

RESEARCH MONOGRAPH . COMPOUND PROFILE

NSI-189

A first-in-class small molecule neurogenic agent discovered through phenotypic screening of human hippocampal neural stem cells, developed for major depressive disorder and under investigation for cognitive impairment, diabetic neuropathy, and post-traumatic stress disorder.

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ABSTRACT

NSI-189 (amdiglurax; ALTO-100) is a benzylpiperazine-aminopyridine small molecule identified through a phenotypic screen of approximately 10,000 compounds against human hippocampal neural stem cells and advanced as a first-in-class hippocampal neurogenesis stimulator for the treatment of major depressive disorder (MDD). The compound was discovered by Karl Johe and colleagues at Neuralstem, Inc. (Germantown, Maryland) and is now under development by Alto Neuroscience (Mountain View, California) under the designation ALTO-100. NSI-189 is mechanistically distinct from all marketed antidepressants: it has no detectable activity at serotonin, norepinephrine, or dopamine transporters, no binding at 52 standard neurotransmitter receptor and ion channel targets, and no activity across a panel of 900 kinases. Instead, the compound stimulates proliferation and neurogenic differentiation of hippocampal neural stem cells in vitro with low-micromolar potency and, on oral administration to rodents at 10 to 30 mg/kg/day, produces dose-dependent increases in hippocampal volume (up to 66 percent at 30 mg/kg in mice), upregulation of brain-derived neurotrophic factor (BDNF), stem cell factor (SCF), glial cell line-derived neurotrophic factor (GDNF), and vascular endothelial growth factor (VEGF), and activation of the TrkB/Akt signaling pathway. The morphological effects are confined to the dentate gyrus of the hippocampus and the subventricular zone; no structural changes have been observed elsewhere in the brain. A bell-shaped dose-response relationship is observed in preclinical hippocampal volume endpoints, with 100 mg/kg producing less

effect than 30 mg/kg, suggesting an optimal range for neurogenic stimulation.

Clinical development has proceeded through Phase 1 (41 healthy volunteers, 2011), Phase 1b (24 MDD patients, Fava et al. 2016, published in *Molecular Psychiatry*), and Phase 2 (220 MDD outpatients, Papakostas et al. 2020, published in *Molecular Psychiatry*). The Phase 1b trial demonstrated safety and tolerability at 40, 80, and 120 mg daily for 28 days, with medium-to-large effect sizes on the Symptoms of Depression Questionnaire (SDQ) and the Cognitive and Physical Functioning Questionnaire (CPFQ). The Phase 2 trial, conducted using a sequential-parallel comparison design across 12 weeks, did not meet its primary endpoint (change from baseline on the Montgomery-Asberg Depression Rating Scale, MADRS) at either 40 mg or 80 mg daily. However, 40 mg daily produced statistically significant improvements on the SDQ (pooled mean difference -8.2; Cohen's d = -0.64 in Stage 2; p = 0.04), the CPFQ (pooled mean difference -1.9; p = 0.03), and several objective cognitive measures on the CogScreen battery (Cohen's d ranging from 0.12 to 1.12 for significant measures). Hippocampal volume was not significantly changed in MDD patients at the studied doses and duration, despite the robust preclinical volumetric signal.

Alto Neuroscience acquired the NSI-189 program in October 2021 and redesignated the compound ALTO-100. A Phase 2b trial (301 adults with MDD, 34 U.S. sites, 6 weeks, biomarker-enriched design using a cognitive memory test) reported topline results in 2024: ALTO-100 did not demonstrate statistically significant improvement in MADRS versus placebo in the biomarker-defined population and did not meet secondary endpoints. The compound was well tolerated, with headache, nausea, and abnormal dreams

as the most common adverse events at rates similar to placebo. A Phase 2b trial in bipolar depression is expected to report in 2026, and the compound remains under investigation for post-traumatic stress disorder.

Preclinical pharmacology extends beyond depression. NSI-189 reverses cognitive and motor deficits in a rat model of ischemic stroke (30 mg/kg oral), ameliorates central and peripheral neuropathy in mouse models of type 1 and type 2 diabetes (10 to 30 mg/kg oral), enhances synaptic plasticity and reverses motor and cognitive impairments in a mouse model of Angelman syndrome through TrkB/Akt pathway activation, and enhances long-term potentiation in hippocampal slice preparations in vitro. The compound has linear pharmacokinetics across the 40 to 120 mg/day clinical dose range, an oral T_{max} of 1 to 2 hours, a plasma elimination half-life of 17.4 to 20.5 hours supporting once-daily dosing, and achieves steady state within 4 to 5 days.

This monograph reviews the chemical identity and synthesis of NSI-189; the discovery through phenotypic screening; the molecular pharmacology and neurotrophic factor cascade; the comprehensive pharmacokinetic profile; the preclinical evidence base across depression, stroke, neuropathy, and Angelman syndrome models; the clinical evidence base from Phase 1 through Phase 2b; sourcing, reconstitution, and handling considerations; stack interactions; the adverse-event and safety signal; and a structured comparative assessment of five neurogenesis-associated compounds (fluoxetine, ketamine, agomelatine, psilocybin, and 7,8-dihydroxyflavone) against NSI-189 on five competency standards. The compound is not approved by any regulatory authority for any indication. It is sold as a research-

grade preparation; investigators should obtain analytical confirmation of identity and purity on every lot.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Amdiglurax.

Development codes: NSI-189 (Neuralstem, Inc.); ALTO-100 (Alto Neuroscience). The development code NSI-189 is the canonical identifier in the published pharmacology and clinical literature and the identifier under which the compound was first described, screened, and advanced through Phase 1 and Phase 2 clinical trials [1, 2].

Chemical Abstracts Service registry numbers: 1270138-40-3 (free base); 1270138-41-4 (phosphate salt). The phosphate salt is the pharmaceutical development form and the material used in all published clinical trials. The free base is available from research chemical suppliers.

International Union of Pure and Applied Chemistry name: (4-benzylpiperazin-1-yl)-[2-(3-methylbutylamino)pyridin-3-yl]methanone.

PubChem CID: 49856040 (free base); 50922680 (phosphate salt).

1.2 Structural class and chemistry

NSI-189 is a benzylpiperazine-aminopyridine small molecule. The structural architecture comprises three linked units: a 2-aminopyridine ring bearing a 3-methylbutylamino (isopentyl amino) substituent at the 2-position and a carbonyl bridge at the 3-position; a piperazine ring connected through the carbonyl to the pyridine; and a benzyl group on the distal piperazine nitrogen. The compound belongs to no established pharmacological class of antidepressant, anxiolytic, or cognitive enhancer; it was identified by phenotypic screening rather than target-based design and has no structural homology to monoamine reuptake inhibitors, serotonin receptor ligands, or known neurotrophin mimetics [1].

Molecular formula: C₂₂H₃₀N₄O (free base); C₂₂H₃₃N₄O₅P (phosphate salt).

Molecular weight: 366.50 g/mol (free base); 464.50 g/mol (phosphate salt).

Physical properties: White to off-white crystalline powder (phosphate salt). The phosphate salt is soluble in dimethyl sulfoxide (DMSO) at concentrations of approximately 50 to 100 mg/mL and has limited aqueous solubility at physiological pH. The free base has moderate lipophilicity. Storage conditions for the solid are room temperature or 2 to 8 degrees Celsius, protected from moisture and light.

Stereochemistry: NSI-189 contains no stereocenters and is supplied as a single achiral entity. No enantiomeric or diastereomeric considerations apply to quality verification.

1.3 Synthesis

The published and patent literature describes the synthesis of NSI-189 through a convergent route involving three principal stages. The first stage is the preparation of the 2-(3-methylbutylamino)pyridine-3-carboxylic acid or its activated ester, achieved by nucleophilic aromatic substitution of a 2-halopyridine-3-carboxylate with 3-methylbutylamine (isoamylamine) under base catalysis. The second stage is the preparation of 1-benzylpiperazine, which is commercially available or prepared by reductive alkylation of piperazine with benzaldehyde. The third stage is amide bond formation between the pyridine-3-carboxylic acid and the piperazine nitrogen, typically mediated by carbodiimide or phosphonium-based coupling reagents (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide with hydroxybenzotriazole, or benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate). The product is purified by silica gel chromatography or recrystallization and characterized by nuclear magnetic resonance spectroscopy, mass spectrometry, and high-performance liquid chromatography. The phosphate salt is prepared by stoichiometric addition of phosphoric acid in an appropriate solvent followed by recrystallization. The synthesis is straightforward and amenable to scale-up; research-grade material is supplied by multiple vendors at greater than 98 percent purity [3].

2. Abstract

NSI-189 (amdiglurax; ALTO-100) is a first-in-class benzylpiperazine-aminopyridine neurogenic compound that stimulates hippocampal neural stem cell proliferation, increases hippocampal volume, and upregulates brain-derived neurotrophic factor and related neurotrophic factors in preclinical models, and has been evaluated in clinical trials for major depressive disorder through Phase 2b [1, 2, 4, 5].

The compound was discovered at Neuralstem, Inc. through a phenotypic screen of human hippocampal neural stem cells. It has no activity at monoamine transporters, monoamine receptors, or kinase panels, and its mechanism of action is distinct from all marketed antidepressants. The molecular target or targets through which NSI-189 drives neurogenesis remain incompletely characterized; the downstream signaling cascade includes upregulation of BDNF, SCF, GDNF, and VEGF, and activation of the TrkB receptor tyrosine kinase and Akt pathway [6, 7].

Clinical development has shown a consistent dissociation between cognitive and mood endpoints. The Phase 1b trial (Fava et al. 2016) demonstrated safety and tolerability at 40 to 120 mg daily in 24 MDD patients over 28 days, with significant improvements on self-rated depression and cognitive functioning measures [4]. The Phase 2 trial (Papakostas et al. 2020) in 220 MDD outpatients over 12 weeks failed its primary MADRS endpoint but showed significant benefits on self-rated depression (SDQ), cognitive functioning (CPFQ), and objective cognitive testing (CogScreen) at 40 mg daily [5]. Alto Neuroscience acquired the program in 2021 and conducted a Phase 2b trial (301 MDD patients, 6 weeks, biomarker-enriched design) that also failed to demonstrate significant improvement on MADRS versus placebo [8].

Preclinical pharmacology extends to ischemic stroke, diabetic neuropathy, and Angelman syndrome, where the compound has demonstrated efficacy in rodent models at oral doses of 10 to 30 mg/kg [9, 10, 11]. The compound has favorable pharmacokinetic properties: oral Tmax of 1 to 2 hours, plasma half-life of 17.4 to 20.5 hours, linear kinetics across clinical doses, and steady-state attainment within 4 to 5 days [4, 5].

The compound is not approved by any regulatory authority. It remains under investigation for bipolar depression and post-traumatic stress disorder. Research-grade material is available from multiple suppliers; investigators should confirm

identity and purity on each lot.

3. Discovery and Development History

3.1 Origin and phenotypic screening

NSI-189 was discovered at Neuralstem, Inc. (Germantown, Maryland; founded 1997 by Karl Johe) through a phenotypic screening program that leveraged Neuralstem's proprietary technology for culturing expandable human neural stem cells from adult hippocampal tissue. Beginning in approximately 2000, Neuralstem developed neural stem cell lines from the human hippocampus and established a screening cascade to identify small molecules capable of stimulating neural stem cell proliferation under growth-factor-free conditions [1]. The primary assay measured cell proliferation (ATP-based luminescence and bromodeoxyuridine incorporation) in human hippocampal neural stem cells cultured without exogenous growth factors; secondary assays assessed neurogenic differentiation, neurite outgrowth, and electrophysiological maturation. NSI-189 was identified from this screen as a hit with potent proliferative activity and neurogenic differentiation capacity.

Neuralstem filed patent applications on NSI-189 and related chemical entities beginning in approximately 2008 to 2009 and was granted United States patents on four first-in-class neurogenic chemical entities. The compound was selected for development on the basis of oral bioavailability, brain penetration, neurogenic potency in vitro and in vivo, and tolerability in preclinical toxicology studies. Early development received support from the Defense Advanced Research Projects Agency (DARPA) and the National Institutes of Health (NIH) [1, 12].

3.2 Phase 1 and Phase 1b clinical trials

Neuralstem filed an investigational new drug (IND) application with the United States Food and Drug Administration and received approval to initiate a Phase 1 trial in 2011. The Phase 1 single ascending dose study enrolled 41 healthy volunteers and established safety and tolerability across the planned dose range [1].

The Phase 1b trial (NCT01520649) was a randomized, double-blind, placebo-controlled, multiple-dose escalation study in 24 patients with MDD, conducted at the Massachusetts General Hospital Clinical Trials Network and Institute under the direction of Maurizio Fava. Three cohorts received 40 mg once daily (n = 6 active, n = 2

placebo), 40 mg twice daily (n = 6 active, n = 2 placebo), or 40 mg three times daily (n = 6 active, n = 2 placebo) for 28 days in an inpatient setting [4]. The trial was completed in July 2014 and results published in *Molecular Psychiatry* in 2016.

3.3 Phase 2 clinical trial

The Phase 2 trial (NCT02695472) was a double-blind, placebo-controlled study using a sequential-parallel comparison design (SPCD) to improve signal detection in the placebo-responsive MDD population. The trial enrolled 220 outpatients with MDD at multiple United States sites and randomized them to NSI-189 phosphate 40 mg daily, 80 mg daily, or placebo for 12 weeks [5]. Topline results, announced in July 2017, indicated that the trial failed to meet its primary endpoint. Full results were published by Papakostas et al. in *Molecular Psychiatry* in 2020.

3.4 Transfer to Alto Neuroscience

Neuralstem, Inc. underwent corporate restructuring beginning in approximately 2019 and ultimately merged into Palisade Bio in 2021. In October 2021, Alto Neuroscience (Mountain View, California) acquired the NSI-189 program from Palisade Bio for up to \$4.9 million and redesignated the compound ALTO-100 [8].

3.5 Alto Neuroscience Phase 2b and ongoing development

Alto Neuroscience conducted a Phase 2b trial of ALTO-100 in MDD (301 adults, 34 U.S. sites, 6-week treatment, biomarker-enriched design using a web-based cognitive memory test to select patients). Topline results reported in late 2024 indicated that ALTO-100 did not demonstrate statistically significant improvement in MADRS versus placebo in the biomarker-defined population or on secondary endpoints [8]. The compound was well tolerated with no new safety signals.

Two additional MDD studies (designated ALTO-203 and ALTO-300) were expected to report results in the first half of 2025. A Phase 2b trial in bipolar depression is expected to read out in 2026. ALTO-100 also remains under investigation for post-traumatic stress disorder (PTSD). The compound is not in active Phase 3 development for any indication as of this monograph revision [8].

4. Mechanism of Action

4.1 Neurogenic phenotypic activity

NSI-189 was identified through a functional screen, not through target-based drug design, and its precise molecular target or targets remain incompletely characterized. The compound has no detectable affinity at 52 standard neurotransmitter receptors and ion channels (including serotonin, norepinephrine, and dopamine transporters; serotonin receptor subtypes 5-HT1A, 5-HT2A, 5-HT2C, 5-HT3; dopamine D1 through D5; adrenergic alpha-1, alpha-2, and beta receptors; muscarinic M1 through M5; histamine H1 through H3; GABA-A and GABA-B; NMDA; AMPA; and opioid receptors). Screening against a panel of 900 kinases produced no significant hits [1, 6].

The primary pharmacological activity observed *in vitro* is stimulation of human hippocampal neural stem cell proliferation and neurogenic differentiation under growth-factor-free conditions. The effect is concentration-dependent, with activity in the low-micromolar range (EC₅₀ approximately 0.1 to 1 micromolar, depending on the assay system). The neurogenic output is accompanied by upregulation of multiple neurotrophic factors in conditioned media, including BDNF, SCF, GDNF, and VEGF, with BDNF and SCF showing the most robust increases [6, 7].

4.2 BDNF and TrkB signaling

The functional antibody-blocking experiments reported in the preclinical literature indicate that the neurogenic effects of NSI-189 are dependent on BDNF and SCF signaling. Antibodies against BDNF and against SCF each partially blocked the proliferative effect of NSI-189 on hippocampal neural stem cells *in vitro*, indicating that the compound acts upstream of these neurotrophic factors and that its proliferative output is mediated through the neurotrophic factor cascade rather than through direct mitogenic receptor activation [6, 7].

The downstream signaling pathway includes activation of TrkB (tropomyosin receptor kinase B, the high-affinity receptor for BDNF), Akt (protein kinase B), and associated survival and plasticity pathways. In a mouse model of Angelman syn-

drome, NSI-189 reversed cognitive and motor deficits through demonstrated TrkB/Akt pathway activation, providing in vivo confirmation of the BDNF-TrkB signaling axis as a mediator of compound activity [11].

4.3 Hippocampal specificity

The morphological effects of NSI-189 are confined to two neurogenic niches: the dentate gyrus of the hippocampus and the subventricular zone (SVZ). No structural, volumetric, or proliferative changes have been observed in other brain regions. This regional specificity distinguishes NSI-189 from broadly neurotrophic interventions and is consistent with the compound acting on neural stem cell populations that are specific to these niches [1, 6].

4.4 Dose-response relationship

Preclinical studies in mice demonstrate a bell-shaped dose-response curve for hippocampal volume. At 10 mg/kg/day oral, hippocampal volume increased by approximately 36 percent; at 30 mg/kg/day, the increase was approximately 66 percent; at 100 mg/kg/day, the effect was less than at 30 mg/kg. This inverted-U pattern is characteristic of compounds that produce optimal effects within a defined concentration window and suggests that excessive stimulation of the neurogenic niche may engage compensatory or inhibitory feedback mechanisms [1, 6].

4.5 Synaptic plasticity

NSI-189 enhances long-term potentiation (LTP) in hippocampal slice preparations in vitro, supporting a direct effect on synaptic strength independent of, or in addition to, the neurogenic mechanism [12]. Enhancement of LTP is a recognized electrophysiological correlate of procognitive activity and may contribute to the cognitive improvements observed in clinical trials at doses and durations that did not produce detectable hippocampal volume changes in humans.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

NSI-189 phosphate is administered orally as a tablet formulation. The compound is well absorbed from the gastrointestinal tract, with a time to peak plasma concentration (T_{max}) of approximately 1 to 2 hours post-administration [4, 5]. Pharmacokinetic analyses from the Phase 1b trial demonstrated linear dose-proportional kinetics across the 40 to 120 mg/day dose range.

5.2 Distribution

Published data on volume of distribution and plasma protein binding in humans are limited. The compound crosses the blood-brain barrier, as evidenced by the central nervous system pharmacodynamic effects observed in both preclinical models (hippocampal volumetric increases, neurogenesis in the dentate gyrus) and in human clinical trials (cognitive improvements). Preclinical brain-to-plasma ratios support adequate central nervous system penetration at clinically relevant doses.

5.3 Metabolism

In vitro data indicate that NSI-189 undergoes limited oxidative and glucuronidated metabolism. The metabolic pathway does not appear to be dominated by a single cytochrome P450 isoform, which reduces the risk of pharmacogenomic variability analogous to the CYP2D6-driven polymorphic phenotype observed with tropisetron and certain other compounds. No active metabolites have been identified in published literature [4].

5.4 Elimination

The plasma elimination half-life of NSI-189 is 17.4 to 20.5 hours, supporting once-daily oral dosing. Steady-state plasma concentrations are reached within 4 to 5 days of daily dosing. The relatively long half-life and attainment of steady state within less than one week are pharmacokinetically favorable for chronic daily administration [4, 5].

5.5 Routes of administration

The only route of administration studied in clinical trials is oral. Preclinical studies have used oral gavage (10 to 30 mg/kg/day in rodents) and, in some in vitro experiments, direct application of the compound to cell culture media. No parenteral formulation has been developed or studied.

5.6 Special populations

No published data address pharmacokinetics in hepatic or renal impairment, in pediatric populations, or in the elderly. The clinical trial populations have been restricted to adults aged 18 to 60 years with MDD. Investigators conducting research in special populations should consider the absence of pharmacokinetic guidance.

6. Preclinical Pharmacology

6.1 Hippocampal neurogenesis in healthy rodents

Oral administration of NSI-189 phosphate at 10 to 30 mg/kg/day to healthy adult mice produces dose-dependent increases in hippocampal volume, with the peak effect (approximately 66 percent increase) at 30 mg/kg/day over 28 days [1, 6]. The volumetric increase is associated with increased bromodeoxyuridine-positive (BrdU+) cells in the subgranular zone of the dentate gyrus, confirming de novo neurogenesis rather than hypertrophy. Newly generated cells express mature neuronal markers (NeuN, doublecortin) and integrate into hippocampal circuitry as assessed by morphological and electrophysiological criteria.

6.2 Neurotrophic factor upregulation

In parallel with the in vivo neurogenic effects, conditioned media from NSI-189-treated hippocampal neural stem cell cultures show robust upregulation of BDNF and SCF, with more modest increases in GDNF and VEGF. The upregulation is concentration-dependent and temporally correlated with the onset of proliferative activity [6, 7].

6.3 Ischemic stroke

Cheng et al. (2017) demonstrated that oral NSI-189 phosphate at 30 mg/kg/day, initiated 24 hours after middle cerebral artery occlusion in rats, significantly improved stroke-induced motor deficits (rotarod, beam walk), reduced neurological deficit scores, and stimulated neurogenesis in the peri-infarct zone and subventricular zone [9]. Brain remodeling was accompanied by increased expression of neuroplasticity markers. The effect was sustained through the 28-day assessment period.

6.4 Diabetic neuropathy

Bhatt et al. (2019) reported that oral NSI-189 at 10 or 30 mg/kg/day ameliorated both central and peripheral neuropathy in mouse models of type 1 (streptozotocin-induced) and type 2 (db/db) diabetes [10]. The compound prevented small-fiber pe-

ripheral neuropathy (assessed by intraepidermal nerve fiber density), promoted hippocampal neurogenesis and synaptic marker expression, protected hippocampal volume, and preserved long-term memory. Motor nerve conduction velocity and sensory nerve action potentials were reversed toward normal values. The neuroprotective effects were associated with increased BDNF expression in both hippocampus and dorsal root ganglia.

6.5 Angelman syndrome

Allen et al. (2018) reported that NSI-189 enhanced synaptic plasticity and reversed impairments in motor and cognitive functions in a mouse model of Angelman syndrome (Ube3a maternal deletion) [11]. The compound activated TrkB/Akt signaling pathways and restored long-term potentiation in hippocampal slices from Angelman model mice. Motor coordination (rotarod), contextual fear memory, and novel object recognition were improved to levels approaching wild-type performance.

6.6 Enhancement of long-term potentiation

Bhatt et al. (2020) demonstrated that NSI-189 enhances long-term potentiation in hippocampal slice preparations from rats, including both naive animals and those with type 2 diabetes-associated cognitive impairment [13]. Enhancement of LTP provides an electrophysiological mechanism for the procognitive effects and supports a synaptic plasticity component of the compound's activity that may be independent of de novo neurogenesis.

7. Clinical Evidence Base

7.1 Phase 1: healthy volunteers

The Phase 1 single ascending dose study enrolled 41 healthy volunteers in 2011 and established safety and tolerability across the dose range subsequently used in MDD trials. The compound demonstrated linear pharmacokinetics and an adverse-event profile similar to placebo. No clinically significant laboratory, electrocardiographic, or vital sign abnormalities were reported [1].

7.2 Phase 1b: major depressive disorder (Fava et al. 2016)

The Phase 1b trial (NCT01520649; Fava et al., Molecular Psychiatry, 2016) was a randomized, double-blind, placebo-controlled, multiple-dose escalation study in 24 MDD patients conducted in an inpatient setting at the Massachusetts General Hospital Clinical Trials Network and Institute [4].

Design: Three sequential cohorts received NSI-189 phosphate 40 mg once daily (n = 6), 40 mg twice daily (n = 6), or 40 mg three times daily (n = 6), each with matched placebo (n = 2 per cohort), for 28 days.

Safety: NSI-189 was well tolerated at all doses. No serious adverse events occurred. The most common adverse events were headache, dizziness, and somnolence.

Efficacy (exploratory): NSI-189-treated patients showed improvements on the Symptoms of Depression Questionnaire (SDQ), the Montgomery-Asberg Depression Rating Scale (MADRS), the Clinical Global Impressions-Improvement (CGI-I) scale, and the Cognitive and Physical Functioning Questionnaire (CPFQ) compared to placebo. Effect sizes (Cohen's d) were medium to large across all measures. Notably, cognitive improvements were pronounced: memory (Cohen's d = 1.12, p = 0.002), working memory (d = 0.81, p = 0.020), and executive function (d = 0.66, p = 0.048).

Hippocampal volume: Structural MRI showed modest numerical increases in left hippocampal volume in the NSI-189 group that did not reach statistical significance (p = 0.12). No significant changes in amygdalar volume were observed.

7.3 Phase 2: major depressive disorder (Papakostas et al. 2020)

The Phase 2 trial (NCT02695472; Papakostas et al., Molecular Psychiatry, 2020) was a double-blind, placebo-controlled study using a sequential-parallel comparison design (SPCD) to reduce placebo response and improve signal detection [5].

Design: 220 MDD outpatients were randomized to NSI-189 phosphate 40 mg daily, 80 mg daily, or placebo for 12 weeks. The SPCD consisted of two stages: placebo non-responders from Stage 1 were re-randomized to active treatment or placebo in Stage 2, increasing statistical power.

Primary endpoint: Change from baseline in MADRS total score. NSI-189 40 mg showed a pooled mean difference of -1.8 versus placebo ($p = 0.22$); 80 mg showed -1.4 ($p = 0.34$). Neither dose met the primary endpoint.

Secondary endpoints: At 40 mg daily, statistically significant improvements were observed on: the SDQ (pooled mean difference -8.2; Cohen's $d = -0.11$ in Stage 1 and -0.64 in Stage 2; $p = 0.04$); the CPFQ (pooled mean difference -1.9; Cohen's $d = -0.28$ in Stage 1 and -0.47 in Stage 2; $p = 0.03$); and several CogScreen cognitive measures (absolute Cohen's d values between 0.12 and 1.12 in favor of NSI-189 for significant measures, p values 0.002 to 0.048). The 80 mg dose did not produce significant secondary endpoint improvements, consistent with the preclinical bell-shaped dose-response relationship.

Subgroup analyses: Post hoc analysis indicated that 80 mg was effective in moderately depressed patients (baseline MADRS less than 30) but not in severely depressed patients (MADRS 30 or greater).

Tolerability: Both doses were well tolerated. Discontinuation rates due to intolerance were lower in the active treatment arms than in the placebo arm.

7.4 Phase 2b: ALTO-100 in major depressive disorder (Alto Neuroscience, 2024)

Alto Neuroscience conducted a Phase 2b trial (301 adults with MDD, 34 U.S. sites, 6-week double-blind treatment) using a biomarker-enriched design in which patients were selected on the basis of a web-based memory test-derived cognitive biomarker [8].

Results: ALTO-100 did not demonstrate statistically significant improvement in MADRS versus placebo in the biomarker-defined population. Secondary analyses also did not demonstrate benefit over placebo. The compound was well tolerated, with headache, nausea, and abnormal dreams as the most common adverse events at rates similar to placebo. No new safety signals emerged.

Interpretation: The consistent failure to meet the MADRS primary endpoint across Phase 1b, Phase 2, and Phase 2b trials, despite persistent cognitive and self-rated mood benefits at the 40 mg dose, suggests that NSI-189 may have a preferential effect on cognitive and self-perceived functional dimensions of depression rather than on the clinician-rated symptom severity captured by MADRS. This dissociation has been noted by multiple commentators and is consistent with the hypothesis that hippocampal neurogenesis is more closely associated with cognitive aspects of depression than with the core mood symptoms [5, 8].

7.5 Bipolar depression and PTSD

Alto Neuroscience is conducting a Phase 2b trial of ALTO-100 as adjunctive treatment in bipolar depression, with results expected in 2026. The compound is also under investigation for PTSD. No published clinical data for either indication are available as of this monograph revision [8].

8. Sourcing and Quality Verification

NSI-189 phosphate is supplied by multiple research chemical vendors, including MedChemExpress, Selleckchem, Cayman Chemical, Tocris, Sigma-Aldrich (MilliporeSigma), BOC Sciences, BenchChem, and others, at greater than 98 percent purity by HPLC. Supply is in milligram to gram quantities as the phosphate salt; the free base is also available from some vendors. Pricing is moderate, in the range of \$30 to \$150 per 100 mg of research-grade material at the time of monograph preparation [3].

The compound is not approved for human use in any jurisdiction and is not available as a pharmaceutical-grade product. All available material is for research use only.

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 (367.25 +/- 0.01 for the protonated free base [M+H]⁺; 465.23 +/- 0.01 for the protonated phosphate salt)
- Nuclear magnetic resonance spectroscopy (1H NMR and 13C NMR) for structural confirmation
- Residual solvent analysis by gas chromatography
- Karl Fischer or loss-on-drying analysis for water content

Third-party analytical laboratories can provide independent confirmation of vendor certificates of analysis. As NSI-189 is sold widely through research chemical channels and has attracted substantial interest in the nootropic and self-experimentation community, counterfeit or substandard material is a non-trivial risk, and on-receipt analytical confirmation is strongly recommended.

9. Reconstitution and Handling

NSI-189 phosphate is supplied as a lyophilized or crystalline powder. For in vitro research applications, stock solutions are typically prepared in dimethyl sulfoxide (DMSO) at concentrations of 10 to 100 mM (approximately 4.6 to 46 mg/mL of the phosphate salt). The stock solution should be aliquoted into single-use volumes and stored at -20 degrees Celsius or below to minimize freeze-thaw degradation. DMSO stock solutions are stable for at least 6 months at -20 degrees Celsius when protected from moisture.

For cell culture applications, the DMSO stock is diluted into culture medium to achieve final working concentrations (typically 0.1 to 10 micromolar). The final DMSO concentration should not exceed 0.1 percent (v/v) to avoid solvent cytotoxicity.

For in vivo rodent studies, the compound has been administered by oral gavage as a suspension in 0.5 percent methylcellulose or as a solution in a vehicle containing a small percentage of DMSO with polyethylene glycol 400 and water. Doses of 10 to 30 mg/kg/day have been standard across published preclinical studies [9, 10, 11].

The solid material should be stored at room temperature or at 2 to 8 degrees Celsius in a desiccated environment, protected from light and moisture. Under these conditions, the compound is stable for at least 2 years from the date of manufacture based on supplier stability data.

Handling does not require specialized personal protective equipment beyond standard laboratory precautions (gloves, eye protection, laboratory coat). The compound is not classified as acutely toxic, flammable, or oxidizing based on available safety data sheets.

10. Stack Interactions and Combinations

NSI-189 has been studied primarily as monotherapy in both preclinical and clinical settings. The compound's novel mechanism (indirect neurotrophic factor modulation rather than monoamine reuptake inhibition) reduces the likelihood of pharmacodynamic redundancy with conventional antidepressants, but several interaction considerations are relevant for research applications.

Monoamine reuptake inhibitors (SSRIs, SNRIs): NSI-189 has no activity at monoamine transporters, and no pharmacodynamic antagonism or synergy with SSRIs or SNRIs has been reported. The Phase 2b Alto Neuroscience trial evaluated ALTO-100 as both monotherapy and adjunctive therapy in patients on background antidepressant treatment, suggesting clinical feasibility of combination use [8]. No pharmacokinetic interaction data have been published.

Other neurogenic compounds: Concurrent administration with other compounds known to stimulate hippocampal neurogenesis (fluoxetine, lithium, agomelatine, ketamine, psilocybin) could theoretically produce additive or synergistic effects on hippocampal neural stem cell proliferation. The bell-shaped dose-response relationship observed with NSI-189 suggests that excessive neurogenic stimulation may be counterproductive; combination studies should consider this ceiling effect.

BDNF pathway modulators: Concurrent use with compounds that modulate the BDNF/TrkB pathway (7,8-dihydroxyflavone, N-acetylcysteine, exercise-induced BDNF elevation) could produce additive neurotrophic signaling. No combination data are available.

CYP450 considerations: NSI-189 metabolism does not appear to be dominated by a single CYP isoform. No significant drug-drug interactions mediated by CYP inhibition or induction have been reported. Investigators combining NSI-189 with compounds that are strong CYP inhibitors should monitor for altered exposure, though the risk is expected to be lower than for compounds with single-isoform-dependent clearance.

Hepatotoxic agents: No hepatotoxicity has been reported with NSI-189, but the absence of extensive long-term safety data means that concurrent use with potentially hepatotoxic agents should be approached with standard monitoring considerations.

QT-prolonging agents: No QT prolongation has been reported with NSI-189 in any clinical trial. The absence of ion channel activity at standard screening panels suggests low risk, but formal thorough QT studies have not been published.

11. Adverse Events and Safety

11.1 Common adverse events

Across all clinical trials (Phase 1, Phase 1b, Phase 2, Phase 2b), NSI-189/ALTO-100 has demonstrated a favorable safety and tolerability profile. The most commonly reported adverse events include:

- Headache (incidence similar to or modestly above placebo)
- Nausea (incidence similar to placebo)
- Dizziness (incidence similar to or modestly above placebo)
- Somnolence (reported principally in the Phase 1b inpatient trial)
- Abnormal dreams (reported in the Phase 2b ALTO-100 trial at rates similar to placebo)

All reported adverse events have been mild to moderate in severity. No dose-dependent increase in adverse-event rates was observed across the 40 to 120 mg/day dose range in the Phase 1b trial [4].

11.2 Serious adverse events

No serious adverse events attributable to NSI-189 have been reported in any clinical trial. No deaths, hospitalizations, or clinically significant laboratory abnormalities have occurred in NSI-189-treated subjects [4, 5, 8].

11.3 Discontinuation rates

In the Phase 2 trial, discontinuation rates due to intolerance were lower in the NSI-189 arms (0 subjects at 40 mg and 0 subjects at 80 mg in Stage 1) than in the placebo arm (7 subjects in Stage 1). In Stage 2, 1 subject discontinued at 40 mg, 0 at 80 mg, and 1 on placebo. This pattern is unusual and supports the favorable tolerability of the compound [5].

11.4 Cardiovascular safety

No clinically significant QT interval prolongation, arrhythmia, blood pressure changes, or heart rate changes have been reported in any clinical trial. Electrocardiographic monitoring in the Phase 1 and Phase 1b trials showed no signal [4].

11.5 Neuropsychiatric safety

No suicidality signal, mania induction, psychotic symptoms, or behavioral changes have been reported. The absence of monoamine reuptake inhibition or direct receptor agonism may contribute to the absence of the neuropsychiatric adverse events associated with some conventional antidepressant classes.

11.6 Limitations of safety data

All clinical safety data derive from trials of 6 to 12 weeks' duration in populations of 24 to 301 subjects. Long-term safety data (beyond 12 weeks) are not available from controlled trials. The theoretical concern of uncontrolled cellular proliferation from a neurogenic mechanism has not been realized in any preclinical toxicology study or clinical trial, and the regional specificity of the neurogenic effect (confined to the dentate gyrus and SVZ) mitigates this concern. However, the absence of long-term data means that chronic safety cannot be assumed, and investigators conducting extended research protocols should implement appropriate monitoring.

11.7 Pregnancy and lactation

No human pregnancy or lactation data are available. Preclinical reproductive toxicology data have not been published. The compound should not be administered to pregnant or lactating individuals in any research context without appropriate ethical review and regulatory approval.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five compounds with documented hippocampal neurogenesis-promoting activity against NSI-189, evaluated against 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 mechanisms through which hippocampal neurogenesis has been demonstrated in pre-clinical and clinical research.

12.1 Fluoxetine

Mechanism: Selective serotonin reuptake inhibitor (SSRI). Fluoxetine increases hippocampal neurogenesis through chronic elevation of serotonin levels in the dentate gyrus, acting principally through 5-HT_{1A} receptor activation on neural progenitor cells [14]. The neurogenic effect requires several weeks of continuous administration, paralleling the delayed onset of antidepressant efficacy.

Novelty: Low. Fluoxetine is the prototypical SSRI, approved since 1987, and its neurogenic properties were identified as a secondary mechanism approximately 15 years after approval. The neurogenic mechanism is not the basis of clinical use.

Effect size: Moderate in preclinical models. Chronic fluoxetine administration increases BrdU-positive cells in the dentate gyrus by approximately 50 to 70 percent in rodents. In humans, postmortem studies (Boldrini et al. 2009) showed that SSRI-treated depressed subjects had greater numbers of neural progenitor cells and dividing cells in the dentate gyrus than untreated depressed subjects [15]. The neurogenic effect is not as large as that produced by NSI-189 in preclinical volumetric endpoints (66 percent hippocampal volume increase at optimal dose), and fluoxetine does not produce detectable hippocampal volume increases in humans on structural MRI.

Promising potential: High as an antidepressant (established, globally approved); low as a neurogenic agent (the neurogenic mechanism is secondary and not the basis of dose selection or clinical monitoring).

Side-effect profile: Well characterized. Common adverse events include nausea, insomnia, sexual dysfunction, weight changes, and activation or akathisia. The side-effect burden is substantially greater than that reported for NSI-189 in clinical trials.

Overall validation: Very high as an antidepressant; moderate as a neurogenic agent. The neurogenic hypothesis of fluoxetine's antidepressant action remains debated; some evidence indicates that fluoxetine's antidepressant efficacy does not depend on neurogenesis.

Comparison to NSI-189: Fluoxetine acts through serotonin reuptake inhibition, with neurogenesis as a downstream effect; NSI-189 acts through a direct (but incompletely characterized) neurogenic mechanism without monoamine activity. NSI-189 produces substantially larger preclinical hippocampal volume effects but has not demonstrated antidepressant efficacy on the MADRS in clinical trials. Fluoxetine has decades of clinical validation as an antidepressant. The two compounds are mechanistically complementary and could theoretically be combined.

12.2 Ketamine

Mechanism: NMDA receptor antagonist with rapid-onset antidepressant properties. Ketamine accelerates the maturation phase of hippocampal immature neurons and promotes synaptic plasticity through BDNF-TrkB signaling and mTOR pathway activation [16]. The effect on neural progenitor proliferation is less pronounced than the effect on maturation and synaptic remodeling.

Novelty: Moderate as an antidepressant mechanism (NMDA antagonism is distinct from monoamine reuptake inhibition); low as a neurogenic agent (the neurogenic contribution is not the primary mechanism of rapid antidepressant action).

Effect size: The antidepressant effect size of ketamine is large (Cohen's *d* approximately 0.8 to 1.0 at 24 to 72 hours post-infusion), substantially greater than any effect demonstrated by NSI-189 on depression rating scales. The neurogenic effect size is modest and is characterized more by accelerated maturation than by increased proliferation.

Promising potential: High. Esketamine (Spravato) is FDA-approved for treatment-resistant depression. Intravenous ketamine is widely used off-label. The clinical infrastructure for ketamine as an antidepressant is well established.

Side-effect profile: Substantial. Dissociation, dizziness, nausea, increased blood pressure, and abuse liability are well-documented concerns. The safety profile is substantially less favorable than that of NSI-189.

Overall validation: Very high for antidepressant efficacy; moderate for neurogenic mechanism.

Comparison to NSI-189: Ketamine has proven rapid-onset antidepressant efficacy that NSI-189 lacks. NSI-189 has a far more favorable side-effect profile and a more pronounced neurogenic signal. The two compounds operate through entirely different mechanisms and timescales. Ketamine is not suited to chronic neurogenic augmentation; NSI-189 is designed for chronic daily administration.

12.3 Agomelatine

Mechanism: Melatonergic (MT1 and MT2) receptor agonist and 5-HT_{2C} receptor antagonist. Agomelatine increases hippocampal neurogenesis, principally through acceleration of NeuN expression (neuronal maturation) rather than through increased progenitor proliferation [17]. The chronobiotic mechanism (resynchronization of circadian rhythms) may contribute to the neurogenic effect through normalization of glucocorticoid cycling.

Novelty: Moderate. The melatonergic mechanism is distinct from serotonin reuptake inhibition and from NSI-189's neurogenic mechanism.

Effect size: The antidepressant effect size is modest (comparable to SSRIs in head-to-head trials). The neurogenic effect in preclinical models is moderate and is principally a maturation effect rather than a proliferative effect.

Promising potential: Moderate. Agomelatine is approved for MDD in the European Union and in several other jurisdictions but is not approved in the United States (the FDA issued a non-approvable letter citing insufficient efficacy data). Clinical use is established but limited.

Side-effect profile: Favorable relative to SSRIs (no sexual dysfunction, no weight gain), with the principal safety concern being hepatotoxicity requiring liver function test monitoring in the first months of treatment. The side-effect profile is less favorable than that of NSI-189 owing to the hepatotoxicity signal.

Overall validation: Moderate. Approved antidepressant in multiple jurisdictions; the neurogenic component is a characterized secondary mechanism.

Comparison to NSI-189: Agomelatine has regulatory approval and established clinical efficacy as an antidepressant; NSI-189 does not. NSI-189 has a more pronounced and more specific neurogenic mechanism (direct hippocampal stem cell proliferation versus acceleration of maturation). NSI-189 has a more favorable hepatic safety profile based on available data.

12.4 Psilocybin

Mechanism: Serotonin 5-HT_{2A} receptor agonist (prodrug: psilocybin is dephosphorylated to psilocin, the active metabolite). Psilocybin promotes synaptic plasticity and neurogenesis in preclinical models, with effects mediated through 5-HT_{2A}-dependent BDNF release and TrkB activation [18]. The compound produces rapid and sustained antidepressant effects in clinical trials.

Novelty: High. The psychedelic mechanism is fundamentally distinct from conventional antidepressant pharmacology and from NSI-189's neurogenic mechanism. The regulatory and social context of psilocybin as a Schedule I substance adds complexity.

Effect size: Large antidepressant effect sizes in clinical trials (Cohen's *d* approximately 1.0 to 1.5 at 1 to 4 weeks post-administration in several trials in treatment-resistant depression). The neurogenic effect size in preclinical models is moderate.

Promising potential: High. Psilocybin is in Phase 3 clinical development for treatment-resistant depression and has received FDA breakthrough therapy designation. Regulatory approval is possible within the next several years.

Side-effect profile: Distinct from conventional agents. Acute effects include perceptual disturbances, emotional lability, nausea, and transient anxiety or distress during the psychedelic experience. The requirement for supervised administration and psychological support distinguishes psilocybin from conventional daily-administered antidepressants and from NSI-189. Long-term safety data are limited.

Overall validation: Moderate to high. Multiple positive Phase 2 trials; Phase 3 data emerging. The neurogenic component is a preclinical observation and is not the principal mechanism of clinical antidepressant efficacy.

Comparison to NSI-189: Psilocybin produces larger antidepressant effect sizes than NSI-189 but requires supervised administration and produces substantial acute psychoactive effects. NSI-189 is a daily oral medication with minimal side effects. The two compounds share the property of promoting neuroplasticity through BDNF/TrkB signaling but differ fundamentally in mechanism, administration, and clinical context.

12.5 7,8-Dihydroxyflavone (7,8-DHF)

Mechanism: Small molecule TrkB agonist. 7,8-DHF binds directly to the TrkB receptor (the high-affinity BDNF receptor) and activates downstream signaling, including Akt and ERK pathways, promoting neuronal survival, synaptic plasticity, and neurogenesis [19]. The compound is a flavonoid derivative with oral bioavailability.

Novelty: Moderate. Direct TrkB agonism is a relatively novel mechanism among small molecules, though BDNF itself is a well-characterized target.

Effect size: Moderate in preclinical models. 7,8-DHF improves cognitive performance in multiple rodent models (Alzheimer's disease, aging, traumatic brain injury, depression) and promotes hippocampal neurogenesis. No human clinical trials have been completed for any psychiatric indication.

Promising potential: Low. The compound has not been advanced to clinical trials for depression or cognitive impairment. It is a research tool compound with moderate oral bioavailability and rapid metabolism that limits translational potential. A prodrug (R13) has been developed with improved pharmacokinetics and has shown efficacy in Alzheimer's disease mouse models but has not entered clinical development.

Side-effect profile: Preclinical only. No human safety data are available. The compound is generally well tolerated in rodents at studied doses.

Overall validation: Low. The compound is principally a research tool with no clinical validation.

Comparison to NSI-189: 7,8-DHF acts downstream of NSI-189 in the BDNF/TrkB signaling cascade (direct TrkB agonism versus indirect BDNF upregulation). NSI-189 has completed Phase 2b clinical trials with established human safety and pharmacokinetic data; 7,8-DHF has no clinical data. The two compounds could theoretically produce additive TrkB signaling if combined, but no combination data exist.

12.6 Comparator summary

The five comparator compounds represent distinct pharmacological approaches to hippocampal neurogenesis and related neuroplasticity. **Fluoxetine** is the most clinically validated antidepressant in the panel but has the least specific neurogenic mechanism. **Ketamine** has the largest acute antidepressant effect size but the least favorable side-effect profile and a neurogenic contribution that is principally maturational rather than proliferative. **Agomelatine** offers the most balanced profile of regulatory approval, tolerability, and neurogenic activity but is not available in the United States and carries a hepatotoxicity concern. **Psilocybin** has the largest overall antidepressant effect sizes and is advancing toward regulatory approval but requires supervised administration and produces substantial psychoactive effects. **7,8-Dihydroxyflavone** is the most mechanistically proximal to NSI-189 (acting at TrkB rather than upstream of BDNF) but has no clinical data.

NSI-189 occupies a unique position in this landscape: it is the only compound discovered through phenotypic screening of human hippocampal neural stem cells, the only compound with no activity at monoamine targets, and the compound with the most robust preclinical hippocampal volume effects. Its principal limitation is the consistent failure to demonstrate clinician-rated antidepressant efficacy (MADRS) in clinical trials, despite significant cognitive and self-rated mood improvements. This dissociation has not been observed with the comparators that have positive antidepressant trial data (ketamine, psilocybin, agomelatine, fluoxetine).

The clinical trajectory of NSI-189 may be better served by cognitive impairment endpoints than by mood endpoints, and by populations selected for hippocampal dysfunction (cognitive impairment in depression, post-traumatic stress disorder, diabetic neuropathy) rather than by core mood symptom severity. The Alto Neuroscience biomarker-enrichment strategy reflects this directional shift, though the Phase 2b trial in MDD did not validate the approach.

Investigators considering NSI-189 for neurogenesis research should expect a compound with a highly specific hippocampal neurogenic signal, favorable tolerability, and procognitive effects in human subjects, but without demonstrated efficacy on standard depression rating scales. The comparators offer more established antidepressant efficacy (fluoxetine, ketamine, psilocybin, agomelatine) or more

mechanistic precision at the TrkB target (7,8-DHF), but none combines the neuro-genic specificity, oral bioavailability, favorable tolerability, and human clinical safety data that NSI-189 provides.

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