

RESEARCH MONOGRAPH . COMPOUND PROFILE

Tofersen

A 2'-O-methoxyethyl gapmer antisense oligonucleotide developed by Ionis Pharmaceuticals and Biogen for intrathecal reduction of SOD1 protein in genetically defined SOD1-associated amyotrophic lateral sclerosis, the first gene-targeted therapy approved for any form of ALS.

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ABSTRACT

Tofersen (BIIB067, marketed as Qalsody) is a 20-base 2'-O-methoxyethyl (MOE) gapmer antisense oligonucleotide that binds to and mediates RNase H-dependent degradation of superoxide dismutase 1 (SOD1) messenger RNA, reducing synthesis of both mutant and wild-type SOD1 protein in the central nervous system. Developed by Ionis Pharmaceuticals and licensed to Biogen, tofersen received accelerated approval from the United States Food and Drug Administration on April 25, 2023, for the treatment of amyotrophic lateral sclerosis (ALS) in adults with a confirmed SOD1 gene mutation, making it the first gene-targeted therapy approved for any form of ALS. Subsequent approvals followed from the European Medicines Agency (May 30, 2024), the Therapeutic Goods Administration of Australia (January 5, 2025), and Health Canada (March 3, 2025). SOD1 mutations, first identified in familial ALS by Rosen et al. in 1993, account for approximately 13 to 20 percent of familial ALS cases and 1 to 2 percent of sporadic ALS cases. Over 200 pathogenic SOD1 variants have been reported, producing a spectrum of disease phenotypes from rapidly progressive (p.A5V, median survival approximately 1 year from onset) to slowly progressive (p.D91A homozygous, median survival exceeding 10 years). The pathogenic mechanism is a toxic gain of function in which mutant SOD1 protein undergoes misfolding, aggregation, and formation of cytoplasmic inclusions in motor neurons and astrocytes, driving neurodegeneration through oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, and impaired protein degradation. Tofersen addresses this mechanism directly by reducing the concentration of the causative toxic protein. The

clinical development program comprises a Phase 1-2 ascending-dose trial (50 participants) establishing dose-dependent CSF SOD1 reduction of up to 36 percent at the 100 mg intrathecal dose; the Phase 3 VALOR trial (108 participants) that did not meet its primary efficacy endpoint of change in the ALS Functional Rating Scale-Revised (ALSFRS-R) at 28 weeks (difference 1.2 points, $p = 0.97$) but demonstrated robust reductions in CSF SOD1 protein (29 percent) and plasma neurofilament light chain (60 percent); and an open-label extension demonstrating that participants who initiated tofersen early had a statistically significant 3.5-point advantage on ALSFRS-R decline compared with those whose treatment was delayed ($p = 0.03$ at 52 weeks). Accelerated approval was based on the reduction in plasma neurofilament light chain as a surrogate endpoint reasonably likely to predict clinical benefit. The ongoing ATLAS trial (NCT04856982) evaluates tofersen initiation in presymptomatic SOD1 variant carriers using plasma neurofilament light chain as a biomarker trigger for randomization. Tofersen is administered intrathecally at a dose of 100 mg in 15 mL, with three loading doses at 14-day intervals followed by maintenance doses every 28 days. The estimated CSF half-life is approximately four weeks, and elimination occurs through exonuclease-mediated hydrolysis without cytochrome P450 involvement. The principal adverse events are procedure-related (pain, headache) and drug-related (CSF pleocytosis in 42 percent of treated participants, fatigue, arthralgia, myalgia). Serious neurologic adverse events including myelitis, radiculitis, papilledema with elevated intracranial pressure, and aseptic meningitis have been reported in approximately 7 percent of tofersen-treated participants. This monograph reviews the chemistry, development history, molecular mechanism, pharmacokinetics, preclinical and clinical

evidence, sourcing and handling, adverse event profile, and a comparative assessment of five alternative ALS therapeutic agents against tofersen.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Tofersen.

Brand name: Qalsody (Biogen Inc.; United States, European Union, Canada, Australia) [1, 2].

Development code: BIIB067 (Biogen); the compound was developed as a next-generation antisense oligonucleotide by Ionis Pharmaceuticals under an internal designation and subsequently licensed to Biogen in December 2018 [3, 4].

Predecessor compound: The first-generation SOD1-targeting antisense oligonucleotide in this development lineage was ASO333611 (also designated ISIS-SOD1Rx, ISIS 333611), a 2'-O-methoxyethyl gapmer that entered the first-in-human Phase 1 trial for SOD1-ALS (NCT01041222) [5, 6]. Tofersen represents a more potent second-generation compound selected from the same structural class [7].

Chemical Abstracts Service registry number: Not consistently reported in the accessible peer-reviewed literature or regulatory databases as of this review. The compound is a biological polymer (oligonucleotide) and is identified principally by its INN, development code, and brand name rather than by CAS number in the clinical pharmacology literature.

1.2 Structural class and chemistry

Tofersen is a synthetic antisense oligonucleotide of the 5-10-5 MOE gapmer class [1, 8]. The compound is a 20-base residue (20-mer) with a mixed backbone consisting of two types of internucleotide linkages and two types of sugar modifications arranged in a gapmer configuration [1, 8].

Backbone chemistry: Of the nineteen internucleotide linkages, fifteen are 3'-O to 5'-O phosphorothioate diesters and four are 3'-O to 5'-O phosphodiester linkages [1, 8]. The phosphorothioate modifications enhance nuclease resistance and extend the biological half-life of the oligonucleotide in the central nervous system, while the retention of four unmodified phosphodiester linkages modulates the overall pharmacokinetic and tolerability profile.

Sugar modifications: Ten of the twenty sugar residues are 2-deoxy-D-ribose (the central "gap" segment), and the remaining ten are 2'-O-(2-methoxyethyl)-D-ribose (2'-MOE), with five MOE nucleosides at each of the 5' and 3' ends flanking the central deoxy gap [1, 8]. The MOE modifications at the flanking positions increase binding affinity for the target mRNA and provide additional nuclease resistance, while the central deoxynucleotide gap is essential for RNase H recognition and cleavage activity.

Base modifications: The cytosine and uridine bases are methylated at the 5-position (5-methylcytosine and 5-methyluridine), modifications that enhance binding affinity and reduce immunostimulatory potential of the oligonucleotide [1, 8].

Target: Tofersen binds to the 3' untranslated region (3' UTR) of SOD1 mRNA [4].

Molecular formula: C₂₃₀H₃₁₇N₇₂O₁₂₃P₁₉S₁₅ [1].

Molecular weight: 7127.86 atomic mass units [1].

1.3 Drug product formulation

Tofersen is supplied as a sterile, preservative-free, clear and colorless to slightly yellow solution for intrathecal injection. The marketed formulation contains 100 mg of tofersen in 15 mL (6.7 mg/mL) in a single-dose glass vial [1]. The solution is formulated in artificial cerebrospinal fluid (aCSF) for compatibility with intrathecal administration.

2. Abstract

Tofersen (BIIB067, Qalsody) is a 20-base 2'-O-methoxyethyl (MOE) gapmer antisense oligonucleotide that mediates RNase H-dependent degradation of SOD1 mRNA, reducing synthesis of mutant and wild-type SOD1 protein in the central nervous system [1, 5, 9]. The compound was developed by Ionis Pharmaceuticals and Biogen for intrathecal administration in amyotrophic lateral sclerosis (ALS) caused by mutations in the superoxide dismutase 1 (SOD1) gene, which account for approximately 13 to 20 percent of familial ALS and 1 to 2 percent of sporadic ALS cases [3, 10].

The preclinical foundation for tofersen development was established by Smith et al. (2006), who demonstrated that continuous intraventricular infusion of a first-generation SOD1 antisense oligonucleotide in SOD1-G93A transgenic rats produced a 50 percent reduction in SOD1 protein levels in the spinal cord and a 37 percent extension of disease duration [5]. McCampbell et al. (2018) subsequently reported that next-generation SOD1 antisense oligonucleotides (the immediate predecessors of tofersen) extended survival by 22 percent in SOD1-G93A mice and by 32 to 39 percent in SOD1-G93A rats, with up to 75 percent reduction in SOD1 mRNA after a single intrathecal injection [7].

The Phase 1-2 ascending-dose trial enrolled 50 adults with SOD1-ALS and demonstrated dose-dependent CSF SOD1 protein reduction, with the 100 mg dose producing a 36 percent reduction relative to placebo [9]. The Phase 3 VALOR trial (n = 108) did not meet its primary endpoint of change in the ALSFRS-R total score at 28 weeks in the faster-progressing subgroup (tofersen, -6.98 points; placebo, -8.14 points; difference, 1.2 points; 95 percent CI, -3.2 to 5.5; p = 0.97) [10]. However, tofersen produced a 29 percent reduction in CSF SOD1 protein and a 60 percent reduction in plasma neurofilament light chain (NfL), a validated biomarker of axonal injury, compared with a 20 percent increase in the placebo group [9, 10]. In the open-label extension, early-start participants showed a statistically significant 3.5-point ALSFRS-R advantage over delayed-start participants at 52 weeks (95 percent CI, 0.4 to 6.7; p = 0.03), with corresponding advantages in slow vital capacity (9.2 percentage points, p = 0.02) and handheld dynamometry (0.28 megascoring points, p = 0.02) [9, 10].

Tofersen received accelerated approval from the United States Food and Drug Administration on April 25, 2023, based on reduction of plasma NfL as a surrogate endpoint reasonably likely to predict clinical benefit [1, 2]. The ongoing ATLAS trial (NCT04856982) evaluates tofersen initiation in presymptomatic SOD1 variant carriers [11]. A systematic review and meta-analysis of tofersen data demonstrated a statistically significant pooled effect on ALSFRS-R decline (standardized mean difference 0.44, $p = 0.03$) and slow vital capacity decline (standardized mean difference 0.53, $p = 0.005$) favoring tofersen [12].

3. Discovery and Development History

3.1 Identification of SOD1 as a therapeutic target

The identification of mutations in the Cu/Zn superoxide dismutase 1 (SOD1) gene as the first genetic cause of familial ALS was reported by Rosen et al. in 1993 [3]. Over 200 pathogenic SOD1 variants have since been identified, with the most common including p.A5V (associated with rapid progression and median survival of approximately 1 year from onset), p.H47R (more common in Japanese populations and associated with slower progression), and p.D91A (most frequently inherited as a recessive trait and associated with a slowly progressive phenotype) [4, 10]. SOD1 mutations account for approximately 13 percent of familial ALS cases in Central European populations and approximately 1 to 2 percent of sporadic ALS cases [10, 13].

The pathogenic mechanism of SOD1-ALS is a toxic gain of function rather than loss of enzymatic dismutase activity. Mutant SOD1 protein undergoes conformational destabilization, misfolding, and aggregation, forming cytoplasmic inclusions in motor neurons and astrocytes [8, 10]. The toxicity is independent of enzymatic activity; there is no clear relationship between the decrease in enzymatic activity and the age at disease onset or the rate of progression [8]. The pathology is distinct from the phosphorylated TDP-43 aggregation that characterizes more than 95 percent of non-SOD1 ALS cases, and SOD1-ALS can be modeled through a prion-like experimental mechanism of templated misfolding [13].

3.2 First-generation SOD1 antisense oligonucleotide

Smith et al. (2006) reported the first demonstration that antisense oligonucleotides targeting SOD1 mRNA could modify disease course in an ALS animal model [5]. Using SOD1-G93A transgenic rats, the investigators continuously infused a 20-mer 2'-O-methoxyethyl phosphorothioate antisense oligonucleotide (SODr/h333611) at 100 micrograms per day into the right lateral ventricle via osmotic pump for 28 days, beginning at postnatal day 65 (approximately 30 days before expected disease onset). The treatment reduced SOD1 mRNA levels to 25 to 40 percent of normal throughout the brain and at all spinal cord levels, and produced a 50 percent reduc-

tion in SOD1 protein in right frontal cortex and lumbar and cervical spinal cord [5]. Disease onset was not significantly delayed (95 plus or minus 10 days in treated animals versus 95 plus or minus 8 days in controls), but survival was extended to 132 plus or minus 7 days compared with 122 plus or minus 8 days in saline-infused controls, representing a 37 percent extension of the typical 27-day disease duration after onset [5].

Winer et al. (2013) subsequently established that CSF SOD1 protein concentration is a viable pharmacodynamic marker for SOD1 antisense oligonucleotide therapy, demonstrating that CSF SOD1 reflects CNS tissue SOD1 levels (brain SOD1 protein correlated with CSF SOD1, $R = 0.54$, $p = 0.02$) and that within-subject CSF SOD1 varied minimally at 7.1 plus or minus 5.7 percent on serial measurements [6]. This work validated CSF SOD1 protein as the primary pharmacodynamic endpoint for clinical development.

3.3 Phase 1 first-in-human trial with ASO333611

The first-in-human trial of an SOD1-targeting antisense oligonucleotide (ISIS-SOD1Rx, ASO333611; NCT01041222) enrolled 32 ALS patients with SOD1 mutations and evaluated intrathecal delivery via lumbar puncture [4, 6]. The trial demonstrated safety and tolerability with no dose-limiting toxic effects, establishing the feasibility of chronic intrathecal antisense oligonucleotide administration in ALS patients [4].

3.4 Next-generation compounds and tofersen selection

McCampbell et al. (2018) reported the development and preclinical characterization of next-generation SOD1 antisense oligonucleotides that were substantially more potent than ASO333611 [7]. These compounds, including the immediate precursors of tofersen, achieved up to approximately 75 percent reduction in SOD1 mRNA after a single intrathecal injection in SOD1-G93A mice. In survival studies, the most potent compound (designated ASO 1) extended median survival by 37 days (22 percent) in SOD1-G93A mice and by 53 days (32 percent) in SOD1-G93A rats; a second compound (ASO 2) extended rat survival by 64 days (39 percent) [7]. In non-human primates, intrathecal bolus administration reduced SOD1 protein levels in CNS tissue by approximately 50 percent [7].

Tofersen (BIIB067), selected from this series for clinical development, was advanced into a Phase 1-2 ascending-dose trial (NCT02623699) that enrolled its first participant in November 2016 [8, 12]. Biogen exercised its option with Ionis Pharmaceuticals to develop and commercialize tofersen in December 2018, based on positive Phase 1 interim data [14].

3.5 Phase 3 and regulatory approval

The Phase 3 VALOR trial (NCT02623699, Part C) enrolled 108 participants with SOD1-ALS across 32 sites in 10 countries from March 2019 to July 2021 [10, 12]. Although the trial did not meet its primary efficacy endpoint, the robust biomarker reductions (CSF SOD1 and plasma NfL) and the delayed-start advantage observed in the open-label extension supported regulatory evaluation [9, 10].

Tofersen received accelerated approval from the United States Food and Drug Administration on April 25, 2023, under the brand name Qalsody, based on the reduction in plasma neurofilament light chain as a surrogate endpoint reasonably likely to predict clinical benefit [1, 2]. The approval included orphan drug, priority review, and fast track designations [2]. European Medicines Agency authorization followed on May 30, 2024 [15]. The Therapeutic Goods Administration of Australia approved tofersen on January 5, 2025, and Health Canada granted marketing authorization with conditions on March 3, 2025 [15].

4. Molecular Pharmacology

4.1 Antisense oligonucleotide mechanism

Tofersen is an antisense oligonucleotide that operates through the RNase H-dependent degradation pathway [9, 10]. After intrathecal administration and distribution into CNS tissues, the compound hybridizes with complementary sequences in the 3' untranslated region of SOD1 mRNA, forming an RNA-DNA heteroduplex [4, 9]. The endogenous endonuclease RNase H1 recognizes the heteroduplex and cleaves the RNA strand (SOD1 mRNA) at the site of hybridization [9]. Following cleavage, the antisense oligonucleotide is released intact and is available to bind additional mRNA molecules, enabling catalytic turnover that amplifies the knockdown effect beyond the stoichiometric concentration of the oligonucleotide [9].

The 5-10-5 gapmer design is essential for this mechanism: the central 10-nucleotide deoxynucleotide gap forms the DNA portion of the RNA-DNA heteroduplex that is recognized by RNase H, while the flanking 5-nucleotide MOE wings enhance binding affinity for the target mRNA (increasing the melting temperature of the duplex by approximately 2 degrees Celsius per MOE nucleotide relative to unmodified DNA) and provide nuclease resistance [4, 8]. The phosphorothioate backbone modifications further extend the biological half-life of the oligonucleotide in CNS tissue, where the estimated half-life is on the order of weeks to months [9, 16].

4.2 Target engagement and SOD1 reduction

The pharmacodynamic effect of tofersen is measured by the reduction in SOD1 protein concentration in cerebrospinal fluid, which has been validated as a surrogate for CNS tissue SOD1 levels [6]. In the Phase 1-2 trial, CSF SOD1 protein reduction was dose-dependent: 1 percent at 20 mg, 27 percent at 40 mg, 21 percent at 60 mg, and 36 percent at 100 mg, compared with a 3 percent decrease in the placebo group [9]. In the Phase 3 VALOR trial at the 100 mg dose, the geometric mean ratio of CSF SOD1 to baseline was 0.62 to 0.70 in the tofersen groups compared with 0.98 to 1.20 in the placebo groups, representing a 29 to 38 percent reduction from baseline [9, 10, 12].

The target engagement is non-selective between mutant and wild-type SOD1 alleles; tofersen reduces expression of both the mutant toxic protein and the normal SOD1 protein [9, 13]. The clinical significance of wild-type SOD1 reduction is unclear but has not produced observable adverse consequences in clinical trials to date, consistent with animal model data showing that SOD1 knockout mice are viable and do not develop motor neuron disease [10].

4.3 SOD1 gain-of-function toxicity

The molecular pharmacology of tofersen is predicated on the toxic gain-of-function hypothesis of SOD1-ALS [3, 8, 10]. Mutant SOD1 protein undergoes structural destabilization that promotes misfolding, oligomerization, and formation of insoluble aggregates within motor neurons and surrounding glial cells [8, 10]. The toxic mechanisms downstream of SOD1 aggregation include mitochondrial dysfunction (through direct association of misfolded SOD1 with the outer mitochondrial membrane), endoplasmic reticulum stress (through interference with the unfolded protein response), oxidative damage (through aberrant redox chemistry of misfolded copper-containing SOD1), impairment of axonal transport, excitotoxicity, and activation of neuroinflammatory cascades in microglia and astrocytes [8, 10, 13].

Importantly, the disease mechanism is independent of the enzymatic superoxide dismutase activity of SOD1. Some pathogenic mutations retain near-normal enzymatic activity (e.g., p.A5V), while others abolish it; the severity of disease does not correlate with the degree of enzymatic impairment [8]. SOD1-ALS pathology is also distinct from the TDP-43 proteinopathy that characterizes more than 95 percent of non-SOD1 ALS cases; SOD1-ALS instead features SOD1-immunoreactive inclusions and can be modeled by a prion-like templated misfolding mechanism [13].

4.4 Neurofilament biomarker pharmacology

A critical pharmacodynamic observation is the reduction in plasma neurofilament light chain (NfL) produced by tofersen, which served as the basis for accelerated approval [1, 2, 9]. NfL is a cytoskeletal protein released from damaged or degenerating axons into the CSF and blood; elevated plasma NfL is a validated biomarker of ongoing neurodegeneration in ALS and other neurodegenerative diseases [9, 10]. In the VALOR trial, tofersen reduced plasma NfL by 60 percent from baseline compared with a 20 percent increase in the placebo group [9, 10]. The reduction in NfL is interpreted as reflecting reduced rates of motor neuron injury and axonal degen-

eration downstream of SOD1 protein reduction, establishing a pharmacological chain from target engagement (SOD1 mRNA degradation), to proximal biomarker (CSF SOD1 protein reduction), to distal biomarker (plasma NfL reduction reflecting reduced neuronal injury) [9, 10, 13].

The SOD1 protein itself has a slow turnover in the central nervous system. Using stable isotope labeling kinetics, SOD1 protein half-life in human CSF was calculated as 25 plus or minus 7.4 days, consistent with the observation that CSF SOD1 reduction reaches steady state only after the three loading doses administered over the initial 28-day period [7, 9].

4.5 Immunological consequences of the phosphorothioate backbone

The phosphorothioate backbone of tofersen, while essential for nuclease resistance and pharmacokinetic stability, has intrinsic immunostimulatory properties [17, 18]. Phosphorothioate antisense oligonucleotides can activate complement through Factor H binding, leading to increased production of complement split products Bb and C3a, and can stimulate innate immune responses through engagement of Toll-like receptors (particularly TLR9) in the CNS [17]. These properties are believed to contribute to the high rates of CSF pleocytosis (42 to 80 percent of treated participants) and elevated CSF protein observed across tofersen clinical trials, and may underlie the serious neuroinflammatory adverse events (myelitis, radiculitis, aseptic meningitis) reported in a subset of patients [17, 18]. The 5-methylcytosine and 5-methyluridine base modifications in tofersen are intended in part to mitigate immunostimulatory potential, but the CSF pleocytosis data indicate that substantial intrathecal immune activation occurs in the majority of treated patients [9, 17].

5. Pharmacokinetics

5.1 Administration and absorption

Tofersen is administered exclusively by intrathecal injection via lumbar puncture, as the compound does not cross the blood-brain barrier in pharmacologically relevant quantities after systemic administration [1, 9, 16]. The approved dose is 100 mg (15 mL) per administration. Prior to injection, approximately 10 mL of cerebrospinal fluid is withdrawn, and the drug is injected slowly over 1 to 3 minutes [1].

The dosing schedule comprises a loading phase of three doses at 14-day intervals (days 1, 15, and 29) followed by a maintenance phase of one dose every 28 days [1, 9]. CSF steady-state tofersen concentrations are achieved following the three loading doses [9, 16].

5.2 Distribution

Tofersen distributes broadly within the central nervous system after intrathecal administration. Cook et al. (2026) characterized the biodistribution of radiolabeled (technetium-99m-conjugated) tofersen using SPECT/CT imaging in Sprague-Dawley rats (n = 2), cynomolgus monkeys (n = 4), and healthy human volunteers (n = 3) [19]. In all three species, broad spinal cord and brain uptake was observed, with the highest radioactivity concentrations in the lumbar and lower thoracic spine near the site of injection [19]. Brain activity ranged from approximately 4 to 10 percent of the injected dose in nonhuman primates [19].

Species differences in clearance kinetics were observed: in rodents and nonhuman primates, time-activity curves showed initial clearance from brain and spine within the first 6 hours followed by a concentration plateau through 24 hours, whereas in humans, clearance from the spinal axis continued throughout the full 24-hour imaging period without reaching equilibrium [19]. Peripheral uptake and clearance occurred primarily through the liver and kidneys across all species, with nonhuman primates displaying relatively higher hepatic and renal uptake [19]. The radiolabeled tofersen remained more than 95 percent intact in all tissues tested at 24 hours postdose, with the exception of CSF where some degradation was observed [19].

5.3 CSF and tissue pharmacokinetics

The estimated CSF half-life of tofersen is approximately 4 weeks [9, 16]. This prolonged half-life reflects the combination of phosphorothioate backbone stability, MOE wing protection from exonuclease degradation, and tissue binding in the CNS. After the loading phase, there is very little or no accumulation of tofersen in the CSF or plasma with monthly maintenance dosing, indicating that the 28-day dosing interval approximates the CSF clearance half-life [16].

In preclinical models, the tissue half-life of tofersen in spinal cord is longer than the CSF half-life, consistent with intracellular accumulation and binding. McCampbell et al. (2018) reported lumbar cord EC50 values of 0.9 micrograms per gram of tissue in mice and 1.4 micrograms per gram in rats for SOD1 mRNA knock-down, with sustained SOD1 mRNA lowering persisting for nearly 10 weeks following a single intrathecal bolus in rodents [7]. In nonhuman primates, a composite EC50 of 20 micrograms per gram of tissue was estimated, with dose-dependent SOD1 protein reduction persisting for approximately 100 days post-administration [7].

5.4 Metabolism and elimination

Tofersen is expected to undergo degradation via 3'-to-5' and 5'-to-3' exonuclease-mediated hydrolysis, the standard metabolic pathway for phosphorothioate oligonucleotides in mammalian tissues [1, 16]. The compound does not undergo hepatic cytochrome P450-mediated metabolism and has shown no evidence of interactions with CYP enzymes, major drug transporters, or other drugs in in vitro studies [1, 9, 16]. Tofersen is not a substrate or inhibitor of major CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4) and is not a substrate or inhibitor of major transporters (P-glycoprotein, BCRP, OAT1, OAT3, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K) [1].

Peripheral clearance of intrathecally administered tofersen occurs primarily through hepatic and renal elimination of oligonucleotide degradation products [19]. Given the intrathecal route and relatively low systemic exposure, renal and hepatic impairment are not expected to substantially alter the pharmacokinetics at the CNS target site, although formal pharmacokinetic studies in these populations have not been conducted [1, 16].

5.5 Special populations

Pharmacokinetic data in specific populations are limited. Of 162 participants who received tofersen across clinical trials, 22 (13.5 percent) were 65 years or older and 2 (1.2 percent) were 75 years or older; no overall differences in safety or effectiveness were observed between these patients and younger patients [1, 16]. Pediatric pharmacokinetics have not been established; ALS is predominantly an adult-onset disease and tofersen is not indicated for pediatric use [1]. Animal reproductive toxicity studies in mice and rabbits at exposures 4 to 20 times the human intrathecal dose showed no adverse embryofetal effects; human pregnancy data are not available [1, 16]. Tofersen was detected in milk of lactating mice after subcutaneous administration; no human lactation data exist [1].

6. Preclinical Pharmacology

6.1 First-generation SOD1 antisense oligonucleotide studies

The preclinical pharmacology program for SOD1-targeting antisense oligonucleotides began with the work of Smith et al. (2006) using the first-generation compound SODr/h333611 in SOD1-G93A transgenic rats [5]. These rats express a high-copy-number human SOD1 transgene bearing the G93A missense mutation and develop progressive motor neuron disease with hindlimb weakness, muscle wasting, and death at approximately 120 to 130 days of age, recapitulating key features of human SOD1-ALS [5].

Continuous intraventricular infusion of ASO333611 at 100 micrograms per day for 28 days, initiated at postnatal day 65 (approximately 30 days before expected disease onset), produced a reduction in SOD1 mRNA levels to 25 to 40 percent of normal throughout the brain and at all spinal cord levels, and a 50 percent reduction in SOD1 protein in right frontal cortex and lumbar and cervical spinal cord [5]. Disease onset (defined by body weight loss) was not significantly delayed (95 plus or minus 10 days in treated animals versus 95 plus or minus 8 days in controls), but survival was extended to 132 plus or minus 7 days compared with 122 plus or minus 8 days in saline controls, representing a 37 percent extension of the 27-day mean disease duration after onset [5]. The authors concluded that antisense oligonucleotide infusion could be "an effective, dosage-regulatable means of treating neurodegenerative diseases, including ALS, where appropriate target proteins are known" [5].

6.2 CSF SOD1 as pharmacodynamic biomarker

Winer et al. (2013) established the translational pharmacology framework for SOD1 antisense oligonucleotide development by demonstrating that CSF SOD1 protein concentration serves as a reliable pharmacodynamic marker of CNS tissue SOD1 levels [6]. In SOD1-G93A rats (low-copy transgenic line surviving beyond 14 months), antisense oligonucleotide treatment decreased CSF SOD1 protein by 42 plus or minus 14 percent, with brain SOD1 protein correlating with CSF SOD1 pro-

tein at $R = 0.54$ ($p = 0.02$) [6]. In human subjects (93 ALS patients, 88 healthy controls, 89 neurological disease controls), within-subject CSF SOD1 varied minimally at 7.1 plus or minus 5.7 percent on repeat measurements over months, with maximum variation of 21 percent between any two timepoints [6]. Power calculations estimated greater than 95 percent probability of detecting a 40 percent or greater reduction in CSF SOD1 in as few as 6 patients, informing the design of subsequent clinical trials [6].

6.3 Next-generation compounds (tofersen predecessors)

McCampbell et al. (2018) reported the preclinical characterization of next-generation SOD1 antisense oligonucleotides that were substantially more potent than ASO333611 and that directly preceded tofersen in the development lineage [7]. Key findings across species:

Mouse studies (SOD1-G93A mice, intraventricular bolus 100 to 300 micrograms): The most potent compound achieved approximately 75 percent maximum reduction of SOD1 mRNA following a single injection [7]. Median disease onset was delayed from 140 days (inactive ASO control) to 183 days (43-day delay, $p < 0.0001$), and median survival was extended by 37 days (22 percent, $p < 0.0001$) [7]. Treated mice maintained rotarod performance 26 days longer than controls, with median age at 50 percent performance decrease of 182 days versus 147 days [7]. A critical functional observation was that initial loss of compound muscle action potential (CMAP) in SOD1-G93A mice was reversed after a single dose of the SOD1 antisense oligonucleotide, indicating that early neuromuscular junction dysfunction is reversible with SOD1 reduction [7]. At necropsy, treated mice maintained innervation of tibialis anterior muscles, whereas control animals showed denervation of more than 75 percent of muscle endplates [7].

Rat studies (SOD1-G93A rats, intrathecal bolus 1000 micrograms): Two lead compounds were evaluated. ASO 1 extended survival by 53 days (32 percent, $p < 0.0001$) and ASO 2 by 64 days (39 percent, $p < 0.0001$) [7]. Disease onset was delayed by 70 days (ASO 1, $p < 0.0001$) and 67 days (ASO 2, $p < 0.001$) [7].

Nonhuman primate studies (cynomolgus monkeys, intrathecal bolus): SOD1 protein levels in CNS tissue were lowered by approximately 50 percent in a dose-dependent manner, with a composite EC₅₀ of 20 micrograms per gram of tissue and duration of effect persisting approximately 100 days postdose [7].

Pharmacokinetic findings: Lumbar cord EC50 values for SOD1 mRNA knock-down were 0.9 micrograms per gram of tissue in mice and 1.4 micrograms per gram in rats, with sustained SOD1 mRNA lowering persisting for nearly 10 weeks following a single intrathecal bolus [7].

6.4 Translational validity

The preclinical pharmacology program established several translational principles that informed the clinical development of tofersen. First, intrathecal administration achieves broad CNS distribution sufficient for target engagement throughout the spinal cord and brain. Second, SOD1 protein reduction in CSF mirrors tissue-level knockdown and serves as a clinically accessible pharmacodynamic endpoint. Third, SOD1 reduction reverses early neuromuscular junction dysfunction (CMAP reversal) even after disease onset has begun, supporting the hypothesis that early intervention may produce greater benefit than treatment after substantial motor neuron loss. Fourth, the magnitude of survival extension in rodent models (22 to 39 percent) is substantial by ALS preclinical standards, although the predictive validity of SOD1-G93A transgenic models for human clinical efficacy is imperfect and has historically overestimated treatment benefit in ALS drug development [7, 13].

7. Clinical Evidence Base

The following section summarizes the published clinical evidence for tofersen. The compound is approved for research-grade and clinical use; investigators should consult regulatory guidance and institutional protocols before initiating any human research application.

7.1 Phase 1-2 ascending-dose trial

Miller et al. (2020) reported a Phase 1-2 randomized, double-blind, placebo-controlled, ascending-dose trial of intrathecal tofersen in adults with ALS due to SOD1 mutations (NCT02623699, Parts A and B) [9]. A total of 50 participants were randomized (38 tofersen, 12 placebo) across four dose cohorts (20, 40, 60, or 100 mg) in a 3:1 ratio, receiving five intrathecal doses over 12 weeks [9, 12].

The primary endpoints were safety and pharmacokinetics. The secondary endpoint was change from baseline in CSF SOD1 concentration at day 85. CSF SOD1 protein reduction was dose-dependent: 1 percent at 20 mg, 27 percent at 40 mg, 21 percent at 60 mg, and 36 percent at 100 mg, compared with a 3 percent decrease in the placebo group [9]. In the 100 mg cohort, exploratory analysis of ALSFRS-R decline showed a numerically favorable trend: tofersen, -1.19 points (95 percent CI, -4.67 to 2.29); placebo, -5.63 points (95 percent CI, -8.90 to -2.36), though this was not a prespecified efficacy endpoint and the trial was not powered for clinical outcomes [9].

Tofersen was generally well tolerated. Most adverse events were related to the lumbar puncture procedure (back pain, post-lumbar-puncture syndrome). Headache occurred in 42 percent, procedural pain in 42 percent, and post-lumbar-puncture syndrome in 34 percent of tofersen-treated participants [9]. CSF pleocytosis (elevated white blood cell count) occurred in 42 percent of the tofersen group compared with 8 percent of the placebo group [9]. Among the 50 participants, two deaths occurred in the tofersen group (one from pulmonary embolism on day 137, one from respiratory failure on day 152) and one in the placebo group (respiratory failure on day 52); all were attributed to underlying ALS disease progression [9].

7.2 Phase 3 VALOR trial

Miller et al. (2022) reported the Phase 3 VALOR trial (NCT02623699, Part C), a multicenter, randomized, double-blind, placebo-controlled study evaluating tofersen 100 mg intrathecal in adults with SOD1-ALS [10]. A total of 108 participants were randomized 2:1 (72 tofersen, 36 placebo), stratified by predicted rate of disease progression based on SOD1 variant type (faster-progressing, n = 60; slower-progressing, n = 48) [9, 10]. Treatment consisted of eight intrathecal doses: three loading doses at 2-week intervals followed by five maintenance doses at 4-week intervals, over a 24-week treatment period [9, 10].

Primary endpoint: The primary endpoint was the change in ALSFRS-R total score from baseline to week 28 in the faster-progressing subgroup. The trial did not meet this endpoint: tofersen, -6.98 points; placebo, -8.14 points; difference, 1.2 points (95 percent CI, -3.2 to 5.5; p = 0.97 on a one-sided test) [9, 10]. In the slower-progressing subgroup, the corresponding values were tofersen, -1.33; placebo, -2.73; difference, 1.4 (95 percent CI, -1.1 to 3.9) [9].

Secondary endpoints: Tofersen produced robust reductions in biomarker endpoints. CSF SOD1 protein was reduced by 29 percent from baseline in the faster-progressing tofersen group (geometric mean ratio to baseline, 0.62; 95 percent CI, 0.49 to 0.78) compared with a 16 percent increase in placebo [9, 10]. Plasma NfL decreased by 60 percent in the tofersen group compared with a 20 percent increase in the placebo group, a highly significant divergence [9, 10]. Slow vital capacity (SVC) showed a numerical trend favoring tofersen: difference 7.9 percentage points (95 percent CI, -3.5 to 19.3) in the faster-progressing subgroup [9]. Handheld dynamometry (HHD) similarly favored tofersen numerically but did not reach statistical significance [9, 10]. Secondary clinical endpoints did not differ significantly between groups at the 28-week timepoint [10].

7.3 Open-label extension

Approximately 88 percent of participants originally randomized to tofersen and 89 percent of those initially randomized to placebo enrolled in the open-label extension (NCT03070119), providing an early-start versus delayed-start comparison at 52 weeks from the VALOR baseline [9, 10].

At 52 weeks, the adjusted mean change in ALSFRS-R was -6.0 in the early-start cohort and -9.5 in the delayed-start cohort (difference, 3.5 points; 95 percent CI, 0.4 to 6.7; non-multiplicity-adjusted $p = 0.03$) [9, 10]. Corresponding advantages favoring early start were observed for slow vital capacity (difference, 9.2 percentage points; 95 percent CI, 1.7 to 16.6; $p = 0.02$) and handheld dynamometry (difference, 0.28 megascoring points; 95 percent CI, 0.05 to 0.52; $p = 0.02$) [9, 10]. Death or permanent ventilation occurred in 12 of 72 early-start participants (16.7 percent) compared with 8 of 36 delayed-start participants (22.2 percent); death alone occurred in 8 of 72 (11.1 percent) versus 6 of 36 (16.7 percent) [9].

The early-start versus delayed-start comparison, while not randomized and subject to the limitations of observational extension analyses, provided the principal evidence for clinical benefit that supported regulatory evaluation. The FDA interpreted the combined biomarker and delayed-start clinical data as consistent with a treatment effect emerging over a timeframe longer than the 28-week VALOR randomized period [1, 2].

7.4 Systematic review and meta-analysis

Hamad et al. (2025) published a systematic review and meta-analysis including 12 studies (2 randomized controlled trials, 5 cohort studies, 1 case series, 4 case reports) encompassing 195 unique tofersen-treated patients [12]. Pooled analysis of tofersen versus placebo demonstrated a statistically significant effect on ALSFRS-R decline favoring tofersen (standardized mean difference, 0.44; 95 percent CI, 0.05 to 0.83; $p = 0.03$) and on slow vital capacity decline (standardized mean difference, 0.53; 95 percent CI, 0.16 to 0.90; $p = 0.005$) [12]. The quality of included studies was assessed as generally moderate [12]. Subgroup analysis for fast-progression patients did not show a significant difference ($p = 0.35$), consistent with the primary VALOR result [12].

7.5 Real-world observational evidence

Meyer et al. (2024) reported an 18-month multicenter observational study of 16 SOD1-ALS patients receiving tofersen through the expanded access program in Germany [20]. Serum NfL decreased in all patients except one heterozygous D91A-SOD1 mutation carrier (mean change, -58 percent; range, -91 to +27 percent; $p < 0.01$) [20]. Seven patients demonstrated increased ALSFRS-R scores during treat-

ment. Slow vital capacity was stable (mean 88 percent, range -15 to +28 percent). Patient-reported global treatment satisfaction was high (mean 83 on the TSQM-9 scale), and the net promoter score for tofersen was +80 [20].

Weishaupt et al. (2024) reported neurofilament response data in 11 ALS patients with the SOD1 p.D91A variant (6 homozygous, 5 heterozygous) treated with tofersen for 4 to 16 months [21]. All homozygous patients displayed a significant reduction in serum NfL starting after the fourth intrathecal injection ($p < 0.05$), with one patient achieving NfL values below 10 percent of baseline after 10 months [21]. Heterozygous patients also showed significant NfL reductions, though with smaller effect sizes, providing evidence that the heterozygous D91A mutation contributes causally to disease pathogenesis [21]. All homozygous patients reported subjective improvement in activities of daily living [21].

7.6 ATLAS presymptomatic trial

Benatar et al. (2022) described the design of the ATLAS study (NCT04856982), the first interventional trial in presymptomatic ALS [11]. The trial enrolls carriers of SOD1 variants associated with high or complete penetrance and rapid progression from a prespecified list of 17 variants (including p.A5V, p.G94A, p.D102G), who are clinically presymptomatic and have plasma NfL below 44 pg/mL [11]. Participants undergo monthly NfL monitoring in Part A (natural history run-in). When plasma NfL reaches 44 pg/mL or above with an increase of 10 pg/mL or more from baseline, participants are randomized 1:1 to tofersen or placebo in Part B (double-blind) [11].

The primary endpoint is the proportion of Part B participants in whom clinically manifest ALS emerges within 12 months of randomization, analyzed using a Bayesian decision rule requiring a posterior probability of at least 0.9 for the alternative hypothesis [11]. Based on NfL trajectory modeling, the average time from reaching the NfL threshold to emergence of clinically manifest ALS is estimated at 3 to 4 months in untreated carriers [11]. Approximately 150 presymptomatic carriers are expected to enroll in Part A by August 2027, with approximately 28 randomized in Part B [4, 11]. ATLAS represents the first trial to use neurofilament as both an enrichment marker for eligibility and a trigger for treatment initiation in ALS [11].

7.7 Ongoing and planned studies

A long-term safety study of tofersen in SOD1-ALS (NCT07259980) is evaluating extended treatment duration and safety monitoring beyond the initial trial periods [15]. Results from the ATLAS presymptomatic trial and longer-term open-label extension data are expected to inform confirmatory evaluation and potential conversion of the accelerated approval to full approval.

8. Sourcing and Quality Verification

Tofersen is a prescription biological product available exclusively as the branded formulation Qalsody (Biogen Inc.) in jurisdictions where it is approved (United States, European Union, Canada, Australia as of the present review) [1, 2, 15]. The compound is not available as a research-grade chemical from standard chemical suppliers, nor is it a generic pharmaceutical; it is manufactured under current Good Manufacturing Practice (cGMP) conditions as a sterile, preservative-free solution for intrathecal injection [1].

Each vial of Qalsody contains 100 mg tofersen in 15 mL of artificial cerebrospinal fluid solution. The drug product is characterized by the manufacturer for identity (sequence verification by oligonucleotide mass spectrometry), purity (anion-exchange and reverse-phase chromatography), potency (bioassay for SOD1 mRNA reduction), sterility, endotoxin content, and particulate matter [1].

Investigators requiring tofersen for non-clinical research applications (in vitro cell-based assays, animal model studies) should note that the compound is a proprietary Ionis/Biogen oligonucleotide and is not commercially available as a bulk research reagent. Potential paths to research access include expanded access programs (where available), collaborative research agreements with the sponsor, or synthesis of the published oligonucleotide sequence by custom oligonucleotide manufacturers with appropriate MOE phosphoramidite chemistry capabilities. Any custom-synthesized material should be fully characterized for sequence identity (mass spectrometry), backbone composition (phosphorothioate content), purity (ion-exchange HPLC, greater than 85 percent full-length product is typical for 20-mer phosphorothioate oligonucleotides), and endotoxin content prior to in vivo use.

Investigators should confirm the SOD1 mutation status of all research subjects or patient-derived cell lines using validated genetic testing. The FDA-approved indication requires a confirmed SOD1 gene mutation; commercially available genetic tests for SOD1-ALS include targeted panel sequencing and whole-exome or whole-genome sequencing offered by multiple clinical laboratories [1, 2].

9. Reconstitution and Handling

Tofersen (Qalsody) is supplied as a ready-to-use sterile solution that does not require reconstitution [1]. Key handling parameters for the drug product:

Storage: Refrigerate at 2 to 8 degrees Celsius (36 to 46 degrees Fahrenheit) in the original carton to protect from light. Do not freeze. Do not shake [1]. Prior to administration, the vial may be allowed to warm to room temperature (approximately 25 degrees Celsius) for up to 4 hours [1].

Visual inspection: Prior to use, the solution should be visually inspected for particulate matter and discoloration. The solution should be clear and colorless to slightly yellow. Do not use if the solution is cloudy, discolored, or contains visible particulate matter [1].

Administration technique: Withdraw approximately 10 mL of cerebrospinal fluid prior to injection using aseptic lumbar puncture technique. Administer the full 15 mL volume of Qalsody (100 mg tofersen) by intrathecal injection over 1 to 3 minutes using a spinal needle of appropriate gauge (typically 20 to 22 gauge) [1]. The injection should be performed by or under the supervision of a healthcare professional experienced in lumbar puncture procedures.

Stability after removal from refrigeration: The solution is stable for up to 4 hours at room temperature (up to 25 degrees Celsius). Any unused drug remaining in the vial should be discarded; the product does not contain preservatives [1].

For investigators conducting preclinical research with custom-synthesized SOD1 antisense oligonucleotides of similar chemistry, stock solutions of phosphorothioate MOE gapmer oligonucleotides are typically prepared in sterile phosphate-buffered saline or artificial cerebrospinal fluid at concentrations of 1 to 10 mg/mL, stored at -20 degrees Celsius for long-term storage, and are stable through at least 5 freeze-thaw cycles without measurable loss of activity. Lyophilized oligonucleotide material is stable at -20 degrees Celsius for extended periods (years).

10. Stack Interactions and Combinations

10.1 Drug-drug interactions

Tofersen has no known pharmacokinetic drug-drug interactions [1, 9, 16]. In vitro, tofersen is not a substrate, inhibitor, or inducer of major cytochrome P450 enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4) and is not a substrate or inhibitor of major drug transporters (P-glycoprotein, BCRP, OAT1, OAT3, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K) [1]. The intrathecal route of administration and the metabolic pathway (exonuclease-mediated hydrolysis rather than hepatic metabolism) render conventional pharmacokinetic drug interactions unlikely [1, 16].

10.2 Concomitant ALS therapies

In the VALOR trial, participants were permitted to receive concomitant riluzole, which was taken by approximately 62 percent of the trial population [9, 10]. No pharmacokinetic or pharmacodynamic interaction between tofersen and riluzole was identified. Tofersen has been administered concurrently with riluzole and edaravone in clinical practice without reported interaction, as expected from the non-overlapping metabolic and mechanistic profiles of these agents [9, 16].

10.3 Immunomodulatory agents

The management of tofersen-related immune-mediated adverse events (myelitis, radiculitis, aseptic meningitis) has involved concomitant use of corticosteroids (intravenous methylprednisolone), plasma exchange, mycophenolate mofetil, and methotrexate [17, 18, 22]. Vidovic et al. (2025) reported that weekly oral methotrexate (10 to 20 mg) with folic acid supplementation provided sustained control of tofersen-induced intrathecal immune activation (reducing CSF pleocytosis and intrathecal IgM synthesis) and allowed continued tofersen dosing over 32 doses, whereas corticosteroids alone provided only temporary benefit [22]. These combinations were used for adverse event management rather than pharmacological en-

hancement; investigators considering long-term tofersen administration should be aware that immunomodulatory co-treatment may be required in a subset of patients.

10.4 Other intrathecal agents

Tofersen should not be mixed with other intrathecal agents in the same injection. No formal compatibility studies with other intrathecally administered drugs (e.g., baclofen, opioids, chemotherapy) have been conducted. Investigators administering tofersen to patients with indwelling intrathecal drug delivery systems should consider the potential for local pharmacokinetic interference and should consult the prescribing information for timing recommendations relative to other intrathecal medications [1].

10.5 Anticoagulants and antiplatelet agents

Lumbar puncture carries inherent risk of epidural or subdural hematoma, particularly in patients receiving anticoagulant or antiplatelet therapy. The chronic lumbar puncture schedule required for tofersen maintenance dosing (monthly) necessitates careful management of anticoagulation. Institutional guidelines for lumbar puncture in anticoagulated patients should be followed; typical practice includes withholding direct oral anticoagulants for 24 to 48 hours and monitoring INR for vitamin K antagonists prior to each procedure [1].

11. Adverse Events and Safety Signal

11.1 Common adverse events

The adverse event profile of tofersen reflects a combination of procedure-related effects (from chronic intrathecal lumbar puncture administration) and drug-related effects (from the pharmacological and immunological properties of the phosphorothioate oligonucleotide) [1, 9, 16].

In the VALOR Phase 3 trial (tofersen, n = 72; placebo, n = 36), adverse events occurring at 5 percent or greater incidence with excess over placebo were: pain (back pain and pain in extremity, 42 percent versus 22 percent), fatigue (17 percent versus 6 percent), arthralgia (14 percent versus 6 percent), CSF white blood cell increased including pleocytosis (14 percent versus 0 percent), myalgia (14 percent versus 6 percent), CSF protein increased (8 percent versus 3 percent), musculoskeletal stiffness (6 percent versus 0 percent), and neuralgia (6 percent versus 0 percent) [1]. Headache (46 percent), procedural pain (57 percent), and falls (24 percent) were also common in the tofersen group [9].

Drug-related adverse events were reported in 28 of 72 tofersen recipients (39 percent) compared with 2 of 36 placebo recipients (6 percent) [9].

11.2 CSF pleocytosis and protein elevation

CSF pleocytosis (elevated white blood cell count above 10 cells per microliter) is the most frequently observed laboratory abnormality with tofersen treatment. In the combined tofersen clinical trial population (n = 147 receiving the 100 mg dose), CSF white blood cell count exceeded 10 cells per microliter in 117 of 147 participants (79.6 percent), and a shift to high CSF protein occurred in 132 of 147 participants (89.7 percent) [17]. In the VALOR trial specifically, CSF pleocytosis was observed in 58 percent of tofersen recipients compared with 6 percent of placebo recipients [9].

CSF pleocytosis and elevated CSF protein were not directly predictive of the development of serious neurologic adverse events; the CSF laboratory findings occurred in the majority of treated participants, whereas serious neurologic events

occurred in approximately 7 percent [17]. The mechanism is believed to involve the proinflammatory properties of the phosphorothioate backbone, including complement activation and innate immune stimulation [17].

11.3 Serious neurologic adverse events

Lovett et al. (2025) reported the most comprehensive analysis of serious neurologic adverse events in tofersen clinical trials [17]. Of 147 participants who received the 100 mg tofersen dose, 10 (6.8 percent) experienced serious neurologic adverse events; no similar events occurred in placebo recipients [17]. The event types and frequencies were:

- **Myelitis:** 4 cases (2.7 percent). Onset occurred after 5 to 7 doses. MRI findings included contrast enhancement and edema at thoracic spinal cord levels [17, 18]. Management included intravenous methylprednisolone (1000 mg daily for 3 to 5 days), oral steroid taper, and in some cases plasma exchange or mycophenolate mofetil [17, 18]. All myelitis cases resolved or improved.
- **Papilledema and elevated intracranial pressure:** 4 cases (2.7 percent). Onset after 9 to 18 doses. Managed with acetazolamide and corticosteroids. Obesity was identified as a potential risk factor (2 of 4 cases had obesity history) [17]. All cases resolved with treatment.
- **Radiculitis:** 2 cases (1.4 percent). Onset after 1 and 24 doses respectively. Managed with analgesics and anti-inflammatory medications [17].
- **Aseptic meningitis:** 2 cases (1.4 percent). Onset after 5 and 7 doses. CSF white blood cell counts reached 144 and 317 cells per microliter respectively [17]. Managed with empiric antibiotics/antivirals (pending culture results), corticosteroids, and analgesics.

All serious neurologic events resolved or improved; none was fatal or life-threatening [17]. Three events led to tofersen discontinuation; all other affected participants continued treatment [17]. AQP4 and MOG antibodies tested negative in myelitis cases, indicating that the events do not represent typical autoimmune demyelinating disease [17].

11.4 Myelitis case reports

Reilich et al. (2023) reported a detailed case of myelitis in a 56-year-old SOD1-ALS patient (heterozygous c.341T>C, p.Ile114Thr) developing on day 115, three days after the sixth tofersen injection [18]. MRI revealed low, partly punctiform contrast uptake and edema at thoracic levels T1, T3/4, T5, and T7 [18]. CSF showed lymphocytic pleocytosis (37 cells per microliter), elevated protein (119 mg/dL), elevated IgM quotient, and CSF-specific oligoclonal bands [18]. Treatment with intravenous prednisolone 1000 mg daily for 5 days followed by an oral taper over 40 days produced symptom regression, though MRI findings persisted at day 196 [18]. The patient declined rechallenge with tofersen [18].

11.5 Immunogenicity

Treatment-emergent anti-drug antibodies (ADAs) developed in 97 of 166 tofersen-treated participants (58.4 percent), of which 14 were transient and 83 were persistent [1]. The clinical significance of ADAs has not been fully characterized; no clear association between ADA development and either reduced efficacy or increased adverse events has been established in the available data [1, 16].

11.6 Hepatic effects

LiverTox assessment classifies tofersen with a likelihood score of E (unlikely cause of clinically apparent liver injury) [23]. In the registration trial, ALT elevation above 3 times the upper limit of normal occurred in 4 percent of tofersen recipients compared with 3 percent of placebo recipients [23]. In longer-term open-label studies, ALT above 3 times ULN was observed in 11 percent and above 5 times ULN in 4 percent, but these elevations were generally transient, mild, and not accompanied by symptoms or jaundice [23]. No discontinuations due to hepatic abnormalities were reported, and no cases of clinically apparent liver injury have been attributed to tofersen [23].

11.7 Mortality and disease progression

In the VALOR trial and open-label extension combined, death or permanent ventilation occurred in 12 of 72 early-start participants (16.7 percent) compared with 8 of 36 delayed-start participants (22.2 percent); death alone occurred in 8 of 72 (11.1 percent) versus 6 of 36 (16.7 percent) [9]. These differences are numerically favor-

able to early tofersen initiation but do not reach statistical significance in the context of the study design and sample size. All deaths were attributed to underlying ALS disease progression [9, 10].

12. Comparative Assessment

This section presents a structured comparative assessment of five therapeutic agents in the ALS or motor neuron disease space against tofersen, evaluated against five competency standards: novelty, effect size, promising potential, side-effect profile, and overall validation. The five comparators were selected to represent the principal approved ALS therapies (riluzole, edaravone), a recently withdrawn ALS therapy (sodium phenylbutyrate-taurursodiol), a closely related antisense oligonucleotide for a different genetic ALS subtype (jacifusen for FUS-ALS), and the foundational intrathecal antisense oligonucleotide platform compound (nusinersen for spinal muscular atrophy).

12.1 Riluzole (Rilutek)

Mechanism: Riluzole is a benzothiazole that modulates glutamatergic neurotransmission through multiple pathways including preferential blockade of tetrodotoxin-sensitive sodium channels associated with damaged neurons, inhibition of presynaptic glutamate release, and direct antagonism at kainate and NMDA glutamate receptors [24, 25]. The mechanism is broadly neuroprotective rather than genotype-directed. No single-target K_i or IC_{50} is established as the therapeutically dominant interaction.

Development trajectory: Developed by Rhone-Poulenc Rorer (now Sanofi). Bensimon et al. (1994) reported the first controlled trial in 155 ALS patients showing a survival advantage at 100 mg daily [25]. Lacomblez et al. (1996) conducted the confirmatory dose-ranging study in 959 ALS patients [24]. FDA approved Rilutek on December 12, 1995, making riluzole the first medication approved for ALS. Generic versions have been available since 2013.

Clinical outcome: In the Lacomblez et al. (1996) dose-ranging study, tracheostomy-free survival at 18 months was 56.8 percent for the 100 mg group versus 50.4 percent for placebo, with a 35 percent reduced risk of death at 100 mg versus placebo ($p = 0.002$) [24]. Cochrane meta-analysis estimates approximately 2 to 3 months of extended median survival and a 9 percent absolute gain in one-year survival probability [24, 25]. Riluzole does not produce improvement in muscle strength, respiratory function, or ALSFRS-R score.

Novelty: 2/5. Riluzole was groundbreaking as the first ALS drug but represents a non-targeted, symptom-modifying approach with no disease-pathway specificity.

Effect size: 2/5. Approximately 2 to 3 months median survival extension with no functional improvement.

Promising potential: 2/5. Riluzole is mature, with limited upside. It remains standard-of-care background therapy in most ALS trials and clinical practice.

Side-effect profile: 3/5. Common adverse events include nausea, asthenia, and hepatic transaminase elevation requiring periodic liver function monitoring. Rare neutropenia and interstitial lung disease have been reported.

Overall validation: 4/5. Well validated by decades of use, multiple confirmatory studies, and inclusion in treatment guidelines worldwide.

Comparison to tofersen: Riluzole is a broad-spectrum oral glutamate modulator given to all ALS patients regardless of genotype. Tofersen is a genotype-specific intrathecal antisense oligonucleotide applicable only to the approximately 2 percent of ALS patients carrying SOD1 mutations. The mechanisms are complementary; approximately 62 percent of VALOR trial participants received concomitant riluzole [9, 10]. Riluzole extends survival by 2 to 3 months without addressing the genetic cause, whereas tofersen reduces the causative toxic protein by 29 to 40 percent in CSF and reduces plasma NfL by up to 60 percent [9, 10].

12.2 Edaravone (Radicava)

Mechanism: Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a free radical scavenger hypothesized to reduce oxidative-stress-mediated neuronal damage. The precise molecular target is not established; the FDA label states that the mechanism by which edaravone exerts therapeutic effects in ALS is unknown [26].

Development trajectory: Developed by Mitsubishi Tanabe Pharma. Originally approved in Japan for acute ischemic stroke. Three ALS clinical trials were conducted in Japan (Studies 16, 18, and 19). The pivotal Study 19 (MCI186-19; NCT01492686) used an enrichment design enrolling only early-stage, well-defined ALS patients. FDA approved Radicava (IV) in May 2017; oral suspension (Radicava ORS) approved May 2022 [26]. The European Medicines Agency rejected authorization in 2019 citing insufficient evidence of generalizability.

Clinical outcome: In the pivotal Study 19, 137 ALS patients meeting strict enrichment criteria (ALSFRS-R score of 4 on all respiratory items, FVC 80 percent or greater predicted, disease duration 2 years or less) were randomized. ALSFRS-R decline at 24 weeks was -5.01 points (edaravone) versus -7.50 points (placebo), a between-group difference of 2.49 points (95 percent CI, 0.99 to 3.98; $p = 0.001$), representing a 33 percent reduction in functional decline [26]. Only approximately 7 percent of screened ALS patients met the enrichment criteria, limiting generalizability.

Novelty: 2/5. Non-specific antioxidant approach without target specificity or biomarker engagement.

Effect size: 3/5. 2.49-point ALSFRS-R advantage over 24 weeks in a highly selected population. No demonstrated survival benefit.

Promising potential: 2/5. Limited generalizability, EMA rejection, mixed real-world results.

Side-effect profile: 3/5. Boxed warnings for hypersensitivity reactions (anaphylaxis) and sulfite sensitivity. IV administration requires 60-minute infusions over 10-14-day on-cycles followed by 14-day off-cycles, creating substantial treatment burden.

Overall validation: 2/5. One positive Phase 3 trial in a narrowly selected population. No confirmatory trial in a broader population.

Comparison to tofersen: Edaravone is a non-specific antioxidant for all ALS patients; tofersen is genotype-specific (SOD1), targeting the causative protein. Edaravone demonstrated a 2.49-point ALSFRS-R advantage over 24 weeks in a selected early-stage population; tofersen showed a 3.5-point ALSFRS-R advantage in the open-label extension (early-start versus delayed-start) and reduced the causative SOD1 protein and neurofilament biomarkers [9, 10, 26]. Tofersen addresses disease pathogenesis in a defined genetic subgroup; edaravone modulates a downstream consequence (oxidative damage) without target engagement biomarkers.

12.3 Sodium phenylbutyrate-taurursodiol (AMX0035, Relyvrio)

Mechanism: AMX0035 is a fixed-dose oral combination of sodium phenylbutyrate (an endoplasmic reticulum stress-reducing chemical chaperone) and taurursodiol (a mitochondrial membrane stabilizer that inhibits cytochrome c release) [27]. The combination targets dual apoptotic pathways without genotype specificity.

Development trajectory: Developed by Amylyx Pharmaceuticals. The Phase 2/3 CENTAUR trial (NCT03127514, n = 137) was published by Paganoni et al. (2020) [27]. FDA granted accelerated approval on September 29, 2022, under the brand name Relyvrio. The confirmatory Phase 3 PHOENIX trial (NCT05021536, n = 664) results announced March 2024 showed no statistically significant difference between AMX0035 and placebo on ALSFRS-R (p = 0.667) or any secondary endpoint. Amylyx voluntarily withdrew Relyvrio from the market effective October 31, 2024 [9, 13].

Clinical outcome: CENTAUR trial: ALSFRS-R decline was 1.24 points per month (AMX0035) versus 1.66 points per month (placebo), a difference of 0.42 points per month (95 percent CI, 0.03 to 0.81; p = 0.03), corresponding to a 2.32-point ALSFRS-R advantage over 24 weeks [27]. Long-term survival analysis suggested a 44 percent lower risk of death (approximately 6.5 months longer average survival) [27]. The PHOENIX Phase 3 trial showed no detectable effect, effectively invalidating the CENTAUR signal [9].

Novelty: 3/5. Dual anti-apoptotic mechanism concept, ultimately unvalidated.

Effect size: 1/5. Phase 3 null result. The CENTAUR signal was not confirmed.

Promising potential: 1/5. Drug withdrawn from market; ALS program discontinued.

Side-effect profile: 4/5. Generally well tolerated. Most common adverse events were diarrhea (approximately 24 percent), abdominal pain, and nausea.

Overall validation: 1/5. Phase 2 signal not confirmed by Phase 3. Represents a cautionary example of accelerated approval based on a single small trial.

Comparison to tofersen: AMX0035 was a broadly applicable oral combination for all ALS patients; tofersen is genotype-specific. AMX0035 demonstrated functional benefit in CENTAUR that did not replicate in PHOENIX, leading to market withdrawal. Tofersen, while also failing its primary ALSFRS-R endpoint in VALOR,

demonstrated robust biomarker engagement (SOD1 and NfL reduction) that provided a mechanistic rationale for ongoing clinical development and earned accelerated approval. The contrast illustrates the value of a mechanistic biomarker that confirms target engagement [9, 10, 27].

12.4 Jacifusen (ION363, ulefnersen)

Mechanism: Jacifusen is a 2'-O-methoxyethyl antisense oligonucleotide that binds FUS (fused in sarcoma) pre-mRNA and promotes its degradation via RNase H1-mediated cleavage, reducing the burden of toxic cytoplasmic FUS protein aggregates in FUS-ALS motor neurons [28]. In treated patients with recent jacifusen administration, FUS protein expression was 66 to 90 percent lower than in untreated FUS-ALS tissue, confirming robust target engagement [28].

Development trajectory: Developed by Ionis Pharmaceuticals. Named after Jaci Hermsstad, a patient with FUS-ALS. An investigator-initiated compassionate-use case series led by Neil Shneider (Columbia University) treated 12 patients via individual expanded-access INDs between June 2019 and June 2023, with results published in *The Lancet* in May 2025 [28]. The Phase 3 FUSION trial (NCT04768972) is ongoing.

Clinical outcome: In the 12-patient case series (median age 26 years; 58 percent female), serial intrathecal injections over 2.8 to 33.9 months demonstrated NfL reduction of up to 82.8 percent after 6 months and reduced FUS protein levels in CSF [28]. Most patients continued to decline functionally, but one patient demonstrated unprecedented objective functional recovery after 10 months of treatment [28]. This was an uncontrolled case series; no placebo comparator was available.

Novelty: 5/5. First ASO targeting FUS and the first genetically targeted therapy for FUS-ALS, the most common cause of juvenile-onset ALS.

Effect size: 3/5. Strong biomarker engagement (66 to 90 percent FUS reduction, up to 82.8 percent NfL reduction) but uncontrolled clinical data.

Promising potential: 4/5. Phase 3 ongoing; FUS-ALS is aggressive (median survival approximately 18 to 24 months in juvenile onset), so any disease modification would be clinically meaningful.

Side-effect profile: 3/5. Most common adverse events were back pain (50 percent), headache (33 percent), nausea (25 percent), and post-lumbar-puncture headache (25 percent). Transient CSF pleocytosis in 50 percent. Two deaths occurred during the study and were attributed to disease progression [28].

Overall validation: 2/5. Strong biomarker evidence from a small open-label case series; Phase 3 data required for definitive assessment.

Comparison to tofersen: Jacifusen and tofersen are the most directly comparable compounds in this analysis. Both are intrathecal 2'-MOE antisense oligonucleotides developed by Ionis Pharmaceuticals targeting gain-of-function mutations in ALS-causative genes (FUS and SOD1 respectively). Both demonstrate robust target protein knockdown (66 to 90 percent FUS reduction for jacifusen; 29 to 40 percent SOD1 reduction for tofersen) and NfL reductions (up to 82.8 percent for jacifusen; up to 60 percent for tofersen). Tofersen has reached FDA approval while jacifusen remains investigational [10, 28]. Together they validate the ASO platform approach for genetically defined ALS subtypes.

12.5 Nusinersen (Spinraza)

Mechanism: Nusinersen is a 2'-O-methoxyethyl phosphorothioate antisense oligonucleotide that modifies splicing of SMN2 pre-mRNA in spinal muscular atrophy (SMA) [29, 30]. It binds an intronic splicing silencer in SMN2 intron 7, promoting exon 7 inclusion and increasing production of full-length, functional SMN protein. This is a splice-switching mechanism (not RNase H-mediated degradation); the drug increases a deficient protein rather than reducing a toxic one [29].

Development trajectory: Co-developed by Ionis Pharmaceuticals and Biogen. The pivotal ENDEAR trial (NCT02193074) enrolled 121 infants with infantile-onset SMA; the CHERISH trial (NCT02292537) enrolled 126 children with later-onset SMA [29, 30]. FDA approved Spinraza on December 23, 2016, as the first treatment for SMA. The NURTURE trial (NCT02386553) evaluated presymptomatic treatment. A higher-dose regimen (28 mg) was approved in 2024 [29, 30].

Clinical outcome: In ENDEAR, 41 percent of nusinersen-treated infants achieved motor milestone response versus 0 percent in the sham group. The trial was stopped early at interim analysis due to overwhelming efficacy [29]. In CHERISH, 57

percent of treated children achieved 3 points or greater increase on HFMSE versus 26 percent of sham controls (least-squares mean difference, 5.9 points; $p < 0.001$) [30].

Novelty: 5/5. First ASO approved for any neurodegenerative or neuromuscular disease and the first treatment for SMA.

Effect size: 5/5. Large and clinically unambiguous (41 percent versus 0 percent motor milestone response; trial stopped early for efficacy).

Promising potential: 5/5. Established, with 8 years of real-world data confirming sustained benefit, particularly with presymptomatic treatment.

Side-effect profile: 3/5. Thrombocytopenia (16 percent developed platelet levels below LLN), renal toxicity (58 percent elevated urine protein versus 34 percent sham), and procedure-related lumbar puncture adverse events. Intrathecal access may require fluoroscopic guidance in patients with severe scoliosis [29, 30].

Overall validation: 5/5. Multiple positive RCTs, approved in over 50 countries, considered standard of care for SMA.

Comparison to tofersen: Nusinersen and tofersen share the Ionis Pharmaceuticals 2'-MOE phosphorothioate ASO platform and intrathecal delivery route, representing the two fundamental ASO strategies: splice switching (increasing a deficient protein) and RNase H-mediated degradation (reducing a toxic protein). Nusinersen achieved dramatic, unambiguous clinical benefit sufficient for standard approval; tofersen demonstrated robust biomarker engagement but did not meet its primary clinical endpoint, earning accelerated approval [10, 29, 30]. The SMA disease context (single genetic cause in more than 95 percent of cases, clear genotype-phenotype correlation, pediatric onset) is more amenable to precision therapy than SOD1-ALS (more than 200 variants, variable penetrance, adult onset, approximately 2 percent of all ALS). Nusinersen's success in establishing the feasibility, safety, and regulatory pathway for chronic intrathecal ASO administration was instrumental to tofersen's development [10, 29].

12.6 Comparator summary

COMPOUND	NOVELTY	EFFECT SIZE	PROMISING POTENTIAL	SIDE-EFFECT PROFILE	OVERALL VALIDATION
Riluzole	2	2	2	3	4
Edaravone	2	3	2	3	2
AMX0035	3	1	1	4	1
Jacifusen	5	3	4	3	2
Nusinersen	5	5	5	3	5

Tofersen occupies a distinctive position in this comparator landscape. It is the first gene-targeted therapy approved for any form of ALS, addressing a specific genetic cause through direct reduction of the pathogenic protein rather than modifying downstream symptoms or pathways (riluzole, edaravone) or general cellular stress (AMX0035). The approval was based on a biomarker surrogate (plasma NfL) rather than a clinical primary endpoint, a regulatory pathway validated by the tofersen precedent. Among the two ASO ALS therapies (tofersen and jacifusen), tofersen has the more advanced regulatory status but targets a slightly more common genetic subtype (SOD1, approximately 2 percent of ALS, versus FUS, approximately 1 percent). The nusinersen comparison illustrates both the potential of the intrathecal ASO platform (dramatic efficacy in SMA) and its current limitations in ALS (biomarker efficacy without primary clinical endpoint success in a 28-week trial). The ATLAS presymptomatic trial and ongoing open-label data will determine whether earlier intervention, analogous to the presymptomatic strategy that has produced the strongest nusinersen results, can deliver clinical outcomes that match the biomarker promise [10, 11, 29].

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