

RESEARCH MONOGRAPH . COMPOUND PROFILE

NA-1

A cell-permeant Tat-conjugated peptide designed to uncouple NMDA receptor activation from downstream neurotoxic signaling by inhibiting the scaffolding protein PSD-95, advanced through Phase 3 clinical trials for acute ischemic stroke neuroprotection.

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COMPOUND CLASS	Synthetic eicosapeptide inhibitor of postsynaptic density protein 95 (PSD-95) with neuroprotective activity mediated by disruption of NMDA receptor excitotoxic signaling

ABSTRACT

NA-1, designated nerinetide in clinical development and also known by its research name Tat-NR2B9c, is a synthetic, cell-permeant eicosapeptide composed of the 11-residue cell-membrane transduction domain of the human immunodeficiency virus type 1 (HIV-1) Tat protein fused to the nine C-terminal residues of the GluN2B (NR2B) subunit of the N-methyl-D-aspartate (NMDA) glutamate receptor. The compound was designed to inhibit the protein-protein interaction between postsynaptic density protein 95 (PSD-95) and the GluN2B subunit, thereby uncoupling NMDA receptor activation from the downstream excitotoxic signaling cascade that drives neuronal injury in ischemic stroke and related conditions, without blocking normal NMDA receptor ion channel function or calcium influx. The foundational discovery was reported by Aarts et al. in *Science* in 2002, demonstrating that perturbation of the NMDA receptor-PSD-95 interaction reduced excitotoxic neuronal death in vitro and focal ischemic brain damage in rats. Subsequent preclinical work extended the neuroprotective efficacy to nonhuman primates, where Cook et al. (2012) demonstrated greater than 50 percent reduction in stroke volume in cynomolgus macaques subjected to transient middle cerebral artery occlusion and treated with Tat-NR2B9c at 2.6 mg/kg intravenous. The compound was advanced into clinical development by NoNO Inc., a Toronto-based biotechnology company founded by the originating investigator Michael Tymianski. The Phase 2 ENACT trial (Hill et al. 2012, *Lancet Neurology*) in 185 patients undergoing endovascular aneurysm repair demonstrated safety and a significant reduction in the number of procedure-related ischemic

infarcts on diffusion-weighted magnetic resonance imaging. The Phase 3 ESCAPE-NA1 trial (Hill et al. 2020, Lancet) in 1105 patients with acute ischemic stroke undergoing endovascular thrombectomy did not meet its primary endpoint of improved 90-day modified Rankin Scale outcomes in the overall population, but a pre-specified subgroup analysis revealed a clinically meaningful 9.5 percent absolute benefit in patients who did not receive concurrent alteplase, with subsequent mechanistic work demonstrating that plasmin generated by alteplase degrades the nerinetide peptide in vivo. The Phase 3 ESCAPE-NEXT trial (2025, Lancet) in 850 patients undergoing thrombectomy without prior thrombolysis did not confirm the subgroup benefit. The Phase 2 FRONTIER trial (2025, Lancet) in 532 patients with suspected acute cerebral ischemia treated by paramedics in the prehospital setting demonstrated safety and feasibility but did not meet primary efficacy endpoints. Pharmacokinetically, nerinetide exhibits a plasma half-life of approximately 5 to 10 minutes following intravenous bolus administration, consistent with rapid peptide clearance by endogenous proteases and tissue distribution. The clinical dose is 2.6 mg/kg intravenous (maximum 270 mg) administered as a single infusion. The safety profile across all clinical trials has been favorable, with adverse event rates comparable to placebo; the only notable signal is a small excess of serious hypotension events immediately following infusion. This monograph reviews the chemistry, structure, and synthesis of nerinetide; the PSD-95 inhibitory mechanism in molecular detail; the pharmacokinetic profile; the preclinical neuroprotection evidence across species; the complete clinical trial record; sourcing and quality considerations; reconstitution and handling; stack-interaction considerations; the adverse event and safety record; and a comparative assessment of five PSD-95

pathway neuroprotective candidates against nerinetide on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Proposed International Nonproprietary Name: Nerinetide.

Development codes: NA-1 (NoNO Inc.). The development code NA-1 is the canonical identifier in the preclinical pharmacology literature and in the Phase 2 and Phase 3 clinical trial registrations. The compound is also identified by the research name Tat-NR2B9c in the foundational academic publications [1, 2].

Chemical Abstracts Service registry number: 500992-11-0.

DrugBank identifier: DB12595.

ClinicalTrials.gov identifiers: NCT00728182 (ENACT, Phase 2), NCT02930018 (ESCAPE-NA1, Phase 3), NCT04462536 (ESCAPE-NEXT, Phase 3), NCT02315443 (FRONTIER, Phase 2).

Amino acid sequence: H-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Lys-Leu-Ser-Ser-Ile-Glu-Ser-Asp-Val-OH. In single-letter code: YGRKKRRQRRRKLSSIESDV. The first 11 residues (YGRKKRRQRRR) constitute the cell-membrane transduction domain of HIV-1 Tat protein. The terminal 9 residues (KLSSIESDV) constitute the C-terminal sequence of the GluN2B (NR2B) subunit of the NMDA receptor, including the terminal tSXV motif that mediates PDZ domain binding to PSD-95 [1, 3].

1.2 Structural class and chemistry

NA-1 is a synthetic linear eicosapeptide (20 amino acid residues) belonging to the class of cell-penetrating peptide conjugates. It is a chimeric construct comprising a cell-penetrating peptide (CPP) domain and a biologically active cargo domain. The CPP domain is derived from the basic domain of the HIV-1 Tat transactivator protein and confers cell membrane permeability and blood-brain barrier penetration through receptor-independent and endocytic uptake mechanisms [4]. The cargo domain mimics the C-terminal PDZ-binding motif of the GluN2B subunit and competitively inhibits the interaction of GluN2B with the second PDZ domain (PDZ2) of PSD-95 [1, 3].

Molecular formula: C₉₆H₁₆₄N₃₆O₂₄ (free peptide, calculated from sequence).

Molecular weight: Approximately 2519.9 g/mol (calculated from amino acid sequence, average isotopic masses).

Physical properties: White to off-white lyophilized powder. Freely soluble in water and in aqueous buffers at physiological pH. The highly cationic character of the peptide (net charge approximately +8 at physiological pH, reflecting the polybasic Tat domain) confers high aqueous solubility and electrostatic affinity for negatively charged membrane surfaces [4].

Stereochemistry: All residues in the clinical formulation are L-enantiomers. A D-enantiomeric variant (D-Tat-NR2B9c) has been reported in the research literature as a plasmin-resistant analog that maintains PSD-95 inhibitory activity while resisting degradation by alteplase-generated plasmin [5].

1.3 Synthesis

NA-1 is manufactured by solid-phase peptide synthesis (SPPS) using standard Fmoc (9-fluorenylmethoxycarbonyl) chemistry on a resin support. The synthesis proceeds from the C-terminal valine residue through sequential coupling of protected amino acids to the growing chain, with deprotection of the Fmoc group at each cycle, activation of the incoming amino acid carboxyl group (typically with HBTU, HATU, or similar coupling reagents), and side-chain deprotection and resin cleavage upon completion of the full sequence. The polyarginine and polylysine character of the Tat domain presents synthesis challenges related to aggregation, incomplete coupling, and deletion sequences; these are managed through double coupling protocols, chaotropic additives (such as dimethylformamide with 1 percent Triton X-100), and pseudoproline dipeptide incorporation where applicable [6]. The crude peptide is purified by reverse-phase high-performance liquid chromatography (RP-HPLC), typically on a C18 column with acetonitrile-water gradient elution containing 0.1 percent trifluoroacetic acid. The purified peptide is lyophilized and characterized by mass spectrometry (matrix-assisted laser desorption ionization time-of-flight or electrospray ionization), analytical RP-HPLC, amino acid analysis, and peptide content determination. Clinical-grade nerinetide is manufactured under Good Manufacturing Practice (GMP) conditions by NoNO Inc. and its contract manufacturing partners [7].

2. Abstract

NA-1 (nerinetide, Tat-NR2B9c) is a first-in-class inhibitor of the postsynaptic density protein 95 (PSD-95), designed to disrupt the ternary protein complex formed by PSD-95 with GluN2B-containing NMDA glutamate receptors and neuronal nitric oxide synthase (nNOS) at the postsynaptic density of central nervous system neurons [1, 3]. The compound represents a mechanistically novel approach to neuroprotection in ischemic stroke: rather than blocking NMDA receptor channel function (as attempted by the earlier generation of NMDA receptor antagonists that failed clinically due to psychotomimetic and cardiovascular adverse effects), nerinetide selectively uncouples the receptor from the downstream excitotoxic signaling pathway that drives neuronal death, while preserving normal NMDA receptor-mediated synaptic transmission and calcium signaling [1, 8].

The molecular mechanism involves competitive inhibition of the PDZ domain-mediated interaction between PSD-95 and the C-terminal tSXV motif of the GluN2B subunit. This interaction normally scaffolds GluN2B-containing NMDA receptors in spatial proximity to nNOS at the postsynaptic density, enabling NMDA receptor-mediated calcium influx to activate nNOS and generate nitric oxide (NO) and, via additional pathways, to activate NADPH oxidase and generate superoxide [9, 10]. Both reactive species contribute to excitotoxic neuronal death during ischemia. By displacing PSD-95 from the GluN2B C-terminus, nerinetide interrupts this signaling cascade at the scaffolding level.

The preclinical evidence base spans cultured neurons, rodent focal ischemia models, and a landmark nonhuman primate study in cynomolgus macaques. In the primate model, nerinetide at 2.6 mg/kg intravenous reduced stroke volume by greater than 50 percent and improved neurological function scores [11]. The clinical development program, conducted by NoNO Inc. under the direction of Michael Tymianski, has produced four completed trials: the Phase 2 ENACT trial in iatrogenic stroke following aneurysm repair (positive imaging endpoints) [12]; the Phase 3 ESCAPE-NA1 trial in acute ischemic stroke with thrombectomy (negative primary endpoint in the overall population; positive subgroup in patients without concurrent alteplase) [13]; the Phase 3 ESCAPE-NEXT trial in acute ischemic stroke with

thrombectomy excluding prior thrombolysis (negative primary endpoint) [14]; and the Phase 2 FRONTIER trial of prehospital paramedic-initiated administration (safety and feasibility demonstrated; efficacy endpoints not met) [15].

A critical pharmacological interaction was identified during the ESCAPE-NA1 trial: alteplase (recombinant tissue plasminogen activator), the standard thrombolytic agent for acute ischemic stroke, generates plasmin that rapidly degrades the nerinetide peptide in vivo, reducing plasma concentrations by approximately 60 percent in patients co-treated with alteplase compared with those receiving thrombectomy alone [5, 13]. This interaction explained the differential treatment effect observed between the alteplase-treated and alteplase-free subgroups and has driven the subsequent clinical strategy of evaluating nerinetide exclusively in patients not receiving prior thrombolysis.

The compound is administered as a single intravenous infusion at 2.6 mg/kg (maximum 270 mg). The plasma half-life is approximately 5 to 10 minutes, reflecting rapid proteolytic clearance. The safety profile across all completed trials is favorable, with adverse event rates comparable to placebo and no dose-limiting toxicities identified at the clinical dose [12, 13, 14, 15].

3. Discovery and Development History

3.1 Origin

The conceptual foundation for NA-1 was established by research at the Toronto Western Hospital Research Institute (now the Krembil Brain Institute at University Health Network) in the laboratory of Michael Tymianski. The approach originated from the recognition that prior clinical attempts at neuroprotection through direct NMDA receptor antagonism (MK-801, selfotel, gavestinel, aptiganel, and others) had uniformly failed in stroke clinical trials, principally because complete NMDA receptor blockade produced unacceptable psychotomimetic, cardiovascular, and neurobehavioral adverse effects and because the therapeutic window was narrow [8, 16]. Tymianski and colleagues hypothesized that the excitotoxic consequences of NMDA receptor activation could be separated from the receptor's normal physiological functions by targeting the downstream scaffolding interactions rather than the receptor itself [1].

3.2 Foundational discovery

The foundational report was published by Aarts et al. in *Science* in 2002 [1]. The investigators demonstrated that a peptide comprising the nine C-terminal residues of the GluN2B subunit (the tSXV motif sequence KLSSIESDV, designated NR2B9c), when rendered cell-permeant by fusion to the HIV-1 Tat transduction domain, could disrupt the PSD-95/GluN2B interaction, reduce excitotoxic neuronal death in cortical cultures, and reduce focal ischemic brain damage in rats when administered either before or one hour after the onset of ischemia. The peptide did not block NMDA receptor-mediated currents, calcium influx, or synaptic transmission, establishing the principle that excitotoxic signaling could be targeted independently of receptor function [1].

3.3 Preclinical advancement

Between 2002 and 2012, the compound was evaluated in multiple rodent stroke models with consistent demonstration of neuroprotection. Sun et al. (2008) demonstrated efficacy in transient focal ischemia with clinically relevant delayed adminis-

tration [17]. Bhatt et al. (2005) and Cook et al. (2012) extended the preclinical program to gyrencephalic nonhuman primates [11, 18]. The Cook et al. study, published in *Science Translational Medicine*, was a blinded, randomized trial of Tat-NR2B9c versus vehicle in 20 cynomolgus macaques subjected to transient middle cerebral artery occlusion, with the peptide administered at 2.6 mg/kg intravenous 60 minutes after occlusion onset. Stroke volume on 24-hour diffusion-weighted MRI was reduced by greater than 50 percent in the nerinetide group, with this effect maintained at day 30, and neurological function scores were significantly improved [11]. This nonhuman primate demonstration was considered a landmark in stroke neuroprotection research, as it addressed longstanding criticisms that rodent-only preclinical data were insufficient for clinical translation.

3.4 Clinical development

Tymianski founded NoNO Inc. in 2003 to advance the clinical development of PSD-95 inhibitors. The first clinical trial was a Phase 1 healthy volunteer safety and pharmacokinetic study (NA-1-001), which established safety at doses from 0.02 to 2.60 mg/kg intravenous with no serious adverse events [7].

The Phase 2 ENACT trial (Hill et al. 2012, *Lancet Neurology*) enrolled 185 patients undergoing endovascular repair of intracranial aneurysms, a population at risk for iatrogenic ischemic stroke from microemboli generated during the procedure [12]. Patients received nerinetide 2.6 mg/kg or placebo intravenously before the procedure. The study demonstrated safety and showed that nerinetide-treated patients sustained fewer ischemic infarcts on diffusion-weighted MRI (adjusted incidence rate ratio 0.53, 95% CI 0.38 to 0.74) and on FLAIR MRI (0.59, 95% CI 0.42 to 0.83), though lesion volumes did not differ between groups [12].

The Phase 3 ESCAPE-NA1 trial (Hill et al. 2020, *Lancet*) was a multicenter, double-blind, randomized controlled trial in 1105 patients with acute ischemic stroke due to large vessel occlusion selected for endovascular thrombectomy [13]. Patients received nerinetide 2.6 mg/kg or placebo intravenous. The primary endpoint (proportion of patients achieving modified Rankin Scale score 0 to 2 at 90 days) was not met: 61.4 percent of nerinetide patients versus 59.2 percent of placebo patients achieved good outcome (adjusted risk ratio 1.04, 95% CI 0.96 to 1.14; $p = 0.35$). However, a pre-specified subgroup analysis in patients who did not receive intravenous alteplase demonstrated a 9.5 percent absolute benefit favoring nerinetide (adjusted risk ratio 1.18, 95% CI 1.01 to 1.38) [13].

Subsequent mechanistic investigation by Bhatt et al. (2021), published in *Science Translational Medicine*, demonstrated that alteplase-generated plasmin cleaves the nerinetide peptide at multiple sites within the polybasic Tat domain, reducing circulating peptide concentrations by approximately 60 percent in patients co-treated with alteplase [5]. The investigators also developed a D-enantiomeric variant that resisted plasmin degradation while maintaining neuroprotective activity.

The Phase 3 ESCAPE-NEXT trial (2025, *Lancet*) enrolled 850 patients with acute ischemic stroke undergoing thrombectomy without prior thrombolysis across 77 centers internationally [14]. The primary endpoint was not met: 45 percent of nerinetide patients versus 46 percent of placebo patients achieved modified Rankin Scale 0 to 2 at 90 days (odds ratio 0.97, 95% CI 0.72 to 1.30; $p = 0.82$) [14].

The Phase 2 FRONTIER trial (2025, *Lancet*) enrolled 532 participants with suspected acute cerebral ischemia within 3 hours of symptom onset, with nerinetide administered by paramedics in the prehospital setting [15]. The trial demonstrated the feasibility of prehospital nerinetide administration and confirmed safety, but did not meet its primary efficacy endpoint in the overall population.

A post-hoc meta-analysis of individual patient data from the three randomized trials (ESCAPE-NA1, ESCAPE-NEXT, and FRONTIER) was published in *Lancet Neurology* in 2025, examining the early treatment window hypothesis [19].

3.5 Current status

As of the most recent monograph revision, nerinetide is not approved by any regulatory authority. NoNO Inc. continues development with a focus on optimizing treatment timing, patient selection, and next-generation plasmin-resistant analogs. The compound remains a research-stage neuroprotective agent with the most extensive clinical dataset of any PSD-95 inhibitor.

4. Mechanism of Action

NA-1 exerts its neuroprotective effect through a single principal molecular mechanism: inhibition of the protein-protein interaction between PSD-95 and the GluN2B subunit of the NMDA receptor at the postsynaptic density. This mechanism operates at the level of intracellular scaffolding rather than at the level of receptor channel function, preserving normal NMDA receptor-mediated neurotransmission while selectively interrupting the downstream signaling cascade that drives excitotoxic neuronal death [1, 3].

4.1 The PSD-95/GluN2B/nNOS ternary complex

PSD-95 (postsynaptic density protein 95, also designated DLG4 or SAP-90) is an abundant scaffolding protein of the membrane-associated guanylate kinase (MAGUK) family, localized at excitatory postsynaptic densities throughout the central nervous system. PSD-95 contains three PDZ domains (PDZ1, PDZ2, PDZ3), an SH3 domain, and a guanylate kinase-like domain. The first two PDZ domains mediate interactions with the C-terminal tSXV motifs of NMDA receptor GluN2 subunits (GluN2A, GluN2B, GluN2C), while PDZ2 also binds neuronal nitric oxide synthase (nNOS) through the nNOS PDZ domain [3, 20].

The functional consequence of PSD-95 scaffolding is the assembly of a ternary signaling complex: GluN2B-containing NMDA receptors are held in spatial proximity to nNOS at the postsynaptic density. When glutamate activates the NMDA receptor during ischemia, calcium influx through the receptor channel is directed to the co-localized nNOS, which generates nitric oxide. In parallel, the PSD-95 complex also facilitates activation of NADPH oxidase (NOX2), generating superoxide [9, 10]. Both nitric oxide and superoxide, and their reaction product peroxynitrite, are primary mediators of excitotoxic neuronal death in the acute phase of ischemic stroke [8].

4.2 Mechanism of PSD-95 inhibition by NA-1

The nine C-terminal residues of nerinetide (KLSSIESDV) replicate the tSXV PDZ-binding motif of the GluN2B subunit. Upon entry into the cell (mediated by the Tat domain), the NR2B9c cargo binds the PDZ2 domain of PSD-95 with high affinity (EC₅₀ approximately 6.7 nM for PSD-95 PDZ2 domain) [21]. This binding competitively displaces the endogenous GluN2B C-terminus from PSD-95, disrupting the ternary complex and physically separating the NMDA receptor from nNOS and NOX2.

The inhibition is selective for the PSD-95-mediated signaling pathway. Nerinetide does not block NMDA receptor channel opening, does not reduce NMDA receptor-mediated calcium influx, does not interfere with synaptic transmission or long-term potentiation, and does not produce the psychotomimetic or hemodynamic effects characteristic of NMDA receptor channel blockers [1, 8]. The selectivity reflects the targeting of a scaffolding interaction rather than the receptor itself.

Nerinetide also inhibits the interaction of PSD-95 with the GluN2A and GluN2C subunits, with IC₅₀ values of approximately 0.5 micromolar and 0.75 micromolar respectively, and blocks the PSD-95/nNOS interaction with an IC₅₀ of approximately 0.2 micromolar [21].

4.3 Cell penetration mechanism

The Tat cell-penetrating peptide domain (YGRKKRRQRRR) enables nerinetide to cross the blood-brain barrier and penetrate neuronal cell membranes to reach the intracellular PSD-95 target. The mechanism of cell entry involves both direct membrane translocation (driven by the electrostatic interaction of the polybasic Tat domain with negatively charged membrane phospholipids) and macropinocytosis-mediated endocytic uptake [4]. Blood-brain barrier penetration has been characterized in vitro and in vivo, though the efficiency of blood-brain barrier transit relative to direct cellular uptake in the neurovascular unit is a subject of ongoing investigation [22].

4.4 Downstream neuroprotective consequences

The disruption of the PSD-95/GluN2B/nNOS complex by nerinetide produces the following downstream neuroprotective consequences:

Reduction of nitric oxide production: nNOS activation by NMDA receptor-mediated calcium influx is diminished because nNOS is physically separated from the calcium source [1, 3].

Reduction of superoxide production: NADPH oxidase activation downstream of the PSD-95 complex is attenuated, as demonstrated by Chen et al. (2015), who showed that Tat-NR2B9c prevents NMDA-induced neuronal superoxide production [10].

Reduction of peroxynitrite formation: the reaction product of nitric oxide and superoxide is reduced as a consequence of reduced production of both parent species.

Preservation of pro-survival NMDA receptor signaling: because the NMDA receptor channel remains functional, calcium-dependent pro-survival signaling through the extracellular signal-regulated kinase (ERK) and cyclic AMP response element-binding protein (CREB) pathways is preserved, providing a mechanistic advantage over direct NMDA receptor antagonism [8].

5. Pharmacokinetics and Routes of Administration

5.1 Route of administration

Nerinetide is administered exclusively by the intravenous route in all clinical trials. The clinical dose is 2.6 mg/kg body weight, up to a maximum of 270 mg, administered as a single intravenous infusion over approximately 10 minutes [7, 12, 13].

5.2 Distribution

Following intravenous administration, nerinetide distributes rapidly from plasma to tissues. The highly cationic character of the Tat domain (net charge approximately +8 at physiological pH) promotes electrostatic binding to negatively charged endothelial glycocalyx and cell membrane surfaces. The compound crosses the blood-brain barrier, as demonstrated by the central nervous system neuroprotective effects observed in preclinical models and by the clinical imaging endpoints in the ENACT trial [11, 12]. The volume of distribution has not been reported in published literature but is expected to be large relative to plasma volume, consistent with the rapid plasma clearance and the extensive tissue distribution of cationic cell-penetrating peptides.

5.3 Metabolism and elimination

Nerinetide is a peptide and is subject to rapid proteolytic degradation by endogenous serine proteases in plasma and in tissues. The plasma elimination half-life is approximately 5 to 10 minutes [7]. The short half-life reflects the vulnerability of the polybasic Tat domain to cleavage by trypsin-like serine proteases and by other circulating peptidases.

A critical metabolic interaction was identified during the ESCAPE-NA1 trial: alteplase (recombinant tissue plasminogen activator), the standard thrombolytic agent for acute ischemic stroke, converts circulating plasminogen to plasmin, a serine protease that cleaves nerinetide at multiple sites within the Tat domain (after residues 3, 4, 5, 6, 7, 9, 11, and 12 from the N-terminus) [5]. In patients receiving

concurrent alteplase, nerinetide plasma concentrations were approximately 60 percent lower than in patients receiving thrombectomy without alteplase [5, 13]. This drug-drug interaction has been established as the mechanistic explanation for the differential efficacy observed in the alteplase-free subgroup of ESCAPE-NA1.

The metabolic products of nerinetide are short peptide fragments and individual amino acids, which are cleared through standard amino acid metabolic pathways. Renal excretion of the intact peptide is not expected to be significant given the rapid proteolytic degradation.

5.4 Dose translation considerations

Teves et al. (2016) demonstrated that the neuroprotective dose of Tat-NR2B9c requires appropriate allometric scaling between species [23]. In mice, the rat-equivalent dose (approximately 7.6 mg/kg) reduced infarct volume by 24.5 to 26.0 percent, while lower doses were ineffective. The clinical dose of 2.6 mg/kg in humans was derived from the nonhuman primate effective dose through standard body-surface-area-based allometric scaling [11, 23].

5.5 Special populations

Specific pharmacokinetic studies in hepatic impairment, renal impairment, and pediatric populations have not been published. Given the proteolytic (rather than hepatic cytochrome P450) clearance mechanism, hepatic and renal impairment are not predicted to substantially alter nerinetide pharmacokinetics. The short half-life and single-dose administration further limit the clinical relevance of population-specific pharmacokinetic variability.

6. Preclinical Pharmacology

6.1 In vitro neuroprotection

The foundational in vitro demonstration was reported by Aarts et al. (2002) [1]. In cultured cortical neurons, Tat-NR2B9c at concentrations of 50 nM to 1 micromolar reduced NMDA-induced excitotoxic cell death in a concentration-dependent manner without affecting NMDA receptor-mediated calcium influx or electrophysiological responses. The peptide was effective when applied either before or after the excitotoxic insult, establishing a post-insult therapeutic window.

Bhatt et al. (2005) extended the in vitro characterization to show that Tat-NR2B9c disrupts the PSD-95/GluN2B complex at the postsynaptic density of hippocampal neurons in culture, with co-immunoprecipitation demonstrating reduced PSD-95/GluN2B association after treatment [18].

Bhatt et al. (2007) further demonstrated that Tat-NR2B9c perturbs the interaction of PSD-95 with multiple PDZ domain partners, including GluN2A, GluN2B, GluN2C, and nNOS, with varying IC50 values [3].

Chen et al. (2015) demonstrated that Tat-NR2B9c prevents NMDA-induced activation of neuronal NADPH oxidase and the resulting superoxide production, identifying a second excitotoxic signaling pathway interrupted by PSD-95 inhibition [10].

6.2 Rodent models

Nerinetide has been evaluated in multiple rodent focal ischemia models. Aarts et al. (2002) demonstrated reduced infarct volume and improved neurological scores in rats subjected to transient middle cerebral artery occlusion (tMCAO) when Tat-NR2B9c was administered either 45 minutes before or 1 hour after occlusion onset [1]. Sun et al. (2008) extended the therapeutic window to 3 hours post-ischemia in the same model [17]. Teves et al. (2016) established that appropriate interspecies dose translation is required, with the mouse-effective dose being approximately three-fold higher on a mg/kg basis than the rat-effective dose [23].

Bhatt et al. (2017) compared nerinetide with the poly-arginine peptide R18 in permanent and transient MCAO models, with both peptides showing neuroprotective efficacy at 2 hours post-stroke administration [24]. Milani et al. (2016, 2017) reported that R18 demonstrated superior neuroprotection to nerinetide in permanent MCAO at 60 minutes post-occlusion, suggesting that the nerinetide mechanism (PSD-95 inhibition) may be more effective in transient (reperfusion) ischemia models than in permanent occlusion models [25, 26].

6.3 Nonhuman primate model

The Cook et al. (2012) study in *Science Translational Medicine* was a blinded, randomized, placebo-controlled trial in 20 cynomolgus macaques subjected to transient middle cerebral artery occlusion under a protocol designed to mimic a clinical stroke trial [11]. Nerinetide at 2.6 mg/kg intravenous or vehicle was administered 60 minutes after occlusion onset; the MCA was reperfused at 90 minutes. Stroke volume on 24-hour diffusion-weighted MRI was reduced by greater than 50 percent in the nerinetide group compared to placebo, with the effect maintained at day 30 MRI. Non-Human Primate Stroke Scores were significantly improved, with significant improvements in both sensory and motor function [11]. This study was the first demonstration of neuroprotective efficacy in a gyrencephalic primate model using a translational trial design and was considered a critical validation step for clinical advancement.

7. Clinical Evidence Base

7.1 Phase 1 healthy volunteer study

The Phase 1 study (NA-1-001) evaluated the safety and pharmacokinetics of ascending single intravenous doses of nerinetide (0.02 to 2.60 mg/kg) in healthy volunteers [7]. No serious adverse events or dose-limiting toxicities were observed. The study established the 2.6 mg/kg dose as the clinical development dose, corresponding to the effective dose in the nonhuman primate model.

7.2 ENACT trial (Phase 2, 2012)

The Evaluating Neuroprotection in Aneurysm Coiling Therapy (ENACT) trial, published by Hill et al. in *Lancet Neurology* in 2012, was a Phase 2, multicenter, randomized, double-blind, placebo-controlled trial in 185 patients undergoing endovascular repair of intracranial aneurysms [12]. Patients were randomized to receive nerinetide 2.6 mg/kg or placebo intravenously following aneurysm coiling. The primary outcome was the number and volume of new ischemic infarcts on post-procedure diffusion-weighted MRI.

Nerinetide-treated patients sustained significantly fewer ischemic infarcts than placebo patients on both diffusion-weighted MRI (adjusted incidence rate ratio 0.53, 95% CI 0.38 to 0.74) and FLAIR MRI (0.59, 95% CI 0.42 to 0.83) [12]. Lesion volumes did not differ significantly between groups. Two minor adverse events were attributed to nerinetide; no serious adverse events were drug-related. The ENACT trial was the first clinical demonstration of neuroprotection in human ischemic brain injury and supported advancement to Phase 3.

7.3 ESCAPE-NA1 trial (Phase 3, 2020)

The Efficacy and Safety of Nerinetide for the Treatment of Acute Ischaemic Stroke (ESCAPE-NA1) trial, published by Hill et al. in the *Lancet* in 2020, was a multicenter, double-blind, randomized controlled trial in 1105 patients with acute ischemic stroke due to large vessel occlusion selected for endovascular thrombectomy at 48 centers across Canada, the United States, Europe, Australia, and Asia [13].

Patients were randomized to receive nerinetide 2.6 mg/kg (maximum 270 mg) or saline placebo intravenous, administered alongside standard endovascular thrombectomy. The primary outcome was the proportion of patients achieving a modified Rankin Scale (mRS) score of 0 to 2 at 90 days.

The primary endpoint was not met: 337 of 549 nerinetide patients (61.4%) and 329 of 556 placebo patients (59.2%) achieved mRS 0 to 2 at 90 days (adjusted risk ratio 1.04, 95% CI 0.96 to 1.14; $p = 0.35$) [13].

A pre-specified subgroup analysis revealed a significant effect modification by alteplase co-treatment. In the 543 patients (49.1% of the total) who did not receive intravenous alteplase before thrombectomy, nerinetide produced a 9.5 percent absolute improvement in the proportion achieving mRS 0 to 2 (adjusted risk ratio 1.18, 95% CI 1.01 to 1.38) [13]. In patients who received alteplase, no treatment effect was observed.

7.4 ESCAPE-NEXT trial (Phase 3, 2025)

The ESCAPE-NEXT trial, published in the Lancet in 2025, was a Phase 3, multicenter, double-blind, randomized controlled trial designed to confirm the subgroup benefit observed in the alteplase-free subgroup of ESCAPE-NA1 [14]. The trial enrolled 850 patients with acute ischemic stroke due to anterior circulation large vessel occlusion undergoing endovascular thrombectomy without prior intravenous thrombolysis, across 77 centers in Canada, the United States, Germany, Italy, the Netherlands, Norway, Switzerland, Australia, and Singapore.

Patients received nerinetide 2.6 mg/kg (maximum 270 mg) or placebo intravenous. The primary outcome was mRS 0 to 2 at 90 days. The primary endpoint was not met: 206 of 454 nerinetide patients (45%) and 181 of 396 placebo patients (46%) achieved mRS 0 to 2 at 90 days (odds ratio 0.97, 95% CI 0.72 to 1.30; $p = 0.82$) [14]. Serious adverse events occurred at comparable rates between groups.

The failure to replicate the ESCAPE-NA1 subgroup finding raised questions about treatment timing, patient selection, and the hypothesis that excluding alteplase alone would be sufficient to demonstrate nerinetide efficacy.

7.5 FRONTIER trial (Phase 2, 2025)

The FRONTIER trial, published in the Lancet in 2025, was a Phase 2, multicenter, randomized, double-blind, placebo-controlled study evaluating the feasibility, safety, and effectiveness of nerinetide when administered by paramedics in the prehospital setting within 3 hours of symptom onset of suspected acute cerebral ischemia [15]. Between March 2015 and March 2023, 532 participants were enrolled: 265 received nerinetide and 267 received placebo. Of the 507 participants in the modified intent-to-treat population, 321 (63%) had confirmed acute ischemic stroke, 93 (18%) had intracranial hemorrhage, 44 (9%) had transient ischemic attack, and 49 (10%) had stroke-mimicking conditions [15].

The trial demonstrated the feasibility of prehospital nerinetide administration by paramedics and confirmed safety across the mixed population of stroke mimics, ischemic stroke, and hemorrhagic stroke. Primary efficacy endpoints were not met in the overall population. The safety data were reassuring: nerinetide did not worsen outcomes in patients with intracranial hemorrhage, supporting the concept that PSD-95 inhibition is well tolerated even when administered to undiagnosed hemorrhagic stroke patients [15].

7.6 Post-hoc meta-analysis (2025)

A post-hoc meta-analysis of individual patient data from the three completed randomized trials (ESCAPE-NA1, ESCAPE-NEXT, and FRONTIER) was published in Lancet Neurology in 2025, examining the efficacy of nerinetide in patients enrolled in the early treatment window [19]. The analysis explored whether earlier administration (closer to symptom onset and before reperfusion) is associated with greater neuroprotective benefit, consistent with the mechanistic hypothesis that PSD-95 inhibition is most effective when the excitotoxic cascade is actively evolving.

8. Sourcing and Quality Verification

Research-grade Tat-NR2B9c is supplied by multiple peptide synthesis and chemical research suppliers, including AbMole BioScience, Selleckchem, GlpBio, Tocris, MedChemExpress, and custom peptide synthesis services (GenScript, Bachem, Creative Peptides, and others). The compound is supplied as the trifluoroacetate salt or as the acetate salt, typically as a lyophilized powder at greater than 95 percent purity by HPLC [21].

Clinical-grade nerinetide is manufactured under GMP conditions by NoNO Inc. and its contract manufacturing partners and is not commercially available outside of clinical trial settings [7].

Investigators acquiring research-grade Tat-NR2B9c should confirm compound identity and purity on every lot through:

- Reverse-phase HPLC area-percent purity analysis (target: greater than 95 percent)
- Mass spectrometry confirmation of molecular weight (expected: approximately 2519.9 Da for $[M+H]^+$ or the corresponding multiply charged species)
- Amino acid analysis to confirm composition and stoichiometry
- Peptide content determination (to account for counterion and moisture content, which can reduce the mass-percent peptide content of lyophilized preparations to 60 to 80 percent of the gross weight)
- Endotoxin testing (Limulus amoebocyte lysate assay) for preparations intended for in vivo administration

The eicosapeptide structure is challenging to counterfeit; however, deletion sequences, truncation products, and racemization during synthesis can reduce functional potency. Investigators should confirm that the supplier provides a certificate of analysis with mass spectrometry, HPLC, and amino acid analysis data for each lot.

9. Reconstitution and Handling

Tat-NR2B9c is supplied as a lyophilized powder. The compound is freely soluble in water, phosphate-buffered saline, and other aqueous buffers at physiological pH. The polybasic character of the Tat domain confers excellent aqueous solubility; stock solutions at concentrations of 1 to 10 mg/mL in water or saline are readily prepared.

For in vitro research applications, stock solutions are typically prepared at 1 to 10 mM (approximately 2.5 to 25 mg/mL) in sterile water or dimethyl sulfoxide (DMSO) and stored as single-use aliquots at minus 20 degrees Celsius or minus 80 degrees Celsius. Aqueous solutions should be prepared fresh or used within 24 hours of preparation at room temperature; frozen aliquots are stable for at least 6 months at minus 80 degrees Celsius.

For in vivo research applications, the compound is reconstituted in sterile normal saline (0.9% sodium chloride) at the required concentration. The clinical formulation uses normal saline as the vehicle for intravenous infusion [7, 12, 13].

The peptide is susceptible to proteolytic degradation in biological matrices. Investigators should avoid prolonged incubation of nerinetide solutions with serum, plasma, or tissue homogenates unless degradation kinetics are the subject of study. Protease inhibitor cocktails may be added to biological samples collected for nerinetide quantification.

Storage of the lyophilized powder at minus 20 degrees Celsius or below in a desiccated environment is recommended. The lyophilized material is stable for at least 2 years under these conditions. Avoid repeated freeze-thaw cycles of aqueous solutions; aliquoting for single use is strongly recommended.

Light protection is not specifically required but is reasonable practice for long-term storage. The compound is not hygroscopic to the degree that humidity-controlled storage is essential, but desiccant storage is preferred for the lyophilized material.

10. Stack Interactions and Combinations

Nerinetide interacts with one pharmacological class through a clinically significant drug-drug interaction that has been demonstrated in vivo, and has theoretical interactions with several additional classes through its mechanism of action.

Thrombolytic agents (alteplase, tenecteplase): This is the single most important drug interaction for nerinetide. Alteplase (recombinant tissue plasminogen activator) generates plasmin from circulating plasminogen. Plasmin cleaves the nerinetide peptide at multiple arginine and lysine residues within the Tat domain, reducing circulating drug concentrations by approximately 60 percent [5, 13]. This interaction was the principal explanation for the failure of the ESCAPE-NA1 trial to demonstrate efficacy in the overall population (which included approximately 50 percent alteplase-co-treated patients). Tenecteplase, a modified variant of alteplase with longer half-life and greater fibrin specificity, would be expected to produce the same interaction through plasmin generation, although this has not been formally characterized. Co-administration of nerinetide with any thrombolytic agent is contraindicated on the basis of current evidence. The temporal separation required to avoid the interaction is a function of nerinetide's short half-life (5 to 10 minutes); if nerinetide is administered and cleared before alteplase is initiated, the interaction is avoided [5].

NMDA receptor antagonists (memantine, ketamine, dextromethorphan): No direct pharmacokinetic interaction is expected. The pharmacodynamic interaction is theoretically complementary: NMDA receptor channel blockade and PSD-95 scaffolding disruption target different elements of the excitotoxic cascade. Preclinical studies have not formally evaluated this combination, and the clinical relevance is unclear.

Anticoagulants and antiplatelet agents: No pharmacokinetic interaction with heparin, warfarin, direct oral anticoagulants, aspirin, or clopidogrel is expected. These agents are routinely co-administered in the stroke thrombectomy setting in which nerinetide has been evaluated, and no adverse interaction signal has been detected in clinical trials [13, 14].

Vasopressors and antihypertensives: A small excess of serious hypotension events was observed in nerinetide-treated patients in the ESCAPE-NA1 trial (6 serious adverse events in the nerinetide group versus 0 in the placebo group) [13]. Investigators should exercise caution with concurrent vasodilators or in patients with compromised hemodynamic reserve.

Other neuroprotective agents: Nerinetide has not been formally evaluated in combination with other investigational neuroprotective agents (edaravone, citicoline, uric acid, hypothermia protocols). The PSD-95 inhibitory mechanism is distinct from the mechanisms of these agents, and additive or synergistic neuroprotection is theoretically possible.

11. Adverse Events and Safety

11.1 Phase 1 safety

In the Phase 1 healthy volunteer study, nerinetide at doses from 0.02 to 2.60 mg/kg was well tolerated with no serious adverse events, no dose-limiting toxicities, and no discontinuations due to adverse events [7].

11.2 ENACT trial safety

In the Phase 2 ENACT trial, two minor adverse events were adjudged to be associated with nerinetide; no serious adverse events were attributed to the drug [12].

11.3 ESCAPE-NA1 trial safety

In the Phase 3 ESCAPE-NA1 trial, rates of adverse events were similar between nerinetide and placebo groups [13]. The principal safety signal was a small imbalance in serious hypotension events immediately following nerinetide infusion: 6 serious adverse events in the nerinetide group (549 patients) versus 0 in the placebo group (556 patients). The mechanism of the hypotensive effect is not fully characterized but may relate to the cationic peptide's interaction with vascular endothelial surfaces or to histamine release associated with polybasic peptide infusion [13].

Rates of mortality, symptomatic intracranial hemorrhage, thromboembolism, seizures, and other serious adverse events were comparable between groups [13].

11.4 ESCAPE-NEXT trial safety

In the Phase 3 ESCAPE-NEXT trial, serious adverse events occurred at comparable rates in the nerinetide and placebo groups [14]. No new safety signals were identified.

11.5 FRONTIER trial safety

In the Phase 2 FRONTIER trial, nerinetide demonstrated safety across a mixed population including patients with confirmed ischemic stroke, intracranial hemorrhage, transient ischemic attack, and stroke-mimicking conditions [15]. No excess of adverse events was observed in any subgroup, including the hemorrhagic stroke patients, supporting the safety of prehospital administration before diagnostic imaging.

11.6 Summary of safety profile

The overall safety profile of nerinetide across four completed clinical trials (approximately 1800 nerinetide-treated participants) is favorable. The compound is well tolerated at the clinical dose of 2.6 mg/kg intravenous. The only identified safety signal is a small excess of acute hypotension events, which were manageable and did not lead to treatment discontinuation. There is no evidence of psychotomimetic effects, cardiovascular toxicity (beyond the acute hypotension), hepatic toxicity, renal toxicity, or delayed adverse events [12, 13, 14, 15].

The safety profile is a notable advantage of the PSD-95 inhibitor mechanism over direct NMDA receptor antagonism, which produced dose-limiting psychotomimetic effects (hallucinations, agitation, catatonia) and cardiovascular instability in clinical trials of MK-801, selfotel, and related compounds [8, 16].

11.7 Immunogenicity

Anti-drug antibody formation has not been reported as a clinical concern for nerinetide, likely reflecting the single-dose administration paradigm and the rapid clearance of the peptide. However, the Tat peptide domain is derived from a viral protein and could theoretically elicit immune responses on repeated administration. Investigators conducting repeat-dose preclinical studies should consider immunogenicity monitoring.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five neuroprotective candidates targeting the PSD-95 pathway or related excitotoxic signaling mechanisms, evaluated against nerinetide on five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation.

12.1 AVLX-144 (formerly UCCB01-144)

Mechanism: Dimeric PSD-95 inhibitor. AVLX-144 is a bivalent ligand that simultaneously engages both the PDZ1 and PDZ2 domains of PSD-95, producing approximately 1000-fold greater binding affinity than the monomeric nerinetide peptide [27, 28]. The compound uses a polyethylene glycol (PEG) linker to bridge two PDZ-binding motifs, and the construct is conjugated to a Tat cell-penetrating peptide for cell entry.

Development trajectory: Developed at the University of Copenhagen and advanced by Avilex Pharma (now Lytix Biopharma). Preclinical studies demonstrated neuroprotection in rodent focal ischemia models at equimolar doses to nerinetide [28]. A Phase 1 clinical trial has been completed. Development status as of 2025 is early clinical.

Novelty: High. The dimeric PDZ-binding approach is mechanistically distinct from nerinetide's monomeric competition and represents a second-generation PSD-95 inhibitor with substantially improved binding affinity and target engagement.

Effect size: In rodent models, AVLX-144 reduced infarct volume to a similar extent as nerinetide at 9 nmol/g. At higher doses (30 nmol/g), AVLX-144 was not neuroprotective, while nerinetide produced acute lethality (28 of 30 mice died), suggesting different dose-response profiles and therapeutic windows [28].

Promising potential: Moderate. The compound is in early clinical development and represents the most advanced second-generation PSD-95 inhibitor. The improved affinity and potential for extended protease resistance are attractive devel-

opment features.

Side-effect profile: Preclinical studies reported an increase in heart rate approximately 80 minutes after administration. The cardiovascular profile was more favorable than nerinetide, which produced an immediate dramatic blood pressure drop at equivalent doses in preclinical models [28].

Overall validation: Low to moderate. Phase 1 clinical data only. The mechanistic rationale is strong and builds on the PSD-95 validation established by the nerinetide clinical program.

Comparison to nerinetide: AVLX-144 has superior in vitro binding affinity and a potentially more favorable preclinical cardiovascular profile. Nerinetide has the advantage of a substantially more developed clinical evidence base (four completed clinical trials, approximately 1800 treated patients) and an established safety record in stroke patients.

12.2 D-Tat-NR2B9c (plasmin-resistant nerinetide)

Mechanism: D-enantiomeric variant of nerinetide in which selected L-amino acid residues in the Tat domain are replaced with their D-enantiomers, conferring resistance to plasmin-mediated degradation while maintaining PSD-95 inhibitory activity [5].

Development trajectory: Described by Bhatt et al. (2021) in Science Translational Medicine as a solution to the alteplase-nerinetide drug-drug interaction [5]. The D-Tat variant maintained neuroprotective efficacy in vitro and in rodent models even when co-administered with alteplase. Clinical development status is preclinical as of the most recent revision.

Novelty: Moderate. The approach is a targeted structural modification of the parent compound rather than a de novo mechanism.

Effect size: Comparable to nerinetide in preclinical models; preserved in the presence of alteplase, which inactivated the parent compound [5].

Promising potential: Moderate to high. If the alteplase interaction was the principal reason for the failure of ESCAPE-NA1 and ESCAPE-NEXT, a plasmin-resistant variant could rescue the neuroprotective hypothesis. Clinical development has not commenced.

Side-effect profile: Preclinical only. D-amino acid peptides may have altered immunogenicity and metabolic handling compared with L-amino acid counterparts.

Overall validation: Low. Preclinical data only; the compound has not entered clinical trials.

Comparison to nerinetide: D-Tat-NR2B9c addresses the single most important limitation identified in the nerinetide clinical program (plasmin susceptibility) and is the natural next-generation candidate if the PSD-95 inhibitor hypothesis is pursued in patients receiving thrombolysis.

12.3 Tat-N-dimer (Tat-NPEG4(IETDV)2)

Mechanism: Novel dimeric PSD-95 inhibitor using a Tat cell-penetrating peptide conjugated to a PEGylated dimer of the IETDV PDZ-binding motif. Binds the tandem PDZ1-2 domain of PSD-95 with high affinity and displays extensive protease resistance in human blood plasma [29].

Development trajectory: Reported in the academic literature as a research tool compound. No clinical development has been announced.

Novelty: Moderate to high. The protease-resistant dimeric design addresses two key limitations of nerinetide (monomeric affinity and protease susceptibility) in a single construct.

Effect size: Preclinical characterization demonstrates high-affinity PSD-95 binding. In vivo neuroprotection data are limited in the published literature.

Promising potential: Low. The compound remains a research tool without clinical development trajectory.

Side-effect profile: Not characterized beyond basic in vitro toxicity.

Overall validation: Low. The compound is principally a proof-of-concept research tool.

Comparison to nerinetide: Tat-N-dimer demonstrates that the protease sensitivity and monomeric affinity limitations of nerinetide can be addressed through peptide engineering. Nerinetide has the advantage of an extensive clinical dataset.

12.4 Edaravone (Radicut)

Mechanism: Free radical scavenger. Edaravone is a low-molecular-weight heterocyclic compound (3-methyl-1-phenyl-2-pyrazolin-5-one) that scavenges hydroxyl radicals, peroxynitrite, and other reactive oxygen and nitrogen species generated during ischemia-reperfusion injury [30]. The mechanism is downstream of the excitotoxic cascade and complementary to PSD-95 inhibition.

Development trajectory: Edaravone is the only neuroprotective agent approved for acute ischemic stroke in any jurisdiction. It has been registered in Japan since 2001 for acute ischemic stroke (30 mg intravenous twice daily for 14 days) and was subsequently approved for amyotrophic lateral sclerosis. The compound has been used in millions of stroke patients in Japan and other East Asian jurisdictions [30].

Novelty: Low. Free radical scavenging is a well-established mechanism that has been the subject of numerous failed clinical trials in Western markets.

Effect size: The Japanese registration was based on clinical trials showing modest improvement in functional outcomes. A Cochrane review and subsequent meta-analyses have characterized the effect size as small. Western regulatory agencies have not approved edaravone for stroke, partly reflecting methodological concerns about the Japanese registration trials [30].

Promising potential: Moderate. The compound is registered and widely used in East Asia. Its positioning as the only approved stroke neuroprotectant gives it practical relevance despite the small effect size and limited Western regulatory acceptance.

Side-effect profile: The compound is well tolerated. Rare adverse events include renal impairment (particularly in patients with pre-existing renal disease), hepatic enzyme elevation, and acute hypersensitivity. The 14-day intravenous dosing regimen is operationally burdensome compared with nerinetide's single-dose administration [30].

Overall validation: Moderate. Regulatory approval in Japan and widespread use in East Asia provide a level of validation, though the Western regulatory and academic assessment is more cautious.

Comparison to nerinetide: Edaravone acts downstream of the PSD-95 complex at the level of reactive species scavenging, whereas nerinetide acts at the scaffolding level to prevent reactive species generation. The two mechanisms are theoretically

complementary. Edaravone has the advantage of regulatory approval and millions of patient-exposures; nerinetide has the advantage of a more selective mechanism that preserves normal NMDA receptor function.

12.5 Memantine

Mechanism: Low-affinity, uncompetitive NMDA receptor channel blocker. Memantine binds within the NMDA receptor ion channel in a use-dependent and voltage-dependent manner, preferentially blocking pathological tonic activation while sparing physiological phasic synaptic transmission [31]. The mechanism addresses excitotoxicity at the level of the NMDA receptor channel rather than at the downstream scaffolding level targeted by nerinetide.

Development trajectory: Memantine is approved for moderate-to-severe Alzheimer's disease in the United States (since 2003), Europe, and globally. It has not been approved for acute ischemic stroke. Preclinical and early clinical studies have explored memantine for stroke neuroprotection, but no Phase 3 stroke trial has been completed [31].

Novelty: Low. The compound has been marketed for over two decades.

Effect size: In Alzheimer's disease, memantine produces modest symptomatic benefit (approximately 1-point improvement on the Severe Impairment Battery). In preclinical stroke models, neuroprotective effects are modest and dose-limited by the narrow therapeutic window between neuroprotection and channel blockade-related adverse effects [31].

Promising potential: Low for stroke. The compound is well established in Alzheimer's disease but has not been advanced for stroke neuroprotection.

Side-effect profile: Well characterized. Principal adverse events are dizziness, headache, confusion, and constipation. The low-affinity, use-dependent binding avoids the severe psychotomimetic and cardiovascular effects of high-affinity NMDA antagonists [31].

Overall validation: High for Alzheimer's disease; low for stroke neuroprotection.

Comparison to nerinetide: Memantine blocks the NMDA receptor channel directly; nerinetide disrupts the downstream scaffolding without affecting channel function. Nerinetide preserves normal NMDA receptor-mediated signaling, includ-

ing pro-survival calcium-dependent pathways, which memantine partially inhibits. For stroke neuroprotection specifically, nerinetide has a substantially more developed clinical evidence base.

12.6 Poly-arginine peptide R18

Mechanism: R18 is an 18-residue poly-arginine peptide (Arg18) with neuroprotective activity that appears to be mediated through multiple mechanisms, including surface charge effects on neuronal membranes, inhibition of calcium influx, and potential intracellular targets [25, 26]. Unlike nerinetide, R18 does not contain a specific PSD-95-binding cargo domain; its neuroprotective activity is attributed to the poly-cationic character shared with the Tat domain of nerinetide rather than to PSD-95 inhibition specifically.

Development trajectory: R18 has been characterized in preclinical rodent stroke models by investigators at the University of Western Australia and Perron Institute [25, 26]. No clinical trials have been initiated.

Novelty: Moderate. The concept that the cationic character of cell-penetrating peptides may itself be neuroprotective (independent of the PSD-95-binding cargo) represents an important mechanistic question for the nerinetide field.

Effect size: In permanent MCAO models in rats, R18 demonstrated superior neuroprotection to nerinetide at 60 minutes post-occlusion [25, 26]. In transient MCAO models, both peptides showed comparable efficacy. The differential suggests that the nerinetide PSD-95-specific mechanism may be particularly relevant in reperfusion settings, while the non-specific cationic peptide mechanism may be effective in both permanent and transient ischemia.

Promising potential: Low. The compound is preclinical only with no announced clinical development.

Side-effect profile: Preclinical only. Expected to share the hemodynamic effects observed with cationic peptides.

Overall validation: Low. Preclinical data only.

Comparison to nerinetide: R18 raises the important question of whether the neuroprotective activity of nerinetide is attributable entirely to PSD-95 inhibition or partly to the non-specific cationic properties of the Tat domain. The comparison

data suggest that both PSD-95-specific and non-specific cationic mechanisms contribute, with the relative contribution depending on the ischemia model.

12.7 Comparator summary

The five compounds evaluated against nerinetide occupy distinct positions in the stroke neuroprotection landscape. **AVLX-144** represents the most mechanistically advanced second-generation PSD-95 inhibitor, with superior binding affinity but limited clinical data. **D-Tat-NR2B9c** directly addresses the alteplase interaction that confounded the ESCAPE-NA1 trial and is the natural next-generation candidate for the PSD-95 inhibitor hypothesis. **Tat-N-dimer** is a proof-of-concept protease-resistant dimeric construct without clinical development. **Edaravone** is the only regulatory-approved stroke neuroprotectant globally, operating downstream of the PSD-95 complex at the level of reactive species scavenging. **Memantine** blocks the NMDA channel directly and is well validated for Alzheimer's disease but undeveloped for stroke. **R18** raises important mechanistic questions about the contribution of cationic peptide properties to nerinetide's activity.

Nerinetide occupies a unique position as the PSD-95 inhibitor with the most extensive clinical development program in stroke neuroprotection. It is the only compound in the comparator panel that has completed Phase 3 clinical trials for acute ischemic stroke, and its safety profile across approximately 1800 treated patients is the most thoroughly characterized. The principal limitation identified through clinical development is the susceptibility to plasmin-mediated degradation during co-treatment with thrombolytic agents. The next-generation development strategy (plasmin-resistant analogs, optimized treatment timing, patient selection) is informed by the mechanistic insights from the ESCAPE-NA1, ESCAPE-NEXT, and FRONTIER trials.

The compound is not approved by any regulatory authority. Its current positioning is as an investigational neuroprotective agent with strong preclinical validation, a favorable safety profile, and an inconclusive clinical efficacy record that may reflect pharmacokinetic limitations (plasmin degradation) and patient selection factors rather than a fundamental failure of the PSD-95 inhibitor mechanism.

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