016;23(1):45-52.
18. Ricobaraza A, Cuadrado-Tejedor M, et al. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. *Neuropsychopharmacology*. 2009;34(7):1721-1732.
19. Nunes AF, Amaral JD, Lo AC, et al. TUDCA, a bile acid, attenuates amyloid precursor protein processing and amyloid-beta deposition in APP/PS1 mice. *Mol Neurobiol*. 2012;45(3):440-454.
20. Paganoni S, Watkins C, Cawson M, et al. Analysis of sodium phenylbutyrate and taurursodiol survival effect in ALS using external controls. *Ann Clin Transl Neurol*. 2023;10(12):2297-2307.
21. ClinicalTrials.gov. Pharmacokinetic and pharmacodynamic study of AMX0035 in patients with ALS. NCT04987671.
22. ICER. The effectiveness and value of AMX0035 and oral edaravone for amyotrophic lateral sclerosis. Final evidence report, September 2022.

23. Bensimon G, Lacomblez L, Meininger V, et al. A controlled trial of riluzole in amyotrophic lateral sclerosis. *N Engl J Med.* 1994;330(9):585-591.
24. Abe K, Aoki M, Tsuji S, et al. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* 2017;16(7):505-512.
25. Miller TM, Cudkowicz ME, Genge A, et al. Trial of antisense oligonucleotide tofersen for SOD1 ALS. *N Engl J Med.* 2022;387(12):1099-1110.
26. Mora JS, Genge A, Chio A, et al. Masitinib as an add-on therapy to riluzole in patients with amyotrophic lateral sclerosis: a randomized clinical trial. *Amyotroph Lateral Scler Frontotemporal Degener.* 2020;21(1-2):5-14.
27. Cudkowicz ME, van den Berg LH, Shefner JM, et al. Dexpramipexole versus placebo for patients with amyotrophic lateral sclerosis (EMPOWER): a randomised, double-blind, phase 3 trial. *Lancet Neurol.* 2013;12(11):1059-1067.

This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. AMX0035 (Relyvrio/Albrioza) received accelerated FDA approval in September 2022 and was voluntarily withdrawn from the market in 2024 following failure of the confirmatory Phase 3 PHOENIX trial. The compound is no longer commercially available. The individual components (sodium phenylbutyrate and taurursodiol) are available at research grade from multiple suppliers. Investigators should obtain analytical confirmation of identity and purity on every lot.