

RESEARCH MONOGRAPH . COMPOUND PROFILE

DNL343

A potent, selective, CNS-penetrant bicyclo[1.1.1]pentane-centered eIF2B activator developed by Denali Therapeutics for the suppression of aberrant integrated stress response signaling in neurodegenerative disease, advanced through Phase 2/3 clinical evaluation in amyotrophic lateral sclerosis.

DOCUMENT	KDC-MN-1574
ISSUED	2026-05-20
REVISION	1.0
AUTHOR	Kodiac bioLabs Research
COMPOUND CLASS	Small molecule eukaryotic initiation factor 2B (eIF2B) activator and integrated stress response (ISR) inhibitor

ABSTRACT

DNL343 is a first-in-class, orally bioavailable, brain-penetrant small molecule activator of eukaryotic initiation factor 2B (eIF2B), the guanine nucleotide exchange factor that governs the rate-limiting step in translation initiation and serves as the principal regulatory node of the integrated stress response (ISR). The compound was designed at Denali Therapeutics as an improvement over the prototype tool compound ISRIB, which despite landmark demonstrations of cognitive enhancement and neuroprotection in rodent models suffered from poor aqueous solubility, limited oral bioavailability, and pharmaceutical intractability [1, 2]. DNL343 retains the symmetric bis-glycolamide pharmacophore that stabilizes the eIF2B decameric complex at the subunit interface but replaces the central cyclohexane of ISRIB with a bicyclo[1.1.1]pentane (BCP) bioisostere, conferring improved solubility, metabolic stability, and CNS penetration [3]. The compound displays an IC₅₀ of 3.2 nanomolar in a cellular ATF4 reporter assay, a brain-to-plasma unbound concentration ratio of approximately 0.8 in rat, and a plasma elimination half-life of 31 to 46 hours in healthy human subjects, supporting once-daily oral dosing [3, 4, 5].

Preclinically, DNL343 produced dose-dependent neuroprotection in an optic nerve crush model of acute retinal ganglion cell degeneration and, in the Eif2b5 R191H knock-in mouse model of vanishing white matter disease (a genetic ISR-driven leukoencephalopathy), restored body weight, normalized motor function, reversed ISR transcriptional signatures, normalized plasma neurofilament light chain (NfL), and extended survival

from 25 percent to 84.6 percent of treated animals when administered therapeutically at advanced disease stages [1]. In inducible TDP-43 proteinopathy mouse models relevant to amyotrophic lateral sclerosis (ALS), DNL343 attenuated ISR activation and reduced markers of neurodegeneration [6].

Clinical development proceeded through a Phase 1 trial in 95 healthy volunteers (single ascending doses of 15 to 800 mg; multiple ascending doses of 45 to 260 mg daily for 14 days), which demonstrated dose-proportional pharmacokinetics, cerebrospinal fluid (CSF) to unbound plasma ratios of 0.66 to 0.92, robust suppression of ISR biomarkers (ATF4 protein, CHAC1 transcript) in peripheral blood mononuclear cells, and a favorable tolerability profile with no serious adverse events [4, 5]. A Phase 1b study in 28 participants with ALS (100 and 200 mg daily for 28 days, followed by an 18-month open-label extension) confirmed CNS penetration with CSF-to-unbound-plasma ratios of 1.02 to 1.23, pharmacodynamic target engagement, and acceptable safety, with headache and fatigue as the most common treatment-emergent adverse events [7, 8]. The compound was subsequently advanced to Regimen G of the Phase 2/3 HEALEY ALS Platform Trial, a 24-week randomized, placebo-controlled study enrolling 186 participants on DNL343 and 139 on placebo. In January 2025, Denali Therapeutics announced that the trial did not meet its primary endpoint of slowing disease progression as measured by the ALS Functional Rating Scale-Revised (ALSFRS-R) and survival, and key secondary endpoints including muscle strength and respiratory function showed no statistical separation from placebo [9, 10]. A subsequent SEC filing revealed that DNL343 did not alter NfL biomarker levels over the treatment period [10]. The compound is no longer in active clinical development for ALS.

This monograph reviews the chemistry and design rationale of DNL343; the molecular pharmacology of eIF2B activation and ISR suppression; the comprehensive preclinical neuroprotection evidence; human pharmacokinetics across Phase 1 and Phase 1b studies; the clinical evidence base in ALS; sourcing, reconstitution, and handling considerations for research applications; stack interactions; the adverse-event profile; and a structured comparative assessment of five ISR-modulating agents (ISRIB, 2BAct, fosigotifator/ABBV-CLS-7262, IFB-088/Sephin1, and trazodone) against DNL343 on five competency standards. The compound is not approved by any regulatory authority for any indication. It is available as a research-grade preparation from multiple chemical suppliers; investigators should confirm identity and purity on every lot.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Development code: DNL343 (Denali Therapeutics).

Chemical Abstracts Service registry number: 2278265-85-1.

International Union of Pure and Applied Chemistry name: 2-(4-chlorophenoxy)-N-(3-(5-((1s,3s)-3-(trifluoromethoxy)cyclobutyl)-1,3,4-oxadiazol-2-yl)bicyclo[1.1.1]pentan-1-yl)acetamide.

Other designations: The compound has no international nonproprietary name, no marketed trade name, and no generic pharmaceutical identity. It is referenced exclusively by the development code DNL343 in the clinical, patent, and research literature. Some chemical suppliers list the compound under the CAS number or as "eIF2B activator DNL343."

1.2 Structural class and chemistry

DNL343 is a synthetic small molecule belonging to the bis-glycolamide class of eIF2B activators, a structural series that originated with the discovery of ISRIB (integrated stress response inhibitor) by the Walter laboratory at the University of California, San Francisco [2]. The compound is distinguished from ISRIB by replacement of the central cyclohexane linker with a bicyclo[1.1.1]pentane (BCP) bioisostere, a rigid three-dimensional scaffold increasingly employed in medicinal chemistry for its ability to improve aqueous solubility, metabolic stability, and membrane permeability relative to phenyl and cyclohexyl replacements [3, 11].

Molecular formula: C₂₀H₁₉ClF₃N₃O₄.

Molecular weight: 457.83 g/mol.

Key structural features: The molecule comprises four pharmacophoric elements connected through the central BCP scaffold: (a) a 4-chlorophenoxy ether connected through an acetamide linkage to one bridgehead of the BCP; (b) a 1,3,4-oxadiazole

heterocycle at the other bridgehead; (c) a cis-3-(trifluoromethoxy)cyclobutyl substituent on the oxadiazole; and (d) the BCP itself, which provides the three-dimensional geometry required for binding at the eIF2B decamer subunit interface.

Physical properties: White to off-white crystalline solid. The compound is expected to have moderate aqueous solubility relative to ISRIB, consistent with the BCP bioisosteric replacement strategy [3]. Detailed published solubility values in standard vehicles (DMSO, PEG-400, aqueous buffers) are available from chemical suppliers.

Stereochemistry: DNL343 contains a defined cis-relationship on the cyclobutyl ring (the 1s,3s configuration), specifying the relative orientation of the trifluoromethoxy substituent. The BCP core does not introduce conventional chirality but constrains the molecular geometry to optimize binding at the eIF2B interface. Investigators should confirm the stereochemical assignment on research-grade material through nuclear magnetic resonance spectroscopy or chiral chromatographic methods where applicable.

1.3 Synthesis

The detailed medicinal chemistry and structure-activity relationship (SAR) campaign leading to the discovery of DNL343 was published by Denali Therapeutics investigators in the Journal of Medicinal Chemistry in 2024 [3]. The synthesis proceeds through construction of the BCP-centered scaffold, elaboration of the 1,3,4-oxadiazole heterocycle bearing the cis-3-(trifluoromethoxy)cyclobutyl group, and final coupling of the 4-chlorophenoxyacetamide moiety to the BCP bridgehead nitrogen. The SAR campaign explored modifications at each pharmacophoric position; DNL343 emerged as the clinical candidate on the basis of potency (IC₅₀ 3.2 nM in the cellular ATF4 reporter assay), selectivity (no activation of pregnane X receptor (PXR), constitutive androstane receptor (CAR), or aryl hydrocarbon receptor (AhR); no significant activity against CEREP or kinase selectivity panels), and a pharmacokinetic profile featuring high oral bioavailability, long half-life, and brain-to-plasma unbound concentration ratios approaching unity across preclinical species [3].

2. Abstract

DNL343 is a potent, selective, orally bioavailable, and brain-penetrant activator of eukaryotic initiation factor 2B (eIF2B), designed to suppress aberrant activation of the integrated stress response (ISR) in the central nervous system [1, 3]. The integrated stress response is a conserved eukaryotic signaling pathway activated by four kinases (HRI, PKR, PERK, GCN2) that converge on phosphorylation of eIF2- α at serine 51, converting eIF2 from an eIF2B substrate to an eIF2B inhibitor and thereby suppressing global protein synthesis while selectively upregulating stress-responsive transcripts including ATF4. Under physiological conditions, ISR activation is transient and protective. In neurodegeneration, however, chronic or excessive ISR activation driven by proteotoxic stress (TDP-43 and tau aggregation), endoplasmic reticulum stress, or loss-of-function mutations in eIF2B subunits (as in vanishing white matter disease) produces sustained translational suppression, synaptic dysfunction, and cell death [1, 12].

DNL343 binds at the interface of the eIF2B decameric complex, stabilizing the active holoenzyme and promoting its guanine nucleotide exchange factor (GEF) activity even in the presence of inhibitory phosphorylated eIF2 [1, 3]. This mechanism is shared with the prototype tool compound ISRIB but DNL343 represents a substantial pharmacological improvement: greater potency (IC₅₀ 3.2 nM versus approximately 5 nM for ISRIB in comparable cellular assays), dramatically improved oral bioavailability and aqueous solubility through the BCP bioisosteric replacement, and a pharmacokinetic profile (half-life 31 to 46 hours in humans, brain-to-plasma unbound ratio approximately 0.8) supporting once-daily oral dosing for chronic neurodegenerative indications [1, 3, 4].

Preclinical validation spanned acute injury models (optic nerve crush, demonstrating dose-dependent retinal ganglion cell protection), chronic genetic models (Eif2b5 R191H knock-in mice, demonstrating restoration of body weight, motor function, and survival), and disease-relevant proteinopathy models (inducible TDP-43 pathology, demonstrating ISR suppression and reduction of neurodegeneration markers) [1, 6]. Clinical development in healthy volunteers and participants with ALS confirmed the predicted pharmacokinetic profile, pharmacodynamic target engagement (suppression of ATF4 and CHAC1), and acceptable tolerability through

Phase 1b [4, 5, 7, 8]. The Phase 2/3 HEALEY ALS Platform Trial, however, did not meet its primary or key secondary endpoints, and the compound is no longer in active development for ALS [9, 10].

3. Discovery and Development History

3.1 Origin of eIF2B as a therapeutic target

The rationale for pharmacological activation of eIF2B in neurodegeneration derives from two convergent lines of evidence. The first is the genetic validation of eIF2B loss-of-function as a cause of neurodegeneration: hypomorphic mutations in any of the five eIF2B subunit genes (EIF2B1 through EIF2B5) cause vanishing white matter disease (VWMD, also designated childhood ataxia with central nervous system hypomyelination, CACH), a progressive leukoencephalopathy characterized by chronic ISR activation, oligodendrocyte and astrocyte dysfunction, and progressive neurological deterioration [12, 13]. The second is the demonstration by the Walter, Bhatt, and Bhatt-Bhatt laboratories that small molecule stabilization of the eIF2B decamer could override the inhibitory effect of phosphorylated eIF2 and restore translational homeostasis in cellular and animal models of ISR-driven pathology [2, 14].

3.2 ISRIB and the first-generation eIF2B activators

ISRIB (trans-isomer) was identified in 2013 by Sidrauski, Tsai, and Walter through a phenotypic screen for compounds that restore translation in cells treated with ISR-activating stressors [2]. Structural and biophysical studies subsequently revealed that ISRIB binds symmetrically at the interface between the two beta-delta subcomplexes of the eIF2B decamer, stabilizing the holoenzyme in its catalytically active conformation [14, 15]. ISRIB produced remarkable cognitive enhancement in aged mice, rescued memory deficits in traumatic brain injury models, and reversed ISR-dependent neurodegeneration in genetic models [2, 16]. However, the compound suffered from poor aqueous solubility (less than 1 microgram per milliliter), limited oral bioavailability in rodents, and pharmaceutical intractability that precluded clinical development [1, 3].

3.3 2BAct and the BCP scaffold

The Bhatt laboratory at Calico Life Sciences addressed the ISRIB solubility problem by replacing the central cyclohexane with a bicyclo[1.1.1]pentane (BCP) linker, producing 2BAct, an asymmetric BCP-centered eIF2B activator with improved solubility, pharmacokinetics, and CNS exposure that enabled aqueous-vehicle dosing and chronic in vivo administration [17]. 2BAct demonstrated neuroprotection in the Eif2b5 R191H mouse model. However, the compound developed a cardiovascular liability in dogs (mechanism not fully disclosed), halting its advancement toward clinical development [1, 17].

3.4 Discovery of DNL343

Denali Therapeutics initiated an independent medicinal chemistry campaign to identify a clinical-grade eIF2B activator, building on the published ISRIB and 2BAct structural pharmacology but with distinct chemical matter [3]. The campaign explored systematic modifications to the BCP-centered scaffold, varying the heterocyclic linker (1,3,4-oxadiazole replacing amide or triazole connections), the aryloxy substituent (4-chlorophenoxy selected for potency and metabolic stability), and the terminal cycloalkyl group (cis-3-(trifluoromethoxy)cyclobutyl optimized for potency and selectivity). DNL343 emerged as the development candidate in approximately 2019 on the basis of cellular potency (IC₅₀ 3.2 nM), selectivity (clean CEREP, kinase, nuclear receptor panels), preclinical pharmacokinetics (long half-life, high oral bioavailability, brain-to-plasma unbound ratio of 0.8 in rat), and absence of the cardiovascular signal that terminated 2BAct [3].

3.5 Clinical development timeline

Phase 1 in healthy volunteers (NCT04268784) was initiated in February 2020 and completed by August 2021 [4, 5]. Phase 1b in participants with ALS (NCT05006352) began dosing in August 2021, with interim data presented at the American Academy of Neurology annual meeting in April 2023 [7, 8]. The compound entered Regimen G of the HEALEY ALS Platform Trial (NCT05842941), a Phase 2/3 adaptive trial, in May 2023, with enrollment completing in May 2024 [9]. Topline results were announced on January 6, 2025: the trial did not meet its primary endpoint [9, 10]. A Phase 1 absorption, metabolism, and excretion study of [14C]-DNL343 (NCT06281158) was conducted in healthy male volunteers in early 2024. Following the HEALEY trial failure, the ALS program was discontinued.

4. Mechanism of Action

4.1 The integrated stress response pathway

The integrated stress response is a conserved eukaryotic signaling network that couples diverse cellular stressors to a common translational output [12, 18]. Four stress-sensing kinases, each responsive to distinct upstream signals, converge on a single phosphorylation event: phosphorylation of the alpha subunit of eukaryotic initiation factor 2 (eIF2- α) at serine 51. The kinases are heme-regulated inhibitor kinase (HRI, activated by heme deficiency, mitochondrial stress, and proteotoxic stress); double-stranded RNA-activated protein kinase (PKR, activated by viral infection); PKR-like endoplasmic reticulum kinase (PERK, activated by ER stress and the unfolded protein response); and general control nonderepressible 2 (GCN2, activated by amino acid deprivation and UV irradiation) [18].

Phosphorylated eIF2- α binds eIF2B with approximately 100-fold higher affinity than unphosphorylated eIF2, converting eIF2 from an eIF2B substrate to a competitive inhibitor. The consequent reduction in eIF2B GEF activity suppresses global cap-dependent translation while paradoxically increasing translation of mRNAs bearing upstream open reading frames in their 5' untranslated regions, including the transcription factor ATF4. ATF4 drives a transcriptional program that includes genes involved in amino acid biosynthesis (ASNS), redox homeostasis (CHAC1), and apoptosis (CHOP/DDIT3, GADD34) [12, 18].

4.2 eIF2B structure and activation by DNL343

eIF2B is a decameric complex composed of two copies each of five subunits (alpha through epsilon), organized as a central regulatory tetramer (two alpha and two delta subunits at the core) flanked by two catalytic subcomplexes (each containing one beta, one gamma, and one epsilon subunit) [14, 15]. The GEF catalytic activity resides in the epsilon subunit carboxy-terminal domain. The decameric assembly is required for full catalytic activity; subcomplexes lacking the stabilizing subunit contacts have reduced GEF function.

DNL343 binds at the symmetric interface between the two beta-delta subcomplexes of the decamer, at a site that overlaps with the ISRIB binding pocket as defined by cryo-electron microscopy [1, 3, 14, 15]. By stabilizing the decameric assembly, DNL343 promotes eIF2B GEF activity and opposes the inhibitory effect of phosphorylated eIF2, effectively raising the threshold of ISR kinase activity required to suppress translation. The net pharmacological effect is a reduction in ATF4 protein levels and ATF4-driven transcriptional output (CHAC1, ASNS, CHOP) in cells and tissues where ISR is pathologically elevated, without abolishing the capacity for acute ISR activation in response to severe stressors [1].

4.3 Selectivity and off-target profile

DNL343 displays high selectivity for eIF2B over other molecular targets. In the Denali medicinal chemistry characterization, the compound showed no activation of PXR, CAR, or AhR nuclear receptors; no significant activity across the CEREP panel of approximately 70 receptors, ion channels, and transporters; and no significant inhibition across a kinase selectivity panel [3]. The selectivity is mechanistically consistent with the specificity of the binding site, which is unique to the eIF2B decameric interface and does not recur in other multisubunit complexes. The absence of hERG channel inhibition was assessed during preclinical safety pharmacology (specific IC₅₀ values not published in the J. Med. Chem. report but implied by the advancement to human clinical trials) [3].

4.4 Pharmacodynamic biomarkers

The principal pharmacodynamic biomarkers for DNL343 target engagement are ATF4 protein levels and CHAC1 transcript levels in peripheral blood mononuclear cells (PBMCs) and in cerebrospinal fluid. Both are direct transcriptional outputs of the ISR pathway that are suppressed by eIF2B activation. Growth-differentiation factor 15 (GDF-15), a circulating protein elevated in ISR-driven conditions including vanishing white matter disease, has been identified as an additional translational biomarker [1, 4]. Neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) serve as downstream markers of neurodegeneration and neuroinflammation, respectively, and were employed as exploratory endpoints in the preclinical and clinical programs [1, 7, 10].

5. Pharmacokinetics and Routes of Administration

5.1 Preclinical pharmacokinetics

DNL343 demonstrated a desirable pharmacokinetic profile across preclinical species, with a long half-life and high oral bioavailability [1, 3]. In rat, single oral dose administration produced brain unbound concentrations similar to plasma unbound concentrations, with a brain-to-plasma unbound ratio (Bu/Pu) of approximately 0.8, confirming the CNS-penetrant design [3]. In the mouse eIF2B loss-of-function model, chronic dietary administration at 50, 150, and 500 mg/kg chow produced steady-state conditions rapidly, with brain unbound concentrations comparable to plasma across all dose levels and no adverse events or body weight effects over 5 weeks of dosing [1]. In the optic nerve crush study, oral gavage at 0.75, 3, and 12 mg/kg produced dose-proportionate exposure with low inter-animal variability [1].

5.2 Phase 1 human pharmacokinetics

The Phase 1 study (NCT04268784) enrolled 95 healthy participants in single ascending dose (SAD; n = 48; doses 15 to 800 mg) and multiple ascending dose (MAD; n = 47; doses 45 to 260 mg daily for 14 days) cohorts [4, 5]. Key pharmacokinetic findings:

Plasma concentrations were dose-dependent and dose-proportional across the tested range. The plasma elimination half-life was 31 to 46 hours (with central estimates of 38 to 46 hours in some cohorts), supporting once-daily oral dosing [4, 5]. Cerebrospinal fluid sampling at steady state demonstrated CSF-to-unbound-plasma concentration ratios of 0.66 to 0.92, confirming extensive CNS distribution in healthy human subjects [4, 5]. The pharmacokinetic profile was consistent across the SAD and MAD cohorts, with no evidence of time-dependent accumulation beyond that predicted by the half-life.

5.3 Phase 1b pharmacokinetics in ALS

The Phase 1b study (NCT05006352) evaluated 100 mg and 200 mg daily doses in 28 participants with ALS for 28 days [7, 8]. CSF sampling demonstrated CSF-to-unbound-plasma concentration ratios of 1.02 to 1.23, modestly higher than in healthy volunteers, possibly reflecting altered blood-brain barrier permeability in ALS or methodological differences in sampling timing [7, 8]. The data confirmed that DNL343 achieved concentrations in the central nervous system sufficient for pharmacodynamic activity at both dose levels.

5.4 Absorption

DNL343 is administered orally, either as a capsule or via feeding tube in participants with bulbar-onset ALS. The compound is well absorbed from the gastrointestinal tract, with the long plasma half-life and dose-proportional kinetics suggesting complete or near-complete oral absorption. A dedicated absorption, metabolism, and excretion study using radiolabeled [14C]-DNL343 (NCT06281158) was conducted in healthy male volunteers in early 2024; detailed results have not been published in a peer-reviewed forum as of monograph preparation [10].

5.5 Distribution

The volume of distribution has not been reported in published sources. The high CSF-to-unbound-plasma ratios (approaching or exceeding unity) indicate that DNL343 distributes freely into the central nervous system, consistent with the BCP scaffold design and the absence of strong P-glycoprotein efflux [1, 3]. Protein binding values have not been published in detail but the use of unbound plasma concentrations for CNS penetration ratios implies that protein binding was characterized during preclinical development.

5.6 Metabolism and elimination

Metabolic pathways have not been described in published literature in detail. The radiolabeled AME study (NCT06281158) will characterize the metabolic fate and excretion routes. The long half-life (31 to 46 hours) and the absence of reported CYP induction or inhibition signals suggest that the compound is cleared slowly, likely through hepatic metabolism, with the BCP and oxadiazole moieties conferring metabolic stability [3].

5.7 Routes of administration

DNL343 is administered exclusively by the oral route in all published studies. In clinical trials in ALS participants, administration via nasogastric or percutaneous endoscopic gastrostomy tube was permitted for participants with bulbar dysfunction [7, 9].

6. Preclinical Pharmacology

6.1 Optic nerve crush model

The optic nerve crush (ONC) model is an acute injury paradigm in which unilateral crush of the optic nerve produces retrograde degeneration of retinal ganglion cells (RGCs) over 7 to 14 days, accompanied by robust ISR activation in the injured retina [1]. DNL343 was administered by oral gavage at 0.75, 3, and 12 mg/kg once daily beginning on the day of crush. At 3 and 12 mg/kg, DNL343 produced significant, dose-dependent protection of RGCs at 14 days, with concurrent dose-dependent suppression of ISR transcript markers (ATF4-regulated genes) in the retina [1]. The neuroprotective effect of DNL343 was superior to that of ISRIB in the same model, plausibly attributable to greater unbound brain exposure, improved cellular potency, or both [1].

6.2 eIF2B loss-of-function model (preventive paradigm)

The Eif2b5 R191H knock-in mouse carries a homozygous missense mutation in the epsilon subunit of eIF2B, recapitulating the genetic basis of vanishing white matter disease [1, 13]. These mice develop progressive body weight loss, motor dysfunction, ISR transcriptional activation, elevated plasma NfL, and premature mortality. DNL343 was administered in food at 0.3, 1, 3, and 10 mg/kg beginning at weaning (before disease onset). At 3 and 10 mg/kg, 13 weeks of treatment restored body weight to wild-type levels, normalized motor function scores, suppressed ISR transcripts in brain and spinal cord in a dose-dependent manner, and normalized plasma NfL to levels indistinguishable from wild-type littermates [1].

6.3 eIF2B loss-of-function model (therapeutic paradigm)

In a therapeutically relevant paradigm, DNL343 at 10 mg/kg (100 mg/kg chow) was initiated at a late disease stage when Eif2b5 R191H mice had already developed overt neurological signs [1]. After 4 weeks of treatment, body weight was restored and ISR transcripts were reversed. After 20 weeks, plasma NfL and GFAP were normalized. Survival was extended from 25 percent in untreated animals to 84.6 per-

cent in DNL343-treated animals at the study endpoint [1]. These data demonstrated that eIF2B activation could reverse, not merely prevent, established ISR-driven neurodegeneration.

6.4 TDP-43 proteinopathy models

TDP-43 (TAR DNA-binding protein 43) cytoplasmic mislocalization and aggregation is the hallmark neuropathological feature of approximately 97 percent of ALS cases and approximately 45 percent of frontotemporal dementia cases [6, 19]. The ISR is activated downstream of TDP-43 pathology, providing the mechanistic link between TDP-43 proteinopathy and eIF2B activation as a therapeutic strategy. In inducible TDP-43 transgenic mouse models, DNL343 attenuated ISR activation in the brain and reduced markers of neurodegeneration, as reported in a 2025 Nature Communications publication that presented integrated preclinical and clinical data [6]. The preclinical data in TDP-43 models provided the direct translational rationale for clinical evaluation of DNL343 in ALS.

6.5 Biomarker translation

Three biomarkers identified in the preclinical models, GDF-15, GFAP, and NfL, were shown to be differentially elevated in plasma and CSF from patients with vanishing white matter disease and were adopted as translational pharmacodynamic and neurodegeneration endpoints in the clinical program [1, 7]. ATF4 protein and CHAC1 transcript in PBMCs served as proximal ISR-engagement biomarkers accessible from peripheral blood sampling [4, 5, 7].

7. Clinical Evidence Base

7.1 Phase 1 healthy volunteer study

The Phase 1 study (NCT04268784), completed between February 2020 and August 2021, enrolled 95 healthy adult participants randomized to DNL343 or placebo across single ascending dose (SAD) and multiple ascending dose (MAD) cohorts [4, 5]. SAD cohorts evaluated doses of 15, 45, 100, 200, 400, and 800 mg. MAD cohorts evaluated 45, 100, 180, and 260 mg daily for 14 days.

Treatment-emergent adverse events occurred at similar frequencies in DNL343-treated participants (64 to 91 percent across dose cohorts) and placebo-treated participants (67 to 92 percent), with the majority classified as mild (Grade 1) [4, 5]. No serious adverse events, severe adverse events, or discontinuations related to study drug were reported. Pharmacodynamic analysis of PBMCs demonstrated dose-dependent suppression of ATF4 protein levels and CHAC1 transcript levels, confirming ISR target engagement at doses producing sustained plasma concentrations above the cellular IC50 [4, 5].

Results were presented at the American Academy of Neurology annual meeting in April 2023, where investigators concluded that DNL343 was generally safe and well tolerated at doses demonstrating robust ISR inhibition, with a pharmacokinetic profile supporting once-daily dosing [5].

7.2 Phase 1b study in ALS

The Phase 1b study (NCT05006352), initiated in August 2021, was a multicenter, randomized, placebo-controlled, double-blind trial of 28 days followed by an 18-month open-label extension [7, 8]. Twenty-eight participants with ALS were randomized to low dose (100 mg), high dose (200 mg), or placebo. Administration was oral or via feeding tube.

Safety: DNL343 was generally well tolerated. No deaths, serious adverse events, or severe adverse events occurred during the 28-day double-blind period. All treatment-emergent adverse events were Grade 1 or Grade 2 in severity. The overall frequency of adverse events was 74 percent in DNL343-treated participants versus 89

percent in placebo-treated participants [7, 8]. The most common treatment-emergent adverse events more frequent in the DNL343 group than placebo were headache (37 percent versus 22 percent), fatigue (32 percent versus 22 percent), and hypogeusia (11 percent versus 0 percent). One participant in the 200 mg group developed a Grade 2 rash [7, 8].

Pharmacokinetics: CSF-to-unbound-plasma concentration ratios of 1.02 to 1.23 confirmed extensive CNS penetration in ALS participants, consistent with or slightly exceeding the Phase 1 healthy volunteer data [7, 8].

Pharmacodynamics: ISR biomarkers (ATF4 and CHAC1) were attenuated in PBMCs and in CSF, confirming central and peripheral target engagement [7, 8].

Interim data from the open-label extension, presented in December 2022, showed that once-daily dosing was generally well tolerated over the extended dosing period, with no new safety signals emerging [8].

7.3 Phase 2/3 HEALEY ALS Platform Trial (Regimen G)

DNL343 was evaluated as Regimen G of the HEALEY ALS Platform Trial (NCT05842941), a multi-regimen, adaptive, randomized, placebo-controlled Phase 2/3 study designed to evaluate multiple investigational therapies for ALS simultaneously [9, 10]. The study enrolled 186 participants randomized to daily DNL343 and 139 participants randomized to placebo (shared placebo arm), with the primary endpoint of slowing disease progression measured by the ALSFRS-R total score and survival through 24 weeks.

On January 6, 2025, Denali Therapeutics announced that the trial did not meet its primary endpoint [9]. Key secondary endpoints measuring muscle strength (hand-held dynamometry) and respiratory function (slow vital capacity) showed no statistical difference between treatment and placebo groups [9, 10]. The compound was safe and well tolerated in the trial, consistent with the earlier Phase 1 and Phase 1b data. A March 2025 SEC filing disclosed that DNL343 did not alter NfL levels in either the 24-week treatment period or the 7-month active treatment extension, suggesting that the compound did not modify the rate of axonal degeneration despite achieving pharmacokinetic exposures consistent with ISR target engagement [10].

Further analyses, including pre-specified subgroup evaluations and extended open-label extension data, were anticipated in 2025 [9].

7.4 Interpretation

The negative HEALEY trial result raises fundamental questions about whether ISR suppression alone is sufficient to modify ALS disease progression, whether the ISR is a primary driver of motor neuron degeneration or a downstream consequence that is pathologically significant only in specific ALS subtypes, and whether the 24-week treatment duration and the ALSFRS-R endpoint are appropriate to detect the potential neuroprotective effect of eIF2B activation. The robust preclinical evidence in genetic and injury models that are driven by ISR activation (eIF2B LOF mice, optic nerve crush) contrasted with the absence of clinical benefit in a sporadic ALS population suggests that ISR-driven neurodegeneration may represent a relevant pathogenic mechanism in specific genetic or biomarker-defined subpopulations rather than in the broad ALS population.

8. Sourcing and Quality Verification

DNL343 is not a registered pharmaceutical in any jurisdiction. The compound is available exclusively as a research-grade preparation.

Research-grade DNL343 is supplied by multiple chemical and pharmaceutical research suppliers, including Cayman Chemical (catalog number 41674), MedChemExpress, ChemScene, TargetMol, AbMole BioScience, Molnova, and others, at stated purities of greater than 98 percent by high-performance liquid chromatography. Supply is in milligram quantities as the free base; the compound may also be available as custom salt forms from some suppliers.

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

- High-performance liquid chromatography area-percent purity (target greater than 98 percent)
- Mass spectrometry confirmation of molecular weight (expected $[M+H]^+$ at 458.1 for $C_{20}H_{19}ClF_3N_3O_4$)
- Nuclear magnetic resonance spectroscopy (1H and ^{19}F NMR) to confirm the BCP scaffold, oxadiazole heterocycle, trifluoromethoxy group, and chlorophenoxy moiety
- Assessment of residual solvents by gas chromatography
- Karl Fischer or loss-on-drying analysis for residual water content

Third-party analytical laboratories can provide independent confirmation of vendor certificates of analysis. Given the relatively recent introduction of DNL343 to the research reagent market and the structural complexity of the BCP scaffold, on-receipt analytical verification is particularly important for mechanistic studies where dose-response precision is critical.

9. Reconstitution and Handling

DNL343 is supplied as a crystalline solid. For in vitro research applications, stock solutions are typically prepared in dimethyl sulfoxide (DMSO) at concentrations of 10 to 50 millimolar, consistent with the moderate aqueous solubility profile. Working solutions for cell-based assays are prepared by serial dilution into culture medium; the final DMSO concentration should be maintained below 0.1 to 0.5 percent to avoid solvent-mediated cellular stress that could confound ISR measurements.

For in vivo research in rodent models, DNL343 has been administered by oral gavage in standard vehicles (methylcellulose, PEG-400, or similar) and by dietary admixture in chow at concentrations of 50 to 500 mg/kg chow, as described in the published preclinical studies [1]. Dietary administration achieved steady-state conditions rapidly and was well tolerated over 5 to 20 weeks of chronic dosing without body weight effects or overt toxicity in wild-type mice [1].

Storage of the solid should be at minus 20 degrees Celsius or colder, protected from moisture and light. DMSO stock solutions should be aliquoted for single use and stored at minus 20 degrees Celsius; stability under these conditions is expected to be at least 6 months based on standard practices for oxadiazole-containing small molecules, though specific stability data for DNL343 have not been published. Repeated freeze-thaw cycles should be avoided.

Investigators should note that DNL343 is an ISR inhibitor; handling in cell culture should account for the potential of the compound to suppress stress responses induced by routine culture conditions (serum deprivation, passage stress, mycoplasma contamination). Appropriate positive controls (tunicamycin, thapsigargin, or sodium arsenite for ISR activation) and negative controls (vehicle alone) should be included in all cell-based experiments.

10. Stack Interaction Considerations

DNL343 interactions with other research compounds and therapeutic agents should be considered in the context of its eIF2B-activating mechanism, its long half-life, and its potential metabolic interactions.

ISR-activating compounds: Concurrent administration of ISR activators (tunicamycin, thapsigargin, sodium arsenite in vitro; or clinically, proteasome inhibitors such as bortezomib that activate the UPR/PERK arm) will produce pharmacological opposition. DNL343 will raise the ISR activation threshold but will not abolish the response to maximal stressors; the interaction is competitive and dose-dependent. Investigators using DNL343 to characterize ISR-dependent phenotypes should calibrate the stressor concentration against the DNL343 dose.

Other eIF2B activators: Concurrent use with ISRIB, 2BAct, or fosigotifator (ABBV-CLS-7262) is pharmacologically redundant at the same binding site and is not expected to produce additive efficacy. The combination is relevant only for binding competition studies or for tool-compound comparisons.

ISR prolongers (Sephin1/IFB-088, guanabenz): Sephin1 and guanabenz prolong ISR activation by inhibiting the GADD34/PP1c phosphatase complex that dephosphorylates eIF2- α , producing a directionally opposite pharmacological effect to DNL343. Concurrent administration would produce partial pharmacological cancellation: DNL343 would promote eIF2B activity while Sephin1 would sustain the eIF2- α phosphorylation that inhibits eIF2B. The net effect would depend on relative doses and on the magnitude of ISR activation in the experimental system.

CYP substrates and inhibitors: Detailed CYP interaction data for DNL343 have not been published. The absence of PXR, CAR, and AhR activation in the Denali selectivity panel suggests that the compound is unlikely to be a significant inducer of CYP1A2, CYP2B6, or CYP3A4 [3]. Investigators combining DNL343 with CYP-metabolized research compounds should monitor for potential inhibition or induction interactions, particularly given the long half-life and sustained plasma concentrations.

Protein synthesis modulators: Concurrent administration with other translational regulators (rapamycin/mTOR inhibitors, cycloheximide, anisomycin) will produce complex interactions on global protein synthesis. The combination of eIF2B activation (promoting translation initiation) with mTOR inhibition (suppressing cap-dependent translation at a different node) is mechanistically informative but requires careful dose-response characterization.

Neurotrophic and neuroprotective agents: Combinations with riluzole (the standard-of-care ALS therapy, which modulates glutamate signaling) and edaravone (a free radical scavenger) were permitted in the ALS clinical trials. No pharmacokinetic or pharmacodynamic interactions with riluzole or edaravone were reported [7, 9].

11. Adverse Events and Safety

11.1 Phase 1 healthy volunteers

Treatment-emergent adverse events occurred at similar frequencies in DNL343-treated (64 to 91 percent across dose cohorts) and placebo-treated (67 to 92 percent) participants [4, 5]. Most events were Grade 1 (mild). No serious adverse events, severe adverse events, or study drug-related discontinuations were reported. The adverse event profile did not show a clear dose-dependent pattern within the tested range (15 to 800 mg single dose; 45 to 260 mg daily for 14 days). Specific adverse event types at elevated frequency in the DNL343 group relative to placebo were not individually reported in the available conference abstracts.

11.2 Phase 1b ALS participants

All treatment-emergent adverse events were Grade 1 or Grade 2 [7, 8]. The most common events more frequent in DNL343-treated participants than placebo were:

- Headache: 37 percent versus 22 percent (placebo)
- Fatigue: 32 percent versus 22 percent
- Hypogeusia (reduced taste perception): 11 percent versus 0 percent
- Rash: one participant in the 200 mg group, Grade 2 severity

No serious adverse events, deaths, or study drug-related discontinuations occurred during the 28-day double-blind period [7, 8]. The open-label extension data through 18 months did not identify new safety signals [8].

11.3 Phase 2/3 HEALEY ALS Platform Trial

DNL343 was reported to be safe and well tolerated in the 24-week Phase 2/3 trial, consistent with the earlier-phase safety profile [9, 10]. Detailed adverse event tabulations from the HEALEY trial have not been published in a peer-reviewed forum as of monograph preparation. No deaths attributable to study drug were reported.

11.4 Theoretical safety considerations

The ISR is a protective pathway under physiological conditions; chronic suppression of the ISR could theoretically impair cellular stress responses to viral infection (PKR-mediated), nutrient deprivation (GCN2-mediated), or endoplasmic reticulum stress (PERK-mediated). The clinical data through 24 weeks of dosing have not identified safety signals consistent with impaired stress adaptation (increased infection rates, metabolic derangement, or exacerbation of ER stress-related pathology), but longer-term safety data for chronic ISR inhibition in humans are not available.

The cardiovascular liability identified with 2BAct in dogs was apparently not recapitulated by DNL343 in preclinical safety pharmacology or in human clinical trials, consistent with the structural and pharmacological differentiation between the two compounds [1, 3].

11.5 Pregnancy, lactation, and special populations

No data on DNL343 in pregnancy or lactation have been published. The ISR pathway is implicated in placental development and embryonic stress responses; use in pregnancy would carry theoretical risk and cannot be recommended. Pediatric and geriatric pharmacokinetic data have not been published. The Phase 1b ALS study enrolled adult participants; age-specific analyses have not been reported.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five ISR-modulating agents against DNL343, evaluated on five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The five comparators were selected to represent the principal pharmacological approaches to ISR modulation that have produced material preclinical or clinical evidence relevant to neurodegeneration.

12.1 ISRIB (trans-ISRIB)

Mechanism: Symmetric bis-glycolamide eIF2B activator. Binds and stabilizes the eIF2B decameric complex at the beta-delta subunit interface, the same site as DNL343. First-in-class tool compound identified by Sidrauski et al. (2013) at the Walter laboratory [2].

Development trajectory: ISRIB is a research tool compound. It has not entered clinical development owing to its poor aqueous solubility (less than 1 microgram per milliliter), limited oral bioavailability in rodents, and pharmaceutical intractability [1, 3]. The compound has been studied extensively in preclinical models of traumatic brain injury, cognitive aging, and neurodegeneration, producing landmark demonstrations of cognitive enhancement and neuroprotection that validated eIF2B as a therapeutic target [2, 16].

Novelty: High at the time of discovery (2013). The compound established the concept of pharmacological eIF2B activation and ISR inhibition as a therapeutic strategy.

Effect size: Substantial in preclinical models. ISRIB improved spatial memory in aged mice, rescued cognitive deficits after traumatic brain injury, and reversed ISR-dependent neurodegeneration in genetic models. Effect sizes in rodent cognitive paradigms (Morris water maze, novel object recognition) were large [2, 16].

Promising potential: None as a clinical candidate. The compound persists as the reference tool for eIF2B activation research and as the structural starting point for clinical-grade analogs.

Side-effect profile: Not characterized in humans. Rodent tolerability is acceptable at studied doses.

Overall validation: High as a target-validation tool. Low as a therapeutic candidate.

Comparison to DNL343: DNL343 was designed specifically to overcome the pharmaceutical limitations of ISRIB while retaining the same molecular target and binding site. DNL343 is approximately equipotent in cellular assays, with dramatically improved solubility, oral bioavailability, CNS penetration, and half-life [1, 3]. DNL343 was superior to ISRIB in the optic nerve crush neuroprotection model, likely reflecting higher unbound brain exposure [1]. ISRIB remains the more extensively studied compound in preclinical models but cannot be advanced to clinical use.

12.2 2BAct

Mechanism: Asymmetric BCP-centered eIF2B activator. Binds the same eIF2B decameric interface as ISRIB and DNL343. Developed at Calico Life Sciences as a second-generation compound addressing ISRIB solubility limitations [17].

Development trajectory: 2BAct demonstrated improved solubility and pharmacokinetics relative to ISRIB, enabling aqueous-vehicle dosing and chronic in vivo administration. In the Eif2b5 R191H mouse model of vanishing white matter disease, 2BAct prevented neurodegeneration and improved survival [17]. However, the compound developed a cardiovascular liability in preclinical dog safety studies (specific mechanism not publicly disclosed), which terminated its advancement toward clinical development [1, 17].

Novelty: Moderate. The BCP bioisosteric replacement strategy was chemically innovative but the target and binding site were unchanged from ISRIB.

Effect size: Substantial in the Eif2b5 R191H mouse model, comparable to DNL343 in the same genetic model [1, 17].

Promising potential: None as a clinical candidate. Development was terminated by the cardiovascular safety signal. The compound serves as a research tool and as an intermediate between ISRIB and DNL343 in the medicinal chemistry lineage.

Side-effect profile: Cardiovascular liability in dogs [1, 17]. Not characterized in humans.

Overall validation: Moderate. The compound advanced the field by demonstrating that BCP-centered eIF2B activators could achieve the pharmacokinetic profile required for chronic in vivo dosing, but the cardiovascular finding limited its translational value.

Comparison to DNL343: DNL343 and 2BAct share the BCP scaffold approach but are chemically distinct compounds developed independently (Denali versus Calico). DNL343 did not recapitulate the cardiovascular liability of 2BAct in preclinical safety pharmacology or in human clinical trials, representing a critical safety differentiation. DNL343 advanced to human clinical evaluation; 2BAct did not.

12.3 Fosigotifator (ABBV-CLS-7262)

Mechanism: Small molecule eIF2B activator developed by Calico Life Sciences in collaboration with AbbVie. Stabilizes the eIF2B decameric complex and boosts GEF activity, including that of complexes carrying pathogenic vanishing white matter mutations [20].

Development trajectory: Phase 1 in healthy volunteers demonstrated safety, tolerability, and suppression of ISR biomarkers in blood cells following dosing for up to 14 days [20]. A Phase 1b/2 study (NCT05757141) in adults and children with vanishing white matter disease began dosing in April 2023 and was the first administration of an eIF2B activator to patients with VWM [20]. In June 2024, fosigotifator was selected for the FDA Support for clinical Trials Advancing Rare disease Therapeutics (START) Pilot Program, reflecting the unmet medical need in VWM and the regulatory support for accelerated development [20].

Novelty: Moderate. The target (eIF2B) and mechanism (decamer stabilization) are shared with ISRIB, 2BAct, and DNL343. The novelty is in the indication (VWM, a genetic eIF2B loss-of-function disease) where the target is directly validated by human genetics.

Effect size: Preclinical data in VWM mouse models demonstrated correction of coordination and movement problems [20]. Human clinical effect size data are not yet available from the Phase 1b/2 study.

Promising potential: Moderate to high. The compound is in active clinical development for VWM, an orphan disease with no approved therapy and direct genetic validation of the target. The FDA START designation supports accelerated regulatory pathways. Unlike DNL343 in ALS, fosigotifator is being evaluated in a disease where the target (eIF2B) is the primary pathogenic factor rather than a downstream mediator.

Side-effect profile: Phase 1 in healthy volunteers reported favorable tolerability [20]. Phase 1b/2 safety data in VWM patients have not been published.

Overall validation: Low to moderate (early clinical stage). The genetic validation of the target in VWM is the strongest of any eIF2B activator indication.

Comparison to DNL343: Fosigotifator and DNL343 share the eIF2B activator mechanism and are being developed by different companies for different indications. The critical strategic difference is the target-disease match: fosigotifator in VWM addresses a genetic eIF2B deficiency directly, whereas DNL343 in ALS addressed a secondary ISR activation downstream of TDP-43 proteinopathy. The HEALEY trial failure for DNL343 in ALS does not predict the outcome of fosigotifator in VWM, where the pathogenic basis for eIF2B activation is more direct.

12.4 IFB-088 (Sephin1)

Mechanism: Selective inhibitor of the stress-induced regulatory subunit of protein phosphatase 1 (PPP1R15A/GADD34), which dephosphorylates eIF2-alpha and terminates the ISR [21, 22]. By inhibiting GADD34, IFB-088/Sephin1 prolongs eIF2-alpha phosphorylation and thereby prolongs the ISR, the directionally opposite pharmacological effect to DNL343. This ISR-prolonging mechanism enhances the protective phase of the ISR (translational pause, chaperone upregulation) while limiting the pro-apoptotic phase through selective targeting of the stress-induced (GADD34) rather than constitutive (CReP) phosphatase [21].

Development trajectory: IFB-088 was developed by InFlectis BioScience. It received FDA orphan drug designation for ALS in 2022 and completed a Phase 2a exploratory clinical trial in 51 patients with bulbar-onset ALS by February 2025, meeting its primary safety and tolerability objective and confirming engagement of the

ISR and oxidative stress pathways [22]. The compound has also demonstrated efficacy in preclinical models of Charcot-Marie-Tooth disease types 1A and 1B, and multiple sclerosis [21, 22].

Novelty: High. The ISR-prolonging approach is mechanistically opposite to eIF2B activation (ISR inhibition) and represents a fundamentally different therapeutic hypothesis: that enhancing, rather than suppressing, the early adaptive ISR can be neuroprotective by promoting proteostatic recovery.

Effect size: Preclinical effect sizes in CMT and ALS models were substantial (reduction of TDP-43 cytoplasmic mislocalization, improved motor neuron survival) [22]. The Phase 2a clinical data met safety and biomarker engagement objectives but efficacy data on clinical endpoints have not been reported.

Promising potential: Moderate. The compound has completed Phase 2a and is seeking partnerships for Phase 2b development. The indication-specific approach (bulbar-onset ALS) is more focused than the broad ALS population studied in the DNL343 HEALEY trial.

Side-effect profile: Phase 2a data in 51 ALS patients indicated that IFB-088 was safe and well tolerated with no meaningful adverse observations [22].

Overall validation: Low to moderate. Phase 2a safety and biomarker data in a small ALS cohort, with broader preclinical support in CMT and MS.

Comparison to DNL343: IFB-088 and DNL343 modulate the same pathway (ISR) in opposite directions: DNL343 inhibits the ISR by activating eIF2B, while IFB-088 prolongs the ISR by inhibiting the GADD34 phosphatase. The two approaches are not interchangeable and represent competing hypotheses about whether ISR suppression or ISR prolongation is the more effective neuroprotective strategy. Both compounds have demonstrated preclinical neuroprotection, and both have produced acceptable safety profiles in early clinical studies. Neither has demonstrated clinical efficacy in a Phase 2/3 ALS trial. The mechanistic opposition makes their parallel clinical development informative for resolving the fundamental question of ISR directionality in neurodegeneration.

12.5 Trazodone

Mechanism: Trazodone is a serotonin antagonist and reuptake inhibitor (SARI) marketed as an antidepressant and hypnotic. The Bhatt laboratory identified trazodone in a screen for clinically approved compounds that reduce ISR activation, finding that trazodone partially inhibits PERK-mediated eIF2-alpha phosphorylation and reduces ATF4 levels in prion-diseased mice [23]. The ISR-modulating activity is mechanistically distinct from the serotonergic pharmacology and occurs at concentrations approximating clinical plasma levels.

Development trajectory: Trazodone is a registered, widely prescribed medicine with decades of clinical safety data. The Halliday et al. (2017) report demonstrated that oral trazodone reduced neuronal loss and clinical disease in prion-infected mice and in the FTD-tau mouse model, acting through ISR inhibition [23]. A clinical trial (TRAVALS, NCT04302870) evaluated trazodone in ALS patients, with results suggesting modest biomarker effects but no definitive clinical benefit [23, 24].

Novelty: Low as an ISR modulator (the ISR activity is an off-target pharmacological effect of an established drug). High as a drug repurposing strategy, leveraging an extensive clinical safety database.

Effect size: Modest in preclinical neurodegeneration models. Trazodone is substantially less potent as an ISR modulator than DNL343 (micromolar versus low-nanomolar cellular IC50). The neuroprotective effect in prion-diseased mice was significant but partial [23].

Promising potential: Low for ISR-mediated neuroprotection specifically. The compound is available for clinical use but at the concentrations required for ISR modulation, sedation (from the serotonergic pharmacology) is the dominant clinical effect, limiting dose escalation.

Side-effect profile: Well characterized from decades of clinical use: sedation, orthostatic hypotension, priapism (rare), and QT prolongation at higher doses. The sedative effect is dose-limiting for the ISR-modulating application.

Overall validation: Moderate. The compound has clinical safety data and preclinical ISR-modulating activity, but the low potency for ISR inhibition and the dose-limiting serotonergic sedation substantially constrain the therapeutic window for neurodegeneration.

Comparison to DNL343: Trazodone and DNL343 both reduce ISR activation but through different molecular mechanisms (PERK inhibition versus eIF2B activation) and at vastly different potencies (micromolar versus low-nanomolar). DNL343 is the superior ISR inhibitor for research applications requiring potent and selective pathway modulation. Trazodone has the advantage of established clinical registration, extensive human safety data, and immediate availability for off-label research-clinical use.

12.6 Comparator summary

The five compounds span the mechanistic landscape of ISR modulation. **ISRIB** established the target and remains the reference tool but cannot be advanced clinically. **2BAct** demonstrated the feasibility of BCP-centered eIF2B activators for chronic dosing but was terminated by a cardiovascular liability. **Fosigotifator** is the most advanced clinical eIF2B activator program currently active, targeting the genetically validated VWM indication. **IFB-088** represents the mechanistically opposite ISR-prolonging approach and has completed Phase 2a in ALS. **Trazodone** offers a repurposing pathway with extensive safety data but limited ISR potency.

DNL343 occupies a unique position as the only eIF2B activator to have completed a Phase 2/3 clinical trial in a neurodegenerative disease, generating the most extensive human safety and pharmacokinetic dataset for this mechanism class. The negative HEALEY trial result in broad ALS does not invalidate the eIF2B activator mechanism but constrains the interpretation: ISR inhibition through eIF2B activation, at the doses and durations studied, was insufficient to modify disease progression in an unselected ALS population. The preclinical efficacy in ISR-driven genetic models (eIF2B LOF, optic nerve crush) remains robust and supports the continued investigation of eIF2B activators in diseases where ISR activation is a primary, genetically validated pathogenic driver rather than a secondary downstream event.

13. References

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This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. DNL343 is not approved by any regulatory authority for any indication. The compound was discontinued from clinical development in amyotrophic lateral sclerosis following a negative Phase 2/3 trial outcome. Compound class generalizations are noted where compound-specific data are sparse; investigators should weight the evidence accordingly. Research-grade DNL343 is available from chemical suppliers; investigators should obtain analytical confirmation of identity and purity on every lot.