

## RESEARCH MONOGRAPH . COMPOUND PROFILE

# ISRIB

*A symmetrical bis-glycolamide that stabilizes the decameric eIF2B holoenzyme, counteracting phospho-eIF2 $\alpha$ -mediated translational repression and reversing age-related, traumatic, and neurodegenerative cognitive deficits in preclinical models without the pancreatic toxicity of upstream PERK kinase inhibitors.*

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## ABSTRACT

ISRIB (integrated stress response inhibitor) is a cell-permeable, brain-penetrant small molecule identified by Sidrauski et al. (2013) at the University of California, San Francisco, through a phenotypic screen for compounds that render cells resistant to the translational consequences of eukaryotic initiation factor 2 alpha (eIF2alpha) phosphorylation [1]. The compound blocks the integrated stress response (ISR) with an IC<sub>50</sub> of approximately 5 nM in ATF4 reporter assays, operating downstream of all four eIF2alpha kinases (PERK, GCN2, HRI, PKR) at the level of the guanine nucleotide exchange factor eIF2B [2]. Cryo-electron microscopy studies (Tsai et al. 2018; Zyryanova et al. 2021) demonstrated that ISRIB binds at the symmetry interface of two eIF2B betagammadeltaepsilon tetrameric subcomplexes, acting as a molecular staple that promotes assembly of the catalytically active decameric holoenzyme and allosterically antagonizes the inhibitory effect of phosphorylated eIF2alpha on the nucleotide exchange reaction [3, 4]. The resulting restoration of ternary complex formation and global protein synthesis rates is partial rather than complete, reaching approximately 50 to 70 percent of unstressed control levels even at saturating compound concentrations, a feature that accounts for the favorable safety profile relative to direct PERK kinase inhibitors such as GSK2606414 that produce pancreatic exocrine destruction [5].

Preclinical pharmacology spans multiple disease-relevant models. In cognition, ISRIB enhances spatial and fear-associated learning in wild-type mice (Sidrauski et al. 2013) [1], reverses cognitive deficits weeks after traumatic brain injury (Chou et al. 2017) [6],

restores age-related memory decline and hippocampal neuronal function within days of treatment in aged mice (Krukowski et al. 2020) [7], and rescues synaptic plasticity in a mouse model of Down syndrome [8]. In neurodegeneration, ISRIB prevents neuronal loss in prion-diseased mice without pancreatic toxicity (Halliday et al. 2015) [5] and stabilizes vanishing white matter disease eIF2B mutant complexes to wild-type catalytic activity (Wong et al. 2018) [9]. Additional preclinical activity has been reported in prostate cancer [10], noise-induced cochlear synaptopathy [11], postinfarct atrial fibrillation [12], and amyotrophic lateral sclerosis models [13]. Pharmacokinetically, ISRIB exhibits good blood-brain barrier penetration and achieves brain concentrations exceeding its IC<sub>50</sub> at intraperitoneal doses of 0.25 to 2.5 mg/kg in mice, but is limited by poor aqueous solubility requiring vehicle formulations with dimethyl sulfoxide and polyethylene glycol 400 [5, 6].

No human clinical trials of ISRIB itself have been conducted. However, the compound served as the pharmacological prototype for two clinical-stage eIF2B activators: DNL343 (Denali Therapeutics), which completed a Phase 2/3 trial in amyotrophic lateral sclerosis (HEALEY platform trial) without meeting primary endpoints [14], and fosigotifator (Calico/AbbVie), which also failed to demonstrate significant slowing of disease progression in the same platform trial, although an exploratory high-dose arm showed signals on muscle strength preservation [15]. This monograph reviews the chemistry, stereochemistry, and synthesis of ISRIB; the molecular pharmacology of eIF2B activation and ISR inhibition; pharmacokinetic properties and formulation challenges; the preclinical evidence base across cognitive, neurodegenerative, oncologic, and inflammatory models; the clinical-translational status through derivative compounds;

sourcing and quality verification for research use; reconstitution and handling; stack interactions; adverse events and safety signals; and a comparative assessment of five ISR-modulating alternatives against ISRIB on five competency standards.

# 1. Compound Identification

## 1.1 Names, synonyms, and registry identifiers

**Common name:** ISRIB (integrated stress response inhibitor). The acronym is the canonical identifier in the research literature and the only name under which the compound was initially reported [1].

**Chemical name (IUPAC):** trans-N,N'-(cyclohexane-1,4-diyl)bis(2-(4-chlorophenoxy)acetamide). The compound is a symmetrical bis-glycolamide constructed on a trans-1,4-diaminocyclohexane core.

**Chemical Abstracts Service registry numbers:** 1597403-47-8 (trans-isomer, the biologically active stereoisomer); 548470-11-7 (cis/trans mixture as originally reported). The trans-isomer is the pharmacologically relevant material and the form supplied by most research chemical vendors.

**PubChem CID:** 1011240.

**Other designations:** trans-ISRIB; SML0843 (Sigma-Aldrich catalog number).

## 1.2 Structural class and chemistry

ISRIB is a symmetrical bis-glycolamide with two identical 4-chlorophenoxy-acetamide arms linked through a trans-1,4-diaminocyclohexane spacer. The structural class is unusual among pharmacological tool compounds in that the molecule possesses a two-fold rotational symmetry axis, a feature that proved central to its mechanism of action at the symmetry interface of the eIF2B decamer [3].

**Molecular formula:** C<sub>22</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub>.

**Molecular weight:** 451.34 g/mol.

**Physical properties:** White to off-white crystalline solid. Poorly soluble in water (less than 1 microgram per milliliter in aqueous buffers at physiological pH). Soluble in dimethyl sulfoxide (DMSO) at concentrations up to 100 mM; moderately soluble in polyethylene glycol 400 (PEG 400) and in mixed DMSO/PEG 400 vehicles. Melting point of the trans-isomer is approximately 201 to 205 degrees Celsius.

**Stereochemistry:** The cyclohexane ring generates cis and trans diastereomers at the 1,4-diamine positions. The trans-isomer (diequatorial amine orientation) is the biologically active form, exhibiting approximately 100-fold greater potency than the cis-isomer in cellular ISR reporter assays [1]. The trans-isomer is achiral (possessing C<sub>2</sub> symmetry) and therefore exists as a single stereoisomeric species. Investigators should confirm trans-isomer identity by nuclear magnetic resonance spectroscopy or by comparison of melting point against published values, as early commercial lots contained variable cis/trans ratios.

## 1.3 Synthesis

The published synthesis of ISRIB proceeds through a convergent two-step route [1, 16]. The key steps are: (1) alkylation of 4-chlorophenol with ethyl bromoacetate or chloroacetyl chloride to generate 4-chlorophenoxyacetic acid or the corresponding acid chloride; and (2) double amide coupling of 4-chlorophenoxyacetic acid (or its activated ester or acid chloride) with trans-1,4-diaminocyclohexane. Standard peptide coupling reagents (carbodiimides with hydroxybenzotriazole catalysis, or direct acid chloride acylation in the presence of a base such as triethylamine) produce the bis-amide product. The trans-1,4-diaminocyclohexane starting material is commercially available and is supplied as the trans-isomer; contamination with the cis-isomer should be verified by the investigator if diastereomeric purity is critical. Purification is by recrystallization from ethanol or ethyl acetate, or by silica gel chromatography for analytical-grade material. Yields in published procedures are typically 60 to 80 percent for the final coupling step. The compound is characterized by proton and carbon-13 nuclear magnetic resonance spectroscopy, high-resolution mass spectrometry, and high-performance liquid chromatography. The synthesis is straightforward for an organic chemistry laboratory and has been reproduced by multiple commercial suppliers; pricing reflects the simplicity of manufacture.

## 2. Abstract

ISRIB (integrated stress response inhibitor) is a cell-permeable, brain-penetrant small molecule that blocks the integrated stress response (ISR) by stabilizing the active decameric form of the guanine nucleotide exchange factor eIF2B [1, 3]. The compound was identified by Sidrauski, Acosta-Alvear, and Walter (2013) in a phenotypic screen at the University of California, San Francisco (UCSF) for inhibitors of PERK-dependent translational attenuation, but was subsequently shown to act downstream of all four eIF2alpha kinases at the level of eIF2B rather than at any single kinase [2].

The ISR is a conserved signaling pathway in which phosphorylation of eIF2alpha at serine 51 by any of four stress-sensing kinases (PERK, GCN2, HRI, PKR) converts eIF2 from a substrate to an inhibitor of its guanine nucleotide exchange factor eIF2B, resulting in global translational downregulation and selective translational upregulation of stress-responsive mRNAs including ATF4, CHOP, and GADD34 [17]. The ISR is protective in acute stress but becomes maladaptive when chronically activated, as occurs in aging, neurodegeneration, traumatic brain injury, and certain cancers. ISRIB counteracts the inhibitory effect of phosphorylated eIF2alpha on eIF2B without affecting basal eIF2B activity in unstressed cells, and its efficacy is confined to a defined window of ISR activation: it suppresses low-to-moderate ISR signaling but does not override strong acute ISR activation, preserving the cytoprotective arm of the pathway [18].

The preclinical evidence base for ISRIB is extensive and spans multiple disease-relevant models. In wild-type mice, a single intraperitoneal injection of 0.25 mg/kg enhances spatial and fear-conditioned memory [1]. In traumatic brain injury models, ISRIB administered weeks after focal cortical impact reverses cognitive deficits and restores long-term potentiation in hippocampal slices [6]. In aged mice (approximately 20 months), a brief course of ISRIB (three daily injections) reverses age-related spatial and working memory decline, restores hippocampal dendritic spine density, normalizes neuronal electrophysiological properties, and reduces interferon-driven and T cell-mediated neuroinflammatory signatures to levels observed in young animals [7]. In prion-diseased mice, ISRIB partially restores protein synthesis rates and prevents neurodegeneration without the pancreatic exocrine toxicity produced by the upstream PERK inhibitor GSK2606414 [5].

Additional activity has been documented in models of vanishing white matter disease [9], Down syndrome [8], prostate cancer [10], noise-induced cochlear synaptopathy [11], atrial fibrillation [12], and amyotrophic lateral sclerosis [13].

ISRIB has not entered human clinical trials directly, principally because of its poor aqueous solubility and limited oral bioavailability. The technology was licensed to Calico Life Sciences in 2015, and the compound has served as the pharmacological prototype for two clinical-stage eIF2B activators with improved pharmaceutical properties: DNL343 (Denali Therapeutics) and fosigotifator (Calico/AbbVie). Both compounds were evaluated in the Phase 2/3 HEALEY ALS Platform Trial and failed to meet their primary efficacy endpoints, although fosigotifator showed a signal on muscle strength preservation at an exploratory high dose [14, 15].

The compound remains the most extensively characterized tool compound for ISR inhibition in mammalian systems and continues to serve as the reference standard against which newer eIF2B activators, PERK inhibitors, and ISR-prolonging agents are benchmarked.

## 3. Discovery and Development History

### 3.1 Origin

ISRIB was identified in 2013 by Carmela Sidrauski in the laboratory of Peter Walter at the University of California, San Francisco, through a phenotypic screen of approximately 100,000 small molecules for compounds that reversed PERK-dependent translational attenuation in a cell-based ATF4-luciferase reporter assay [1]. The screen was designed to identify chemical tools for dissecting the contribution of the ISR to proteostasis and stress signaling. ISRIB emerged as the most potent hit, with an IC<sub>50</sub> of approximately 5 nM in the reporter assay and favorable pharmacokinetic properties including blood-brain barrier penetration.

The initial report (Sidrauski et al. 2013, eLife) demonstrated that ISRIB enhanced spatial and fear-associated learning in wild-type mice, establishing the compound as a cognitive enhancer operating through the ISR pathway [1]. This finding attracted substantial attention because it suggested that the ISR, previously studied primarily in the context of cellular stress and unfolded protein responses, was a constitutive regulator of memory-relevant synaptic plasticity and translation.

### 3.2 Mechanistic characterization

The molecular target of ISRIB was not immediately apparent from the phenotypic screen. Initial work established that the compound acted downstream of eIF2 $\alpha$  phosphorylation (it did not affect phospho-eIF2 $\alpha$  levels) and did not inhibit any of the four eIF2 $\alpha$  kinases directly [1]. The key mechanistic advance came in 2015 when Sidrauski et al. demonstrated that ISRIB promotes the dimerization and activation of eIF2B, the guanine nucleotide exchange factor that catalyzes GDP-to-GTP exchange on eIF2 and is the rate-limiting step in translation initiation [2]. This finding reframed ISRIB from a kinase-pathway inhibitor to an assembly activator of a multisubunit enzyme complex.

A companion study by Sidrauski et al. (2015, eLife) further demonstrated that ISRIB reverses the translational effects of eIF2 $\alpha$  phosphorylation, blocks stress granule formation induced by eIF2 $\alpha$  phosphorylation, and promotes the rapid

disassembly of pre-formed stress granules, liberating sequestered mRNAs into the actively translating pool [16].

### 3.3 Structural biology

The structural basis of ISRIB action was resolved in 2018 by two independent cryo-electron microscopy studies published in *Science*. Tsai et al. (2018) reported the cryo-EM structure of ISRIB bound to human eIF2B at near-atomic resolution, revealing the compound in a deep hydrophobic pocket at the symmetry interface between two eIF2B betagammadeltaepsilon tetrameric subcomplexes, effectively stapling them together into the catalytically active octameric assembly that subsequently recruits the dimeric alpha subunit to form the fully active decameric holoenzyme [3]. The two-fold symmetry of ISRIB matches the two-fold symmetry of the eIF2B interface, explaining the unusual structure-activity relationship in which the symmetrical bis-glycolamide scaffold is required for potency.

Zyryanova et al. (2018), working in the laboratory of David Ron at the University of Cambridge, reported complementary structures and demonstrated that chemogenetic mutations at the ISRIB binding site confirmed the functional importance of the pocket [19].

Subsequent work by Zyryanova et al. (2021) demonstrated that ISRIB also operates allosterically within fully assembled decameric eIF2B: phosphorylated eIF2alpha and ISRIB bind at physically distant sites on the decamer, and ISRIB antagonizes the conformational change (a rocking motion of the regulatory subcomplex) that phospho-eIF2alpha induces to inhibit nucleotide exchange [4]. This allosteric mechanism explains how ISRIB can counteract ISR activation without preventing eIF2alpha phosphorylation per se.

### 3.4 Licensing and clinical translation

The ISRIB technology was licensed by UCSF to Calico Life Sciences (a subsidiary of Alphabet) in 2015. Sidrauski joined Calico to lead medicinal chemistry efforts aimed at developing drug candidates with improved pharmaceutical properties. Calico's program produced fosigotifator, an oral eIF2B activator co-developed with AbbVie [15]. Independently, Denali Therapeutics developed DNL343, a brain-penetrant eIF2B activator with a distinct chemical scaffold [14]. Both compounds entered clinical trials for amyotrophic lateral sclerosis and other neurodegenerative

indications. As of the most recent revision of this monograph, neither program has produced a positive Phase 2/3 readout, and the clinical translation of the eIF2B activator mechanism remains unvalidated in humans.

## 4. Mechanism of Action

ISRIB modulates a single molecular target, the guanine nucleotide exchange factor eIF2B, through two related but mechanistically distinct activities: promotion of eIF2B decamer assembly and allosteric antagonism of phospho-eIF2alpha inhibition within the assembled decamer. Both activities converge on the restoration of ternary complex (eIF2-GTP-Met-tRNAi) formation and the relief of translational repression imposed by the integrated stress response.

### 4.1 The integrated stress response

The ISR is a conserved eukaryotic signaling network that adjusts global protein synthesis rates and gene expression in response to diverse cellular stresses [17]. Four kinases sense distinct stress signals and phosphorylate a single convergent substrate, eIF2alpha, at serine 51: PERK (endoplasmic reticulum stress and unfolded protein accumulation), GCN2 (amino acid deprivation and UV irradiation), HRI (heme deficiency and mitochondrial stress), and PKR (double-stranded RNA and viral infection). Phosphorylated eIF2alpha (eIF2alpha-P) binds eIF2B with increased affinity and acts as a competitive inhibitor of nucleotide exchange, reducing the availability of eIF2-GTP ternary complex. The resulting global translational attenuation is accompanied by selective translational upregulation of mRNAs bearing upstream open reading frames, including the transcription factor ATF4, which activates downstream effectors (CHOP, GADD34, asparagine synthetase) that mediate stress adaptation, apoptosis, and feedback dephosphorylation of eIF2alpha.

The ISR is protective in acute and transient stress, enabling cells to conserve resources and mount a coordinated adaptive response. However, chronic or excessive ISR activation is pathological: it underlies neuronal dysfunction in aging [7], traumatic brain injury [6], prion disease [5], vanishing white matter disease [9], and amyotrophic lateral sclerosis [13], and it contributes to treatment resistance in certain cancers [10].

## 4.2 eIF2B assembly and activation by ISRIB

eIF2B is a decameric complex composed of two copies each of five subunits (alpha, beta, gamma, delta, epsilon) [3]. The catalytic activity resides in the epsilon subunit, but the full decameric assembly is required for maximal nucleotide exchange activity. Under basal conditions, eIF2B exists in an equilibrium between tetrameric (betagammadeltaepsilon), octameric (betagammadeltaepsilon)<sub>2</sub>, and decameric (alpha<sub>2</sub>betagammadeltaepsilon)<sub>2</sub> forms. The octameric and decameric forms are substantially more active than the tetrameric form.

ISRIB binds at the symmetry interface of two betagammadeltaepsilon tetramers, stabilizing the octameric assembly and shifting the equilibrium toward the fully active decameric holoenzyme [2, 3]. The binding pocket is a deep, hydrophobic cleft lined by residues from the beta and delta subunits of each tetramer. The two 4-chlorophenoxy arms of ISRIB extend symmetrically into equivalent hydrophobic subsites on opposite sides of the interface, and the cyclohexane core bridges the two tetramers. Mutations at the binding interface (eIF2B delta H160D, eIF2B beta V164A) confer resistance to ISRIB in cellular assays and in structural studies [2].

The functional consequence of enhanced decamer assembly is an increase in the steady-state pool of active eIF2B, which accelerates GDP-to-GTP exchange on eIF2 and increases ternary complex availability. In unstressed cells (where eIF2B is already predominantly decameric), the effect of ISRIB is minimal. In stressed cells (where phospho-eIF2alpha-dependent inhibition shifts the equilibrium toward less active assemblies), ISRIB counteracts the stress-induced loss of decameric eIF2B and partially restores translation [18].

## 4.3 Allosteric antagonism of phospho-eIF2alpha

Zyryanova et al. (2021) demonstrated that ISRIB also antagonizes the inhibitory effect of phospho-eIF2alpha on fully assembled eIF2B decamers through an allosteric mechanism [4]. Cryo-EM structures revealed that phospho-eIF2alpha binding induces a conformational rocking motion in the eIF2B regulatory subcomplex that propagates to the catalytic site and reduces nucleotide exchange activity. ISRIB binding at the distant symmetry interface rigidifies the decamer and opposes this conformational change, maintaining the catalytic site in an active configuration even when phospho-eIF2alpha is bound.

This dual mechanism (assembly promotion plus allosteric antagonism) explains several features of ISRIB pharmacology: the compound is effective across all four eIF2alpha kinase pathways (because it acts downstream at eIF2B); its efficacy is partial rather than complete (because it does not prevent eIF2alpha phosphorylation or eliminate phospho-eIF2alpha binding to eIF2B); and its activity is confined to a defined window of ISR activation (strong ISR signaling overwhelms the allosteric antagonism) [18].

## 4.4 Selectivity and off-target pharmacology

ISRIB has no characterized activity on kinases, G protein-coupled receptors, ion channels, or other pharmacological targets outside the eIF2B complex. The compound does not inhibit any of the four eIF2alpha kinases and does not affect eIF2alpha phosphorylation status [1]. In broad-panel selectivity screens, ISRIB has not produced significant hits at concentrations up to 10 micromolar. The selectivity is consistent with the unusual molecular target (a multi-subunit enzyme complex rather than a conventional receptor or kinase) and with the highly specific binding mode revealed by structural biology.

# 5. Pharmacokinetics and Routes of Administration

## 5.1 Absorption and bioavailability

ISRIB is poorly soluble in aqueous media (less than 1 microgram per milliliter at physiological pH), and this poor solubility is the principal limitation to its pharmaceutical development. In preclinical studies, the compound is administered intraperitoneally in rodents using DMSO/PEG 400 vehicle formulations or by direct central nervous system infusion through implanted cannulae [5, 6, 7].

Oral bioavailability in mice has not been formally reported in the published literature but is understood to be low, consistent with the aqueous insolubility. The absence of a viable oral formulation was the principal motivation for the development of derivative compounds (DNL343, fosigotifator) with improved solubility and oral absorption [14, 15].

## 5.2 Distribution

ISRIB readily crosses the blood-brain barrier. Pharmacokinetic studies in wild-type mice given a single intraperitoneal dose of 2.5 mg/kg demonstrated plasma and brain T<sub>max</sub> of approximately 6 hours, C<sub>max</sub> of approximately 180 ng/mL in plasma and 100 ng/mL in brain, and C<sub>24h</sub> of approximately 55 ng/mL in plasma and 33 ng/mL in brain [5]. The brain-to-plasma ratio is approximately 0.55 to 0.60 across the concentration-time curve, indicating good central nervous system penetration without evidence of strong P-glycoprotein-mediated efflux. At the 0.25 mg/kg dose used for cognitive enhancement studies, brain concentrations at 8 and 24 hours exceeded the cellular IC<sub>50</sub> of 5 nM (approximately 2.3 ng/mL), providing pharmacokinetic support for the observed behavioral effects [1].

Volume of distribution data have not been formally reported, but the brain penetration and lipophilicity (calculated logP approximately 3.5) suggest moderate-to-large tissue distribution.

Plasma protein binding has not been formally characterized in the published literature.

## 5.3 Metabolism and elimination

Hepatic metabolism of ISRIB has not been comprehensively characterized. The compound contains two aromatic chlorine substituents and two amide bonds; expected metabolic pathways include oxidative dechlorination, aromatic hydroxylation, and amide hydrolysis, but the specific cytochrome P450 isoforms responsible have not been reported. The sustained brain concentrations at 24 hours post-dose suggest a relatively slow elimination profile consistent with the lipophilic physicochemical properties. The absence of detailed metabolic characterization reflects the compound's status as a research tool rather than a clinical candidate.

## 5.4 Routes of administration

Three principal routes are used in preclinical research:

**Intraperitoneal injection** (the most common): 0.25 to 2.5 mg/kg in DMSO/PEG 400 vehicle (typically 1:1 v/v DMSO:PEG 400, with the compound dissolved in DMSO first and then diluted with PEG 400). This route produces reliable brain exposure and is the standard for behavioral and neuroprotection studies [1, 5, 6, 7].

**Intracerebroventricular or intra-amygdala cannulation:** Used in studies requiring localized central nervous system delivery. Single amygdala administration of 25 ng in 0.5 microliters immediately following auditory fear conditioning significantly enhances long-term memory in rats [1].

**Subcutaneous injection:** Used in some chronic dosing studies; vehicle formulation is similar to the intraperitoneal route.

**Oral gavage** has been used in limited studies but is not the standard route owing to solubility limitations.

## 5.5 Special populations

No special population pharmacokinetic data exist. All published pharmacokinetic data are from wild-type C57BL/6 mice and Sprague-Dawley rats. Sex-dependent differences in ISRIB pharmacokinetics have not been systematically characterized, although sex-dependent differences in ISR activation and in ISRIB efficacy have been reported in some models [11].

## 6. Preclinical Pharmacology

### 6.1 Cognitive enhancement in wild-type animals

The original Sidrauski et al. (2013) report demonstrated that ISRIB enhances long-term memory in wild-type mice [1]. A single intraperitoneal injection of 0.25 mg/kg administered after training in the Morris water maze produced significant enhancement of spatial memory consolidation, measured by probe trial performance 24 hours later. In contextual fear conditioning, ISRIB (0.25 mg/kg intraperitoneal) enhanced 24-hour freezing responses to the conditioned context without affecting short-term (1-hour) memory, consistent with a specific effect on long-term memory consolidation rather than on acquisition or short-term retention.

The mechanism of cognitive enhancement was attributed to partial relief of constitutive low-level ISR activity in hippocampal neurons, enabling increased translation of synaptic plasticity-relevant mRNAs. This interpretation was supported by the observation that ISRIB reduced ATF4 protein levels in hippocampus without affecting phospho-eIF2alpha levels, consistent with action at the level of eIF2B rather than upstream kinases [1].

### 6.2 Traumatic brain injury

Chou et al. (2017) demonstrated that ISRIB reverses cognitive deficits in two mouse models of traumatic brain injury (TBI), even when administered weeks after the initial injury [6]. In a controlled cortical impact model, mice exhibited persistent deficits in radial-arm water maze performance beginning 28 days after injury. Three daily injections of ISRIB (2.5 mg/kg intraperitoneal) beginning at day 28 restored maze performance to levels indistinguishable from uninjured controls, and the cognitive restoration persisted for at least one week after the final injection. In a modified Barnes maze using a closed-head concussive injury model, four daily ISRIB injections beginning 14 days post-injury produced comparable cognitive restoration.

At the cellular level, ISRIB restored long-term potentiation (LTP) in hippocampal slices from TBI animals [6]. The ISR was found to be chronically activated in hippocampal neurons after TBI, with elevated phospho-eIF2alpha and ATF4 levels per-

sisting for weeks after injury. ISRIB treatment normalized these molecular markers and restored the translational capacity required for LTP-dependent memory formation.

The finding that ISRIB can reverse TBI-induced cognitive deficits when administered in the chronic post-injury phase (rather than requiring acute neuroprotective intervention) is particularly noteworthy, as the majority of TBI patients present for cognitive treatment well after the acute injury phase.

## 6.3 Age-related cognitive decline

Krukowski et al. (2020) reported that a brief course of ISRIB reverses age-related cognitive decline in aged (approximately 20-month-old) mice [7]. Three daily intraperitoneal injections of ISRIB (2.5 mg/kg) restored spatial memory (radial-arm water maze) and working memory (Y-maze) to levels comparable to young (approximately 4-month-old) controls. The cognitive restoration was accompanied by cellular-level rejuvenation in the hippocampus: neuronal intrinsic excitability and dendritic spine density were restored to youthful levels, and the age-related increase in interferon-driven and T cell-mediated neuroinflammatory gene signatures was reversed.

Cognitive improvement persisted for at least three weeks after cessation of the three-day treatment course, suggesting that ISRIB reverses a maladaptive steady state rather than simply masking symptoms during treatment [7].

The immune system effects extended beyond the brain: ISRIB treatment also improved the function of aged T cells in peripheral immune assays, suggesting that chronic ISR activation contributes to immunosenescence and that ISR inhibition can rejuvenate immune function [7]. These findings were published in eLife and received substantial attention as evidence that age-related cognitive decline may be pharmacologically reversible through ISR pathway modulation.

At the molecular level, phosphorylated eIF2 $\alpha$  and ATF4 levels were elevated in the aged hippocampus relative to young animals, and ISRIB treatment reduced both markers, confirming that chronic ISR activation contributes to age-related cognitive decline and that eIF2B activation is sufficient to reverse it.

## 6.4 Prion neurodegeneration

Halliday et al. (2015) characterized ISRIB in a mouse model of prion disease (Rocky Mountain Laboratory prion strain), a rapidly progressive neurodegeneration in which chronic ISR activation and translational repression precede neuronal loss [5]. ISRIB treatment (2.5 mg/kg intraperitoneal, daily from clinical onset) partially restored protein synthesis rates in hippocampus and cortex (to approximately 70 percent of control levels) and significantly delayed neurodegeneration. The study was notable for demonstrating that ISRIB produced no detectable pancreatic toxicity: pancreatic weight, histological architecture, and exocrine and endocrine function were preserved, in contrast to the PERK kinase inhibitor GSK2606414, which produced approximately 50 percent reduction in pancreatic weight and extensive acinar cell destruction in the same model [5]. The differential toxicity was attributed to the partial (rather than complete) restoration of protein synthesis by ISRIB, which maintained sufficient ISR-mediated translational control in the pancreas to prevent the loss of proteostatic surveillance that PERK inhibition produces.

## 6.5 Vanishing white matter disease

Wong et al. (2018) demonstrated that ISRIB rescues the stability and catalytic activity of eIF2B complexes bearing vanishing white matter disease (VWM) mutations [9]. VWM is a fatal childhood leukodystrophy caused by mutations in any of the five eIF2B subunit genes. The mutations destabilize the decameric holoenzyme and reduce nucleotide exchange activity, resulting in constitutive ISR activation, impaired myelination, and progressive white matter loss. ISRIB stabilized multiple VWM mutant eIF2B complexes in the decameric form and restored residual catalytic activity to wild-type levels in biochemical assays. In mice bearing the Eif2b5 R132H/R132H VWM mutation, ISRIB inhibited ISR activation and normalized translation [9, 20]. These findings provided proof of concept for eIF2B activation as a therapeutic strategy in VWM and motivated clinical development of derivative compounds for this indication.

## 6.6 Down syndrome

Zhu et al. (2019) reported that the ISR is chronically activated in the hippocampus of the Ts65Dn mouse model of Down syndrome, contributing to hippocampal LTP failure and cognitive impairment [8]. ISRIB treatment restored LTP and improved performance in hippocampal-dependent learning tasks. Elevated phospho-

eIF2alpha levels and ATF4 expression in the Ts65Dn hippocampus were normalized by ISRIB, confirming ISR hyperactivation as a pharmacologically tractable contributor to cognitive disability in Down syndrome.

## 6.7 Cancer

Nguyen et al. (2018) reported that ISRIB causes selective death of treatment-resistant prostate cancer cells that have adapted to chronic ISR activation as a survival mechanism [10]. In xenograft models of PTEN-null, eIF2alpha-overexpressing prostate cancer, ISRIB treatment reduced tumor growth and extended survival. The mechanism involved disruption of the adaptive ISR that cancer cells use to maintain translation of pro-survival mRNAs under conditions of chronic proteotoxic stress. The finding illustrates the context-dependent nature of ISR modulation: while ISR inhibition is generally cytoprotective in neurons, it can be selectively cytotoxic in cancer cells that depend on chronic ISR activation for survival.

## 6.8 Cochlear synaptopathy

Bhatt et al. (2020) demonstrated that ISRIB protects against noise-induced cochlear synaptopathy in a sex-dependent manner [11]. Mice pretreated with ISRIB prior to noise exposure were protected against noise-induced synapse loss between inner hair cells and auditory nerve fibers. The protection was more pronounced in male mice, suggesting sex-dependent differences in ISR activation in the cochlea.

## 6.9 Cardiac pathology

ISRIB has been reported to reduce susceptibility to postinfarct atrial fibrillation in rats, operating through ISR inhibition in cardiac tissue and reducing fibrosis and inflammatory signaling [12].

## 6.10 Amyotrophic lateral sclerosis

ISR activation is a feature of TDP-43 proteinopathy, the dominant neuropathological signature of ALS. ISRIB has been characterized in cellular and animal models of ALS with variable results [13]. The eIF2B activator mechanism was the rationale for the clinical development of DNL343 and fosigotifator for ALS [14, 15]. In a cellular

model, ISRIB improved neuronal survival in SOD-1-expressing motor neurons by fine-tuning the unfolded protein response without abolishing the protective arm of the ISR [13].

## 7. Clinical Evidence Base

### 7.1 Direct clinical studies of ISRIB

No clinical trials of ISRIB in humans have been conducted or registered. The compound has not entered any phase of formal clinical development. The principal barriers to clinical translation are poor aqueous solubility, limited oral bioavailability, and the availability of derivative compounds with superior pharmaceutical properties [14, 15].

### 7.2 DNL343 (Denali Therapeutics)

DNL343 is a brain-penetrant, orally bioavailable eIF2B activator developed by Denali Therapeutics as a clinical successor to ISRIB [14]. The compound completed a Phase 1 trial in healthy volunteers, demonstrating safety, tolerability, dose-proportional pharmacokinetics, and cerebrospinal fluid penetration with once-daily oral dosing. DNL343 was subsequently evaluated in the Phase 2/3 HEALEY ALS Platform Trial (Regimen G), enrolling 186 participants with ALS against 139 receiving placebo. The trial did not meet its primary endpoint (change in ALS Functional Rating Scale-Revised over 24 weeks), and additional analyses did not demonstrate a treatment effect on neurofilament light chain (NfL) levels in either the 24-week trial or a 28-week open-label extension [14]. DNL343 was generally well tolerated; the principal adverse events were gastrointestinal (nausea, diarrhea) and were predominantly mild.

Pharmacodynamic data from the trial demonstrated ISR pathway engagement: ISRIB-class target engagement was confirmed by reduction of the ISR biomarker GDF-15 in cerebrospinal fluid and modulation of ISR-responsive transcripts in peripheral blood mononuclear cells [14].

### 7.3 Fosigotifator (Calico/AbbVie)

Fosigotifator is an oral eIF2B activator co-developed by Calico Life Sciences and AbbVie [15]. The compound was evaluated in a separate arm of the HEALEY ALS Platform Trial and also failed to meet its primary endpoint of slowing disease pro-

gression. However, an exploratory high-dose arm was associated with a statistically significant slowing of muscle strength decline relative to placebo, providing a preliminary signal that higher eIF2B activation may be required for clinical effect in ALS [15]. An open-label Phase 1b/2 study of fosigotifator in vanishing white matter disease was initiated in March 2023, representing the first clinical test of eIF2B activation in the rare genetic disorder most directly caused by eIF2B deficiency. The clinical development status beyond these trials has not been fully disclosed as of the most recent monograph revision.

## 7.4 Interpretation

The negative Phase 2/3 outcomes for DNL343 and fosigotifator in ALS do not invalidate the eIF2B activator mechanism for other indications. ALS is a rapidly progressive neurodegenerative disease with heterogeneous molecular pathology; ISR hyperactivation may be a contributing rather than a driving factor in disease progression, and the 24-week trial duration may have been insufficient to detect a disease-modifying effect. The preclinical evidence for ISR inhibition in traumatic brain injury, aging, and vanishing white matter disease remains robust. The clinical translation of the eIF2B activator mechanism to indications with stronger preclinical support (VWM, cognitive aging, TBI-associated cognitive decline) has not been tested and represents an unresolved research-clinical question.

## 8. Sourcing and Quality Verification

Research-grade ISRIB (trans-isomer) is supplied by multiple chemical and pharmaceutical research vendors, including Sigma-Aldrich (catalog SML0843), Selleck Chemicals, Cayman Chemical, MedChemExpress, Tocris Bioscience, Focus Biomolecules, and Santa Cruz Biotechnology. Supply is in milligram to gram quantities as the free base (trans-isomer). Pricing is moderate, typically in the range of \$100 to \$500 per 5 to 50 milligrams of research-grade material at greater than 98 percent purity by HPLC.

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

- High-performance liquid chromatography area-percent purity (greater than 98 percent, trans-isomer)
- Mass spectrometry confirmation of molecular weight (451.34 g/mol;  $[M+H]^+ = 452.12$ )
- Proton nuclear magnetic resonance spectroscopy to confirm trans-isomer identity (the trans-isomer shows diagnostic symmetry-related peak patterns distinct from the less symmetrical cis-isomer; coupling constant analysis of cyclohexane ring protons distinguishes the diequatorial trans-isomer from the equatorial-axial cis-isomer)
- Melting point determination (201 to 205 degrees Celsius for the trans-isomer)
- Certificate of analysis review confirming that the supplied material is the trans-isomer (CAS 1597403-47-8), not the less active cis-isomer or unresolved mixture (CAS 548470-11-7)

The critical quality attribute is diastereomeric purity: early commercial lots contained variable proportions of the cis-isomer, which is approximately 100-fold less active [1]. Investigators should request and verify trans-isomer purity from the vendor certificate of analysis and confirm by NMR if the cis/trans ratio is not explicitly stated. Third-party analytical laboratories (Janoshik Analytical, Anabolic Lab) can provide independent confirmation of vendor certificates of analysis. The research-grade ISRIB market is well established; counterfeit material is uncommon, but lot-

to-lot variability in diastereomeric composition has been observed, and on-receipt analytical confirmation is appropriate for any investigation where dose-response precision is important.

## 9. Reconstitution and Handling

ISRIB is poorly soluble in water and requires organic solvent-based vehicle formulations for both in vitro and in vivo applications.

**Stock solution preparation (in vitro):** Dissolve in DMSO at 10 to 100 mM (4.5 to 45 mg/mL). Stock solutions in DMSO are stable at minus 20 degrees Celsius for at least 6 months with protection from moisture. Aliquot into single-use volumes to avoid freeze-thaw cycles; published stability data indicate measurable degradation after more than 3 to 5 freeze-thaw cycles. For cell culture applications, dilute DMSO stock into culture medium immediately before use; final DMSO concentration should not exceed 0.1 percent (v/v) to avoid solvent toxicity. At working concentrations of 100 to 500 nM, the DMSO concentration from a 10 mM stock is well below this threshold.

**In vivo formulation (intraperitoneal injection, mice):** Dissolve ISRIB in DMSO with gentle warming (37 to 40 degrees Celsius) to achieve a clear solution, then dilute 1:1 (v/v) with polyethylene glycol 400 (PEG 400). The final injection vehicle is 50 percent DMSO / 50 percent PEG 400 (v/v) [5, 6, 7]. Alternative formulations include DMSO/PEG 400/Tween-80 mixtures at 5:45:5:45 (DMSO:PEG 400:Tween-80:saline, v/v/v/v) to reduce DMSO content. Injection volumes are typically 5 to 10 microliters per gram body weight. The solution should be prepared fresh or used within 24 hours of preparation; precipitation may occur on standing, particularly at higher compound concentrations.

**Storage of solid compound:** Store at minus 20 degrees Celsius in a desiccated container, protected from light and moisture. The compound is stable under these conditions for at least 2 years per vendor specifications.

**Handling precautions:** ISRIB is classified as a research compound with no established human safety profile. Standard laboratory personal protective equipment (gloves, laboratory coat, eye protection) should be used during handling. The compound is not volatile under standard laboratory conditions and does not require fume hood containment for routine weighing and dissolution, but prudent practice recommends minimizing inhalation of any research powder.

## 10. Stack Interactions and Combinations

ISRIB is a modulator of a single pathway (the ISR, via eIF2B) with no characterized off-target receptor or enzyme interactions. Stack interaction considerations therefore center on pharmacodynamic interactions with other ISR-pathway modulators and with agents that affect the upstream or downstream components of the pathway.

**PERK kinase inhibitors (GSK2606414, AMG PERK Inhibitor 44):** Concurrent administration of ISRIB and a PERK kinase inhibitor produces pharmacological redundancy for the PERK-driven component of the ISR but does not provide the pancreatic safety advantage of ISRIB alone. The combination is not recommended because the upstream kinase inhibitor reintroduces the toxicity that ISRIB was designed to avoid [5].

**eIF2alpha phosphatase modulators (Sephin1/IFB-088, salubrinal):** Sephin1 inhibits the phosphatase complex (PPP1R15A/GADD34-PP1c) that dephosphorylates eIF2alpha-P, thereby prolonging rather than inhibiting the ISR. The combination of ISRIB and Sephin1 produces opposing pharmacodynamic effects and is generally not useful except in mechanistic dissection studies. Salubrinal similarly prolongs ISR activation and functionally antagonizes ISRIB.

**Trazodone:** Halliday et al. (2017) identified trazodone hydrochloride as a repurposed drug that, like ISRIB, acts downstream of eIF2alpha-P to restore translation in prion-diseased mice [21]. The mechanism appears to involve partial eIF2B activation at high concentrations. Concurrent use with ISRIB in research would be expected to produce additive ISR inhibition; no published combination studies exist.

**GCN2 inhibitors (GCN2-IN-1 and related compounds):** Concurrent administration would produce additive ISR suppression in contexts where GCN2 is the activated kinase. The interaction has research utility for pathway dissection but has not been evaluated for safety in chronic dosing.

**mTOR pathway modulators (rapamycin, torin):** The ISR and mTOR pathways converge on translational control. Rapamycin (which inhibits mTORC1 and reduces cap-dependent translation initiation) may produce complex interactions with ISRIB depending on the relative contributions of each pathway to the translational state. No systematic combination pharmacology has been published.

**Cholinesterase inhibitors (donepezil, rivastigmine) and memantine:** These Alzheimer's disease treatments operate through distinct mechanisms (cholinergic enhancement and NMDA receptor antagonism, respectively). No pharmacokinetic or pharmacodynamic interaction with ISRIB is expected. The combination could be of interest in translational research on neurodegenerative cognitive impairment.

**DMSO vehicle considerations:** Because ISRIB requires DMSO for dissolution, investigators using the compound in cell-based assays should control for DMSO effects on cellular physiology, particularly at DMSO concentrations above 0.1 percent. In vivo, the DMSO content of the injection vehicle should be reported, as DMSO itself has anti-inflammatory and membrane-permeabilizing properties that may confound pharmacological interpretation at high concentrations.

**CYP enzyme modulators:** Because the specific CYP isoforms responsible for ISRIB metabolism are not characterized, the potential for metabolic drug-drug interactions cannot be assessed from published data.

# 11. Adverse Events and Safety Signal

## 11.1 Preclinical safety profile

ISRIB has been administered to mice and rats in multiple published studies at doses of 0.25 to 5 mg/kg intraperitoneal, with dosing durations ranging from single injections to daily treatment for 3 to 4 weeks. The compound has been generally well tolerated across these studies, with no overt behavioral toxicity, weight loss, or organ-system adverse events at doses up to 2.5 mg/kg [1, 5, 6, 7].

## 11.2 Pancreatic safety

The most important safety observation for ISRIB is the absence of pancreatic toxicity, in direct contrast to the upstream PERK inhibitor GSK2606414 [5]. PERK is constitutively active in pancreatic acinar cells (which have a high secretory protein synthesis load), and complete PERK inhibition produces rapid acinar cell death, exocrine pancreatic destruction, and approximately 50 percent reduction in pancreatic weight in mice. ISRIB treatment at equivalent ISR-inhibitory doses produced no detectable change in pancreatic weight, histological architecture, or exocrine and endocrine function. The mechanistic basis for the differential toxicity is the partial restoration of translation by ISRIB (approximately 50 to 70 percent of control levels, versus near-complete restoration by PERK inhibition), which maintains sufficient translational control in pancreatic cells to preserve proteostatic surveillance [5].

Multi-organ histopathology has been examined in studies employing chronic ISRIB dosing (14 to 28 days at 2.5 mg/kg intraperitoneally) in mice, with no inflammatory cell infiltration or histopathological changes in heart, liver, spleen, lung, or kidney attributable to treatment.

## 11.3 High-dose toxicity

One report noted excessive mortality in an Alzheimer's disease mouse model at the 5 mg/kg dose, suggesting a narrow therapeutic index at higher exposures. The specific cause of death was not characterized. Effective and non-lethal dosing sched-

ules at lower doses (0.25 to 2.5 mg/kg) have been well established across multiple laboratories and multiple mouse strains [1, 5, 6, 7].

## 11.4 Theoretical safety considerations

Because the ISR is a fundamental cytoprotective pathway, systemic ISR inhibition carries theoretical risks of impaired cellular adaptation to stress, including viral infection (PKR-dependent ISR), amino acid deprivation (GCN2-dependent ISR), and heme deficiency (HRI-dependent ISR). The confined window of ISRIB activity (suppression of low-to-moderate ISR but not strong acute ISR) partially mitigates these risks [18], but long-term chronic dosing in conditions of concurrent cellular stress has not been systematically evaluated.

Cancer implications are context-dependent: the ISR is activated in many solid tumors as an adaptive survival mechanism. ISR suppression by ISRIB could impair tumor adaptation to hypoxia and nutrient stress (potentially antitumor, as observed in prostate cancer models [10]), or could impair ISR-mediated tumor cell death under extreme stress conditions. The net effect of ISR inhibition on tumor biology is incompletely characterized.

No reproductive or developmental toxicology studies of ISRIB have been published.

## 11.5 Human safety data

No human safety data for ISRIB itself exist. The derivative compounds DNL343 and fosigotifator were generally well tolerated in Phase 1 and Phase 2/3 clinical trials, with the principal adverse events being mild gastrointestinal symptoms (nausea, diarrhea) [14, 15]. The intermediate derivative 2BAct was discontinued due to cardiovascular QT prolongation in canine preclinical safety studies, indicating that the eIF2B activator scaffold is not exempt from off-target pharmacology and that each derivative requires independent safety characterization. These data provide indirect evidence that the eIF2B activator mechanism is tolerable in humans at ISR-modulating doses, although the structural differences between ISRIB and the clinical derivatives preclude direct extrapolation.

## 12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five ISR-modulating compounds against ISRIB, evaluated against five competency standards: novelty, effect size, promising potential (current development state and trajectory), side-effect profile (preclinical or clinical), and overall validation. The five comparators were selected as the principal pharmacological alternatives for modulating the ISR pathway, with priority on compounds that have produced substantive preclinical or clinical evidence.

### 12.1 GSK2606414 (PERK kinase inhibitor)

**Mechanism:** Selective ATP-competitive inhibitor of the PERK kinase (EIF2AK3). Blocks phosphorylation of eIF2alpha at the level of the ER-stress-sensing kinase. IC50 approximately 0.4 nM against PERK in cell-free assays [22].

**Development trajectory:** GlaxoSmithKline tool compound. Advanced through preclinical neuroprotection studies in prion-diseased mice (Moreno et al. 2013), where it demonstrated potent neuroprotective effects, but development was halted due to severe on-target pancreatic toxicity (exocrine acinar cell destruction, approximately 50 percent reduction in pancreatic weight) [5, 22].

**Novelty:** Moderate. First reported selective PERK inhibitor; mechanism is well characterized but the single-kinase approach is inherently limited to PERK-driven ISR activation.

**Effect size:** Strong neuroprotection in prion mice (near-complete prevention of neuronal loss), but confounded by lethal pancreatic toxicity that limits interpretability of chronic dosing studies.

**Promising potential:** Very low. Development terminated due to toxicity. The compound persists as a research tool for acute in vitro PERK inhibition studies where pancreatic toxicity is not relevant.

**Side-effect profile:** Severe. Pancreatic exocrine destruction, weight loss, and hyperglycemia on chronic dosing in mice. The toxicity is on-target: PERK is required for proteostatic surveillance in high-secretory-load cells [5, 22].

**Overall validation:** Low as a therapeutic strategy; high as a research tool for understanding PERK-specific ISR biology.

**Comparison to ISRIB:** GSK2606414 operates upstream of ISRIB (at PERK rather than at eIF2B) and produces more complete ISR inhibition, which is both its strength (greater efficacy in acute neuroprotection) and its weakness (on-target pancreatic destruction). ISRIB's partial ISR inhibition and downstream point of action produce a dramatically superior safety profile. ISRIB is effective across all four eIF2alpha kinase pathways; GSK2606414 blocks only the PERK-driven component. For research applications requiring ISR pathway inhibition, ISRIB is strongly preferred over GSK2606414 except in contexts specifically requiring PERK-selective pharmacology.

## 12.2 DNL343 (Denali Therapeutics eIF2B activator)

**Mechanism:** Oral, brain-penetrant eIF2B activator with improved solubility and pharmacokinetic properties relative to ISRIB. Promotes eIF2B decamer assembly through a mechanism analogous to ISRIB but with a distinct chemical scaffold [14].

**Development trajectory:** Completed Phase 1 in healthy volunteers. Advanced to Phase 2/3 in the HEALEY ALS Platform Trial (Regimen G, 186 participants). Failed to meet primary endpoint (ALSFRS-R change over 24 weeks). Additional analyses did not demonstrate treatment effect on NfL biomarkers. Generally well tolerated [14].

**Novelty:** Moderate. The mechanism is analogous to ISRIB; the novelty is in the pharmaceutical improvement (oral bioavailability, solubility) rather than in the pharmacological mechanism.

**Effect size:** Not assessable for cognition (not studied in cognitive indications). No significant effect on ALS disease progression in Phase 2/3. Pharmacodynamic biomarker engagement confirmed in cerebrospinal fluid.

**Promising potential:** Low for ALS. Unclear for other indications. The compound has demonstrated that the eIF2B activator mechanism is tolerable in humans but has not demonstrated clinical efficacy.

**Side-effect profile:** Favorable. Mild gastrointestinal events (nausea, diarrhea) were the most common adverse events. No pancreatic toxicity, no serious adverse events attributed to the compound [14].

**Overall validation:** Moderate. The compound has validated the tolerability of eIF2B activation in humans but has not validated the therapeutic hypothesis in ALS.

**Comparison to ISRIB:** DNL343 is the clinical-stage evolution of the ISRIB mechanism with improved pharmaceutical properties. For preclinical research, ISRIB remains the preferred tool compound due to its extensive characterization, lower cost, and wide availability. For clinical translation, DNL343 has superior properties but has not demonstrated efficacy.

## 12.3 Fosigotifator (Calico/AbbVie eIF2B activator)

**Mechanism:** Oral eIF2B activator co-developed by Calico Life Sciences and AbbVie. Specific mechanism and binding site relative to ISRIB have not been fully disclosed publicly [15].

**Development trajectory:** Evaluated in the HEALEY ALS Platform Trial. Failed to meet primary endpoint. An exploratory high-dose arm showed a statistically significant signal on muscle strength preservation, suggesting possible dose-dependent efficacy [15]. Phase 1b/2 study in vanishing white matter disease initiated in 2023.

**Novelty:** Moderate. Shares the eIF2B activator mechanism with ISRIB and DNL343.

**Effect size:** Exploratory high-dose arm showed signal on muscle strength in ALS. Primary endpoint not met. VWM efficacy data pending.

**Promising potential:** Low to moderate. The high-dose signal provides a rationale for dose escalation studies. The VWM indication targets the disease most directly caused by eIF2B deficiency and has stronger preclinical support than ALS.

**Side-effect profile:** Generally well tolerated in reported clinical data. The predecessor compound 2BAct was discontinued for cardiovascular QT prolongation in canine preclinical studies, warranting attention to cardiovascular safety monitoring.

**Overall validation:** Low to moderate. Similar to DNL343, tolerability has been demonstrated but efficacy has not been confirmed.

**Comparison to ISRIB:** Like DNL343, fosgotifator represents a pharmaceutical improvement over ISRIB for clinical applications. The high-dose signal is potentially informative for the broader eIF2B activator class, suggesting that higher levels of eIF2B activation may be required for clinical effect in rapidly progressive neurodegeneration.

## 12.4 Trazodone (repurposed ISR modulator)

**Mechanism:** Clinically approved antidepressant (serotonin antagonist and reuptake inhibitor) that, at high concentrations, produces partial ISR inhibition downstream of eIF2 $\alpha$ -P, possibly through low-affinity eIF2B activation or through indirect effects on translational control [21]. The ISR-modulatory activity is distinct from and occurs at concentrations higher than the serotonergic activity.

**Development trajectory:** Clinically approved and widely prescribed for depression and insomnia. Halliday et al. (2017) reported neuroprotection in prion-diseased mice at oral doses corresponding to clinically achievable concentrations [21]. Clinical investigation of trazodone for neurodegenerative indications is being explored but has not produced formal efficacy trial results.

**Novelty:** Low for the serotonergic mechanism; moderate for the ISR-modulatory activity, which represents an unexpected off-target pharmacology of a known drug.

**Effect size:** Neuroprotective in prion mice. Clinical ISR-modulatory effect size in humans at tolerable doses is unknown and may be insufficient for ISR-dependent therapeutic effect.

**Promising potential:** Moderate as a repurposed drug due to established human safety data and oral bioavailability, but limited by the substantially lower potency for ISR modulation relative to ISRIB and clinical-stage derivatives.

**Side-effect profile:** Well characterized at antidepressant doses (sedation, orthostatic hypotension, dry mouth, rare priapism). The additional serotonergic pharmacology introduces confounds for ISR-specific therapeutic development.

**Overall validation:** Low for ISR-modulatory indication; high for antidepressant indication.

**Comparison to ISRIB:** Trazodone has the advantage of an established clinical safety profile and oral bioavailability, but its ISR-modulatory potency is orders of magnitude lower than ISRIB, and the additional multi-target pharmacology (5-HT<sub>2A</sub> antagonism, alpha-1 adrenergic antagonism, serotonin reuptake inhibition) makes it unsuitable for mechanistic ISR research. For preclinical ISR research, ISRIB is strongly preferred. For clinical translation of ISR inhibition, trazodone offers an accessible but pharmacologically imprecise option.

## 12.5 Sephin1 (IFB-088; ISR prolonger)

**Mechanism:** Selective inhibitor of the stress-induced eIF2alpha-P phosphatase complex PPP1R15A (GADD34)/PP1c. Prolongs the ISR by preventing the feedback dephosphorylation of eIF2alpha-P, in contrast to ISRIB which inhibits the ISR [23]. The mechanism is pharmacologically opposite to ISRIB.

**Development trajectory:** InFlectis BioScience advanced IFB-088 (the clinical form of Sephin1) into clinical trials for Charcot-Marie-Tooth type 1A (CMT1A) and potentially for other protein-misfolding diseases where ISR prolongation is protective. Phase 2 data in CMT1A have been reported with preliminary positive signals [23].

**Novelty:** High. The ISR-prolonging strategy is mechanistically opposite to ISR inhibition and targets a distinct set of diseases (those in which ISR activation is protective and insufficiently sustained, such as protein-misfolding disorders of the peripheral nervous system). This is the most mechanistically distinct compound in the comparator panel.

**Effect size:** Positive in Charcot-Marie-Tooth and in protein-misfolding models. Not applicable to the cognitive enhancement, TBI, or aging indications where ISRIB has been characterized.

**Promising potential:** Moderate to high for CMT1A and protein-misfolding diseases. Not relevant for ISR-inhibitory indications.

**Side-effect profile:** Phase 2 data suggest acceptable tolerability. Theoretical risk of excessive ISR activation in tissues that require translational flux, but not observed clinically [23].

**Overall validation:** Moderate. Phase 2 clinical data in CMT1A support the concept; the compound validates the ISR-prolonging arm of ISR pharmacology.

**Comparison to ISRIB:** Sephin1 and ISRIB modulate the ISR in opposite directions. They are complementary research tools for dissecting the contribution of ISR timing and magnitude to disease pathology. They are not alternatives for the same indication but rather represent the two pharmacological poles of ISR modulation. For cognitive enhancement, TBI recovery, and neurodegenerative translation rescue, ISRIB (ISR inhibition) is the relevant pharmacology; for protein-misfolding diseases and myelination protection, Sephin1 (ISR prolongation) may be preferred.

## 12.6 Comparator summary

The five comparator compounds span the pharmacological space of ISR modulation. **GSK2606414** validates the concept that ISR inhibition is neuroprotective but is unusable for chronic dosing due to pancreatic toxicity; its failure as a therapeutic agent underscores the importance of the partial, downstream mechanism of ISRIB. **DNL343** and **fosigotifator** are pharmaceutical improvements of ISRIB that have validated the tolerability of eIF2B activation in humans but have not yet demonstrated clinical efficacy; the negative ALS results do not invalidate the mechanism for other indications. **Trazodone** offers an accessible, clinically approved compound with weak ISR-modulatory activity that is insufficient for mechanistic ISR research but may represent a pragmatic clinical option in the absence of approved eIF2B activators. **Sephin1** represents the opposite pharmacological strategy (ISR prolongation) and validates the broader principle that the ISR is a pharmacologically tractable pathway with disease-specific directionality requirements.

ISRIB occupies a unique position in this landscape as the most extensively characterized ISR-inhibitory tool compound, the prototype for the eIF2B activator mechanism, and the standard against which all other ISR modulators are benchmarked. Its limitations (poor solubility, no oral formulation, no human safety data) are pharmaceutical rather than pharmacological, and the development of DNL343 and fosigotifator demonstrates that these limitations are addressable through medicinal chemistry optimization. The principal unresolved question is whether eIF2B activation produces sufficient clinical benefit in any human disease to justify continued development; the negative ALS results have reduced but not eliminated enthusiasm, and indications with stronger preclinical ISR rationale (cognitive aging, TBI, VWM) remain untested in human studies.

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