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Small extracellular vesicles as emerging biomarkers and therapeutic targets in neurodegenerative diseases

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ABSTRACT

Small extracellular vesicles (sEVs) have rapidly emerged as versatile mediators of intercellular communication with significant potential to transform the diagnosis and treatment of neurodegenerative diseases (NDDs). Increasing evidence shows that sEVs not only participate in the propagation of pathogenic proteins but also serve as accessible, CNS-informative carriers of molecular signatures that reflect neuronal, glial, and systemic disease processes. This dual role positions sEVs at the intersection of biomarker discovery and therapeutic innovation. In the diagnostic domain, advances in immunoaffinity capture, single-vesicle analysis, and multi-omics profiling have enabled increasingly precise characterization of neuron-, astrocyte-, and microglia-derived sEVs, revealing candidate markers for Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and related disorders. However, translation remains limited by methodological heterogeneity, a lack of large-scale validation, and the need for standardized pre-analytical and analytical pipelines aligned with the ISEV/MISEV guidelines. On the therapeutic front, native and engineered sEVs, particularly those derived from mesenchymal and neural stem cells, demonstrate promising neuroprotective effects, including the modulation of neuroinflammation; the enhancement of synaptic resilience; and the delivery of antioxidant, anti-amyloid, or gene-modifying cargo across the blood–brain barrier. Scalable GMP manufacturing, cargo-loading strategies, targeting specificity, and long-term safety remain key challenges for clinical translation. This narrative review synthesizes current advances in sEV-based biomarkers and therapeutics, outlines technological and regulatory barriers, and proposes a translational roadmap spanning mechanistic discovery, platform standardization, and integration into precision-medicine frameworks. Collectively, emerging data position sEVs as powerful tools capable of reshaping the diagnostic and therapeutic landscape of NDDs, provided that coordinated multidisciplinary efforts address the remaining gaps in validation, scalability, and regulatory readiness.

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1. Introduction

Neurodegenerative diseases (NDDs), such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), are chronic, progressive disorders characterized by the selective loss of neuronal populations and synaptic dysfunction, leading to cognitive, motor, and behavioral decline [1–3]. The incidence of this disease is increasing sharply with increasing global population age, creating urgent medical, social, and economic burdens. Despite advances in symptomatic management, current therapies largely fail to halt or reverse disease progression, underscoring the need for earlier diagnosis, mechanism-based biomarkers, and disease-modifying interventions [4–6]. Traditional biofluid and neuroimaging biomarkers, while informative, often lack sensitivity for preclinical stages, require invasive sampling (e.g., cerebrospinal fluid), or are constrained by cost and accessibility, limiting their utility for population-level screening and longitudinal monitoring [7–9].

In this context, small extracellular vesicles (sEVs), a size-defined subset of extracellular vesicles (EVs) of approximately 30–150 nm, have emerged from being viewed as cellular waste products to being recognized as active mediators of intercellular communication and key players in NDD biology [10,11]. EVs are lipid bilayer–encapsulated particles released by virtually all cell types that lack self-replication capacity and carry complex cargoes of proteins, lipids, and nucleic acids reflecting the (patho)physiological state of their cell of origin. According to recent ISEV-aligned frameworks, EVs are operationally classified by size and biogenesis into small EVs (sEVs; <200 nm), larger microvesicles, and apoptotic bodies, with sEVs typically arising from multivesicular bodies and displaying enriched regulatory cargo such as microRNAs (miRNAs), mRNAs, and long non-coding RNAs [12].

Within the central nervous system (CNS), sEVs are released by neurons, astrocytes, microglia, oligodendrocytes, and other brain-associated cells and participate in processes ranging from neuronal development and synaptic plasticity to neuroinflammation and repair [13–15]. Crucially, sEVs can cross the blood–brain barrier (BBB) bidirectionally, allowing brain-derived vesicles to be detected in peripheral biofluids such as plasma, serum, and saliva and enabling peripherally administered vesicles to access CNS targets [15]. These properties position sEVs as an attractive interface between brain pathology and minimally invasive “liquid biopsy” strategies.

Accumulating evidence implicates sEVs in the propagation of hallmark pathogenic proteins across neural circuits. Misfolded or aggregation-prone species, including amyloid-β and tau in AD, α-synuclein in PD, TDP-43 in ALS, and mutant huntingtin in HD, have been detected within sEVs and other EV subtypes, supporting a vesicle-mediated, prion-like transfer of toxic cargo that may underlie stereotyped spatiotemporal spreading of pathology [16,17]. sEV secretion can be upregulated as a compensatory response to lysosomal or proteostatic stress, facilitating the removal of misfolded proteins while inadvertently exporting pathogenic material to recipient cells. Parallel alterations in EV-associated miRNA profiles, including disease- and vesicle- size–specific signatures, further suggest a role for sEVs in dysregulating gene expression networks relevant to ubiquitin-proteasome function, innate immune signaling, and synaptic maintenance. Thus, sEVs play dual roles as both propagators of neuropathology and potential endogenous mechanisms of detoxification or neuroprotection, depending on context, cellular origin, and cargo composition [18].

From a biomarker perspective, the molecular content of sEVs offers several advantages over conventional soluble analytes. sEV membranes shield embedded proteins and nucleic acids from enzymatic degradation, improving their stability and enabling the detection of low–abundance, disease–relevant species. Cell- type–enriched vesicle populations, such as neuron–derived or astrocyte–derived sEVs immunocaptured from blood, can report on compartment–specific pathophysiology within the CNS and have been shown to differentiate NDD patients from controls and to track cognitive or motor impairment [19]. Importantly, emerging data suggest that sEV-based biomarkers may outperform traditional cerebrospinal fluid markers for early detection and longitudinal monitoring in some NDD contexts.

Concurrently, sEVs are being intensively explored as therapeutic tools and targets. Mesenchymal stem cell–derived sEVs and other stem cell–derived vesicles recapitulate many of the neuroprotective, immunomodulatory, and pro-regenerative properties of their parental cells while exhibiting lower immunogenicity, easier storage and handling, and avoidance of the safety and ethical concerns associated with cell transplantation [20]. In preclinical models of AD, PD, and other NDDs, unmodified or engineered sEVs have been used to deliver miRNAs, siRNAs, neurotrophic factors, proteases (e.g., Aβ-degrading enzymes), and other therapeutic cargos across the BBB, leading to reduced protein aggregation, attenuated neuroinflammation, and improved functional outcomes [20]. Moreover, strategies that inhibit the release, uptake, or pathological cargo loading of disease-promoting sEVs are being evaluated as novel disease-modifying approaches.

Despite these advances, major translational challenges remain, including standardization of sEV isolation and characterization, scalable manufacturing with robust quality control, precise definition of vesicle identity and potency, and harmonization of regulatory frameworks across agencies. Innovative solutions such as CRISPR-based barcoding for in vivo tracking of vesicle biodistribution, AI-assisted analytics for cargo profiling, and evolving MISEV-2023 guidelines for minimal reporting standards are beginning to address these gaps and will be critical for clinical translation. Therefore, small extracellular vesicles have emerged as a convergent focus for NDD research, integrating mechanistic insights into disease propagation with opportunities for biomarker discovery and targeted therapy. The present narrative review synthesizes current knowledge on sEV biology in neurodegeneration, emphasizing their roles as emerging biomarkers and therapeutic targets across major NDDs and highlighting conceptual and practical priorities for advancing sEV–based strategies toward clinical impact.

2. Biogenesis, Classification, and Molecular Cargo of Small Extracellular Vesicles

Small extracellular vesicles (sEVs), which often operationally overlap with exosomes (~30–150 nm), are generated through endosomal trafficking and multivesicular body (MVB) formation. In neurons and glia, inward budding of the late endosomal membrane produces intraluminal vesicles (ILVs); subsequent fusion of MVBs with the plasma membrane releases these ILVs as sEVs into the extracellular space [21,22]. In contrast, larger microvesicles/ectosomes are shed by outward budding of the plasma membrane, and apoptotic bodies are derived from the blebbing of dying cells, providing a size– and biogenesis–based classification framework for extracellular vesicles (EVs) in the nervous system [23,24].

In experimental neurodegeneration models, EV biogenesis is dynamically regulated. After spinal cord injury in mice, proteins involved in EV formation are upregulated at the lesion site, and tetraspanin markers (CD9, CD63, and CD81) increase in multiple CNS cell types, indicating enhanced vesicle production under neuroinflammatory stress [25]. Similarly, in Alzheimer's disease (AD) models, acid sphingomyelinase (ASM) and its product ceramide lipid regulators of EV budding and cargo association are enriched in brain-derived sEVs, particularly in females, which is consistent with ceramide-dependent facilitation of Aβ binding to vesicle membranes. The inhibition of ASM reduces Aβ–ceramide complex formation and alters sEV output in astrocytes, directly linking lipid-dependent biogenesis mechanisms to disease-related vesicle composition [26].

Within the CNS, sEVs are released by neurons, astrocytes, oligodendrocytes, microglia, and neural stem or progenitor cells and can be recovered from brain tissue, cerebrospinal fluid, and peripheral blood [27]. Quantitative nanoparticle tracking and electron microscopy in AD mouse brains revealed median sEV diameters of approximately

110–120 nm and high particle numbers, with proteomic enrichment of canonical EV markers and endocytosis-related pathways, confirming their endosomal origin. Novel isolation methods from unfixed frozen brains further demonstrated that CNS sEVs meet current size, morphology, and marker criteria, with minimal contamination from intracellular organelles [26].

The molecular cargo of sEVs is highly complex and disease-modulated. High–resolution proteomics of brain-derived EVs from AD transgenic mice revealed 3444 unique proteins enriched with neuron-, astrocyte-, oligodendrocyte-, and microglia–specific molecules as well as endocytic and synaptic components. AD-model sEVs present increased levels of presenilin-1, amyloid precursor protein (APP), and microglial Itgax (a disease-associated microglia marker), implicating the Aβ-processing machinery and neurodegenerative microglia in vesicle secretion. Proteomic analysis of sEVs from neuropathic pain and stress models similarly revealed disease–specific enrichment of complement proteins, adhesion molecules, and astrocytic cytosolic proteins, reflecting selective sorting rather than passive shedding [28,29].

Pathogenic proteins central to neurodegenerative diseases are frequently detected in EV cargo. Brain- and CSF-derived EVs from AD and familial AD systems carry Aβ and tau, whereas EVs from APP/PS1 or post-mortem AD brains transmit tau and Aβ and can induce memory impairment or aberrant tau phosphorylation when injected into the hippocampus of wild-type mice [30]. In Parkinson's disease (PD), CSF-derived EVs contain soluble and aggregated α-synuclein; these vesicles catalyze α-synuclein nucleation and are more efficiently taken up and more toxic than free protein, supporting vesicle-mediated prion-like spread [31]. Astrocyte-derived EVs in HIV-associated neurocognitive disorders shuttle amyloid species and induce synaptodendritic injury and behavioral deficits in vivo, with HIF-1α signaling regulating both EV biogenesis and amyloid cargo loading.

Nucleic acids constitute a second major cargo class. Plasma or tissue sEVs from CNS injury and neurodegenerative models show robust changes in microRNA (miRNA) profiles. After SCI, circulating $CD81^{+}$ sEVs exhibit miRNA signatures resembling those of activated astrocytes, and their intracerebral injection alters inflammatory and reactive astrocyte gene expression in the cortex. Mesenchymal stem cell–derived sEVs modulate microglial polarization and neuroinflammation via specific miRNAs (e.g., miR-467f, miR-466q, and miR-486) that target the MAPK and PTEN/Akt pathways, promoting M2-like phenotypes and neurogenesis in experimental autoimmune encephalomyelitis and ischemic stroke [32,33]. In PD models, circulating EVs carry tRNA-derived fragments such as tRF-02514, which regulate autophagy and microglial pyroptosis; inhibition of this EV-borne RNA protects dopaminergic neurons and delays disease progression [34]. Neural stem cell-s and umbilical cord–derived sEVs similarly transport miR-124-3p and other neurogenic or synaptic miRNAs, enhancing neuronal differentiation, mitochondrial function, and synaptic protein expression in AD and PD models [35].

Together, primary experimental data show that sEVs in neurodegenerative and neuroinflammatory conditions arise predominantly from the endosomal pathway, are classified within the small EV/exosome size range, and carry a selectively sorted cargo of pathogenic proteins, immunomodulatory lipids, and regulatory non-coding RNAs that both mirror and modulate disease processes across the CNS and periphery.

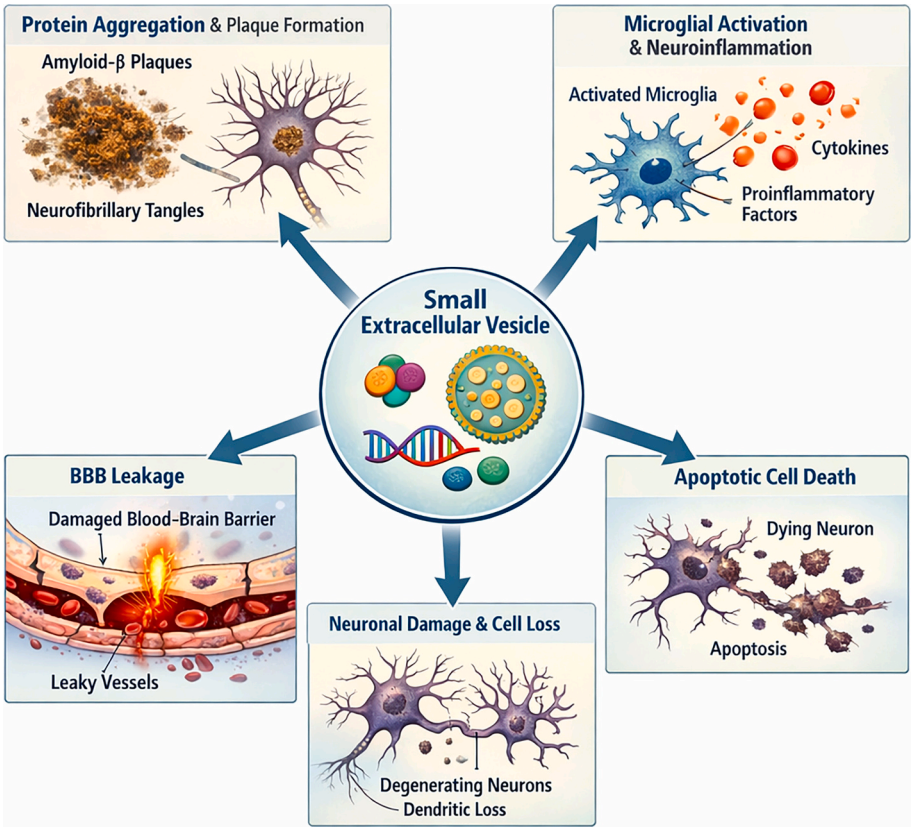

Fig. 1. Pathological effects of small extracellular vesicles (sEVs) in the brain. Small extracellular vesicles released under pathological conditions contribute to multiple neurodegenerative processes. sEVs can propagate misfolded proteins, promoting amyloid-β plaque deposition and neurofibrillary tangle formation. They stimulate microglial activation and amplify neuroinflammation through cytokine and proinflammatory factor release. sEV cargo also triggers apoptotic pathways, leading to neuronal cell death. In parallel, they exacerbate neuronal damage and loss, including dendritic degeneration. Additionally, sEV-mediated signaling disrupts the blood–brain barrier (BBB), resulting in vascular leakage and impaired neurovascular integrity. Collectively, these mechanisms illustrate the multifaceted role of sEVs in driving neurodegenerative pathology.

3. Mechanisms of sEV-mediated Pathogenesis in Neurodegenerative Disorders

Experimental studies have shown that small extracellular vesicles (sEVs) contribute to neurodegeneration through several interconnected mechanisms: propagation of misfolded proteins, induction of neuroinflammation and glial activation, disruption of autophagy and cell death pathways, and modulation of synaptic and mitochondrial function (Fig. 1).

In Parkinson's disease (PD), sEVs provide a catalytic microenvironment that promotes α-synuclein misfolding and prion-like propagation. Cerebrospinal fluid (CSF)-derived EVs from PD patients contain soluble and aggregated α-synuclein; these vesicle-bound oligomers are taken up more efficiently and are more toxic to recipient cells than free α-synuclein, and EVs enhance the nucleation and aggregation of the protein in vitro [31]. Short intranasal administration of PD CSF-EVs to healthy mice induces widespread midbrain α-synuclein aggregation, dopaminergic neurodegeneration, neuroinflammation, and autophagy alterations, along with motor and non-motor PD–like symptoms, demonstrating that human disease-derived EVs can initiate and propagate pathology in vivo.

Environmental toxins can further aggravate this process. Manganese exposure in α-synuclein–expressing dopaminergic neurons drives the secretion of misfolded α-synuclein in exosomes; these exosomes transfer α-synuclein to microglia and neurons, accelerating cell-to-cell aggregation and dopaminergic neurotoxicity in mouse models, and serum exosomes from manganese-exposed welders show increased misfolded α-synuclein levels, which are linked to metal exposure, exosomal trafficking, and the risk of synucleinopathy [36].

Tau pathology is also EV-mediated. Brain-derived EVs from human tau transgenic mice contain high levels of human tau capable of seeding aggregation; genetic deletion of the neuronal protein Arc markedly reduces tau loading into EVs and abolishes their seeding capacity, indicating that Arc-dependent EV packaging is a key mechanism for intercellular tau transmission [37]. EVs from Alzheimer's disease (AD) and frontotemporal dementia (FTD) brains carry tau and Aβ and, when injected into the hippocampus of wild-type or humanized tau mice, induce memory impairment and are associated with proteomic changes in synapse-related proteins, supporting a direct contribution of disease EVs to synaptic dysfunction and cognitive decline [22].

sEVs can cross the blood–brain barrier (BBB) and modulate glial cells. Peripheral blood sEVs from aged mice, but not from young animals, enter the brains of young mice and rapidly alter the expression of glial activation genes; the astrocytic marker GFAP is significantly upregulated, indicating robust astrocyte activation and linking age-related peripheral vesicles to central neuroinflammation [38]. Plasma exosomes injected into the hippocampus of AD model mice diffuse through the hippocampus and cortex, preferentially target microglia, and cluster around β-amyloid plaques, where they are engulfed by activated microglia, suggesting that circulating exosomes participate in plaque–microglia dynamics and local inflammatory responses [39].

In PD, serum-derived EVs carrying the tRNA-fragment tRF-02514 promote microglial pyroptosis and inflammation by targeting ATG5 and inhibiting autophagy, which accelerates neuronal loss; inhibition of this EV-borne RNA reverses these effects, decreases inflammatory factor release, and protects dopaminergic neurons in vivo [34]. Misfolded SOD1 in ALS models localizes to vacuoles containing EV markers and the necroptosis effector pMLKL in vulnerable motor neurons, supporting a mechanism in which EVs released from damaged neurons via secretion or necroptotic disruption act as triggers for early microgliosis, glial-mediated neurotoxicity, and disease spread [40].

Proteomic profiling of brain-derived EVs from AD model mice revealed enrichment of APP, presenilin-1, and the disease-associated microglial marker Itgax, linking Aβ-processing complexes and neurodegenerative microglia to EV secretion [23]. EVs from AD and FTD brains show altered synapse-related proteomes, and their intracerebral injection causes memory impairment, indicating that EV cargo can directly disrupt synaptic regulation and plasticity [22].

Neural stem cell–derived EVs in APP/PS1 mice improve cognition without reducing the Aβ burden but increase the expression of mitochondrial biogenesis regulators (PGC-1α, NRF1/2), SIRT1, and synaptic proteins (GAP43, synaptophysin, PSD95) while lowering oxidative damage and microglial markers, indicating that disease-modifying actions of EVs can occur through the restoration of mitochondrial and synaptic homeostasis and the suppression of neuroinflammation, even as endogenous pathogenic EVs propagate toxic proteins [35].

sEVs are also involved in systemic inflammatory cascades relevant to neurodegeneration. In SOD1 ALS mice, spinal cord proteomics and transcriptomics show marked overactivation of complement and coagulation cascades and NF-κB signaling; therapeutic intranasal sEV administration suppresses these pathways and glial responses, improving motor neuron survival and function, underscoring that the same vesicular routes used for pathogenic signaling can also be harnessed to counteract disease-driving inflammation [41].

4. sEVs from Distinct CNS and Peripheral Cell Types: Neurons, Glia, and Beyond

Small extracellular vesicles (sEVs) in neurodegenerative contexts arise from multiple CNS cell types, diverse glial populations, neural stem cells and peripheral tissues, each contributing a characteristic molecular signature and functional profile. The cell-s of- origin strongly shape the sEV proteome and transcriptome, determining how vesicles influence disease progression and repair. Human iPSC-derived excitatory neurons secrete EVs enriched in the neuronal membrane and synaptic proteins such as ATP1A3 and NCAM1, which were identified as neuron-specific EV markers in an unbiased proteomic screen. Network analysis of brain-derived EVs from controls, mild cognitive impairment patients, and Alzheimer's disease (AD) patients revealed that neuron-enriched EV modules were relatively depleted as astrocyte-enriched modules increased with pathology, suggesting a shift in EV contribution from neurons to glia during disease [42].

In primary murine cultures, neuronal sEV transcriptomes contain thousands of full-length mRNAs, which are selectively packaged relative to bulk cell lysate. These mRNAs include synaptic signaling and ribosomal transcripts, with ~80% of neural sEV mRNAs being full-length and potentially translatable in recipient cells. In the AD brain, neuronal sEVs are relatively depleted of synaptic mRNAs compared with those in the non-diseased brain, indicating the loss of neuron-derived homeostatic signaling [43].

Astrocyte-like cells produce EVs distinguished by surface proteins such as LRP1 and ITGA6 and an astrocyte-enriched proteome. When these cell-type signatures are projected onto human brain-derived EV datasets, an astrocyte-EV module is the one most strongly associated with AD pathology and cognitive impairment. Within this module, the hub protein integrin-β1 (ITGB1) is significantly elevated in astrocyte-enriched EVs from AD brains and is correlated with Aβ and tau burdens in independent human cohorts, directly linking astrocyte-derived EV signaling to disease severity [42].

Astrocyte sEVs also carry distinct mRNA repertoires. In murine primary astrocytes, sEVs are enriched in transcripts related to lipid metabolism, cytokine signaling, and the extracellular matrix, differing clearly from the neuronal and microglial sEV transcriptomes [43]. These cargo patterns support a role for astrocyte-derived sEVs in modulating neuroinflammation, synaptic support, and vascular interactions.

Microglia- such as iPSC–derived cells release EVs marked by ITGAM and LCP1, forming a microglia-specific EV signature. In AD brain-derived EVs, microglial markers are prominent within inflammatory modules, supporting microglial vesicles as major carriers of immune mediators [42].

Cell–specific cultures combined with long-read sequencing revealed that microglial sEVs are enriched in full-length mRNAs related to

cytokine production, interferon responses, chemokines, and immune receptors [43]. This immune-skewed transcriptome aligns with evidence that microglial sEVs in AD brains present increased interferon-γ/β response transcripts and high levels of ApoE, MS4A4A, and MS4A6A mRNAs, genes strongly linked to neuroinflammatory risk [44]. These data support microglial sEVs as key vectors of inflammatory and lipid-handling signals in neurodegeneration.

Oligodendrocyte-like cells secrete EVs containing markers such as LAMP2 and FTH1, forming an oligodendrocyte-specific signature distinct from that of neuronal or astrocytic EVs. Mapping these signatures onto human brain EV proteomes suggests that oligodendrocyte-EV modules participate in myelin and iron-handling pathways that become altered across the AD continuum [42].

In addition to classical brain glia, Müller glia in the retina also generate neuroactive sEVs. In a mouse optic nerve crush model, intravitreal administration of Müller glia-derived sEVs preserved retinal ganglion cell (RGC) survival and visual function while suppressing microglial activation. Cargo analysis revealed that miR-125b-5p and miR-16-5p are key miRNAs that target Cx3cl1; MG-sEV treatment reduced retinal Cx3cl1 and pro-inflammatory cytokines and modulated Cx3cl1–Cx3cr1 signaling in microglia, demonstrating that specialized glia-derived sEVs can restrain neuroinflammation and degeneration in vivo [45].

Neural stem cells (NSCs) release sEVs that regulate the neurogenic niche, influencing endogenous NSCs, neurons, astrocytes, microglia, and vascular endothelial cells. Under denervation, NSC-sEVs show the upregulation of specific circular RNAs; circAcbd6 acts as a sponge for miR-320-5p, increasing oxysterol-binding protein-related protein 2 expression and promoting NSC differentiation, revealing an intrinsic NSC-sEV program that fosters repair [46].

Across models of ischemic stroke and demyelination, NSC-sEVs modulate neuroinflammation, promote neuronal and oligodendroglial differentiation, and support angiogenesis, with reduced immunogenicity and enhanced blood–brain barrier crossing compared with whole-cell transplantation [46].

Although most mechanistic work has focused on CNS-resident cells, peripheral sEVs can reach and influence the brain. Aging alters the composition of circulating sEVs, which then enter the CNS and shift glial gene expression toward activated states, whereas stem-cell-derived peripheral sEVs can deliver neuroprotective cargo, attenuating complement and NF-κB activation in neurodegenerative models [3].

5. Circulating sEVs as Minimally Invasive Biomarkers in Major Neurodegenerative Diseases

Circulating small extracellular vesicles (sEVs) in blood offer a window into CNS pathology while avoiding invasive cerebrospinal fluid (CSF) collection. Original clinical and translational studies of Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD) support their potential for diagnosis, staging, and prognosis. A key advance is the simultaneous enrichment of multiple brain cell–derived sEV subtypes from plasma (neurons, astrocytes, microglia, oligodendrocytes, pericytes, and endothelial cells), followed by targeted miRNA analysis. In older adults with normal cognition, mild cognitive impairment (MCI), MCI converting to AD dementia (MCI-AD), and AD dementia, miRNAs in specific sEV subtypes were differentially expressed and distinguished dementia stages with an area under the curve (AUC) > 0.90. These miRNA signatures are also correlated with temporal cortical thickness on MRI, directly linking blood sEV signals to structural neurodegeneration [47].

Broader plasma EV studies have shown that small and large EV fractions carry distinct miRNA profiles that distinguish AD patients from healthy controls and from those with other neurodegenerative diseases. SEV-associated miRNAs converge on pathways including ubiquitin-mediated proteolysis and Toll-like receptor signaling, which is consistent with AD-related protein homeostasis and innate immune activation [15].

Work on blood brain-derived EV enrichment protocols highlights the feasibility and challenges of CNS-specific biomarker capture. A systematic comparison of polymer-based and L1CAM immunoprecipitation–based enrichment in age-related neurodegenerative diseases revealed that most AD blood studies use ExoQuick for total EVs and L1CAM for presumed neuronal EVs, yet centrifugation and antibody protocols vary substantially, underscoring the need for harmonization to make AD sEV biomarkers clinically robust [48].

Plasma neural-derived sEVs carrying α-synuclein (α-syn) and DJ-1 are repeatedly elevated in PD patients. In a large case–control study, L1CAM-positive neuronal sEVs were isolated from the plasma of 267 PD patients and 214 controls; the neuronal sEV α-syn concentration was significantly greater in PD patients than in HCs and strongly correlated with clinical severity. A second study revealed that both α-syn and DJ-1 in plasma neural-derived sEVs were upregulated in early- and advanced-stage PD patients compared with controls, although these markers did not reliably differentiate early from late PD patients [49].

Crucially, circulating neuronal sEV α-syn can aid in differential diagnosis. In plasma from patients with PD and multiple system atrophy (MSA), neuronal sEV α-syn was approximately two-fold lower in MSA patients than in PD patients. By combining cohorts (735 participants total), investigators identified a neuronal sEV α-syn threshold of 14.0 ng/L that consistently differentiated PD from MSA, suggesting practical cut-offs for clinical use [49].

In addition to proteins, plasma-derived sEV miRNAs also show diagnostic potential. In a multi-disease study, miRNA profiles from small and large EVs discriminated PD patients from controls and from AD patients, ALS patients, and FTD patients, with disease–specific signatures hint at pathway-level differences [15].

The same plasma EV miRNA sequencing study demonstrated that both LEV and SEV fractions from ALS and FTD patients harbor distinct miRNA signatures compared with those of controls. Pathway analysis suggested that SEV-associated miRNAs are enriched for the ubiquitin-proteasome and Toll-like receptor pathways, whereas LEV-associated miRNAs are more closely linked to neurotrophin signaling and glycosphingolipid biosynthesis, implying that different circulating EV size classes may be involved in different aspects of ALS/FTD pathobiology [15]. These disease– and vesicle–specific patterns support the use of circulating sEV miRNA panels as multi-disease biomarker tools.

Blood-based brain EV biomarkers face two central technical issues: how to optimally enrich CNS-derived vesicles from a complex plasma background and how to standardize protocols between laboratories. A systematic assessment of brain-derived EV (BEV) enrichment for blood biomarkers across AD and other age-related neurodegenerative diseases revealed that 82% of studies use polymer-based EV precipitation and that 92% rely on L1CAM for neuronal EV immunocapture. Only 26.8% of the 82 proteins used for BEV enrichment or post-enrichment characterization were CNS- specific, emphasizing that better marker panels are needed to confirm the CNS origin [48].

Despite these challenges, accumulating evidence indicates that (i) detecting AD with high sensitivity/specificity via miRNAs in cell-specific sEVs; (ii) reflecting structural brain atrophy; (iii) distinguishing PD patients from controls and from MSAs via neuronal sEV α-syn and DJ-1 [49]; and (iv) providing disease–specific miRNA fingerprints across AD, PD, ALS, and FTD (Table 1) [15].

6. Therapeutic Targets of Pathogenic sEV Pathways in Neurodegeneration

Therapeutic strategies for treating neurodegeneration increasingly aim either to neutralize **pathogenic sEV signaling** or to exploit engineered sEVs as precision delivery systems to restore homeostatic pathways. Original experimental studies in Alzheimer's disease (AD), Parkinson's disease (PD), and related models highlight several concrete

Table 1
Circulating sEVs as Minimally Invasive Biomarkers in Major Neurodegenerative Diseases.

Disease/condition	Biofluid & sEV type	Main sEV biomarker(s) measured	Key diagnostic/clinical finding	References
Glaucoma (RGC degeneration)	Plasma-derived sEVs	miR-29 family in circulating sEVs	PDEV-miR-29 s upregulated in glaucoma; levels associate with visual field defects and protect RGCs in vivo	[50]
Parkinson's disease	Salivary sEVs	Total sEV concentration; sEV α-synuclein	Fluorescence-tagged salivary sEVs and sEV α-syn discriminated PD and prodromal PD from controls with ~94% sensitivity	[51]
Alzheimer's disease & MCI	Plasma-derived sEVs	Aβ1–42, p-Tau, synaptophysin, IL-1β, TNF-α, GFAP	Higher PsEV counts and Aβ1–42/p-Tau in MCI/AD; synaptophysin↓, inflammatory markers↑; high AUC for AD vs controls	[52]
AD, PD, ALS, FTD	Plasma LEVs & SEVs	Global mRNA cargo	Disease-specific and shared mRNA signatures in SEVs/LEVs; ALS/FTD SEVs enriched for mRNA-processing pathways	[53]
Parkinson's disease (older adults)	Serum sEVs	Mitochondrial proteins (ATP5A, NDUFS3, SDHB) & tetraspanins	PD showed more sEVs but reduced mitochondrial proteins; combined sEV and inflammatory profile classified PD vs controls	[54]
AD, PD, ALS, FTD	Plasma LEVs & SEVs	miRNA profiles (NGS)	Distinct miRNA signatures in LEVs and SEVs that discriminate each ND from controls; pathways linked to proteolysis and TLR signaling	[15]
Parkinson's disease	Plasma & plasma-sEVs	miR-23b-3p (plus 3 other miRNAs)	miR-23b-3p up in plasma but down in plasma-sEVs in PD; both matrices yield good ROC AUC vs age-matched controls	[55]
Parkinson's disease	Plasma EV subtypes (pure small EVs)	miR-34a-5p	miR-34a-5p significantly upregulated in pure small EVs from PD; AUC ≈ 0.74 and correlates with disease duration and severity	[56]
Amyotrophic lateral sclerosis	Serum sEVs	miR-23c, miR-192-5p	Distinct sEV miRNA profile in ALS; up-regulation of miR-23c and down-regulation of miR-192-5p validated as potential biomarkers	[57]
Dementia with Lewy bodies	Serum sEVs	Total RNA (mRNA, miRNA, others)	846 DE RNAs (incl. 30 miRNAs); serum sEV RNAs mirror brain changes, implicating UPS, DNA repair, RNA modification pathways	[58]
ALS, FTD, PSP, bvFTD	Plasma EVs (small-EV enriched)	TDP-43; 3R/4R tau isoform ratio	EV TDP-43 and 3R/4R tau ratios distinguish ALS, tauopathies, and FTD with AUC > 0.9; correlate with neurodegeneration and severity	[59]
Early Alzheimer's disease	Plasma neuron-derived EVs	p181-Tau, Aβ42, BDNF/proBDNF, GluR2, PSD95, GAP43, syntaxin-1	NDEVs show elevated p181-Tau/Aβ42 and reduced synaptic proteins; multianalyte model correctly classifies most AD vs controls	[60]
Glioblastoma (brain tumor)	Serum sEVs	circSMARCA5, circHIPK3 (circRNAs)	Both circRNAs reduced in GBM-sEVs; each achieves AUC ≈ 0.82–0.86; combination with blood ratios improves diagnostic accuracy	[61]
Brain tumors (GBM, metastases, meningioma)	Serum sEVs	MMP-9 protein	sEV-MMP-9 reflects tumor aggressiveness; low sEV-MMP-9 (<28 ppm) associates with ~8-month survival advantage in GBM	[62]
Traumatic brain injury	Plasma brain-specific sEVs	CCL2 on ATP1B2/EAAT2-positive sEVs	Brain-specific sEV-CCL2 markedly increased in TBI; single-sEV SERS platform reaches near-perfect AUC for TBI vs controls	[63]

approaches. In toxin-based PD models, enteric neurons exposed to rotenone release α-synuclein-loaded sEVs that traffic along the vagus nerve to brainstem nuclei, providing a mechanistic route for pathology propagation. Surgical vagotomy or pharmacologic blockade of sEV tetraspanins markedly reduces the central accumulation of α-synuclein, directly indicating that interfering with vesicle-mediated transport can attenuate prion-like spread in vivo [1]. In LRRK2-G2019S PD mutation carriers, phosphorylated Rab12 (pSer106-Rab12) is elevated in urinary and plasma sEVs and rapidly decreases after one week of treatment with the LRRK2 inhibitor MLi-2, demonstrating that targeting a kinase upstream of sEV–associated trafficking proteins can both modify vesicle signaling and provide real-time pharmacodynamic read-outs [1]. This illustrates a second mode of targeting: inhibiting regulators of vesicle biogenesis/trafficking rather than the vesicles themselves.

Human Wharton's jelly mesenchymal stem cell (MSC)-derived sEVs have been tested in an in vitro AD model using hippocampal cells from 5xFAD transgenic mice. After addition to cultures, these sEVs were non-toxic, efficiently internalized by neurons and astrocytes, reduced extracellular Aβ peptide levels, and increased synaptic density [64]. These data suggest that MSC-sEVs can acutely shift amyloid handling and synaptic integrity toward a less pathogenic state.

In vascular dementia–relevant models, stromal or mesenchymal cell–derived sEVs attenuate microglial-driven neuroinflammation by inhibiting TLR2/IRAK1/NF-κB signaling and increasing the M2/M1 microglial ratio. Hypoxia-preconditioned MSC-sEVs enriched with miR-216a-5p downregulated the TLR4/NF-κB/PI3K/AKT pathway, further facilitating M2 polarization. Adipose stem cell–derived sEVs carrying miR-188-3p inhibited CDK5-dependent autophagy and NLRP3-mediated inflammasome activation, conferring neuroprotection in preclinical models [65]. Collectively, these interventions **reprogram innate immune and cell- death pathways** through vesicle-delivered miRNAs.

A direct demonstration of therapeutic sEV engineering involves delivery of the ROCK inhibitor SR3677 in a PD mouse model. Macrophage-derived sEVs were loaded with SR3677 and administered intranasally to Parkin Q311X(A) mice. Compared with free SR3677, sEV-SR3677 increased the expression of mitophagy– and mitochondrial function-related genes, decreased ROCK2 expression, reduced inflammatory factor levels, and elevated brain dopamine levels more effectively. Sham EVs had modest benefits, indicating that both the pharmacologic cargo and the vesicle scaffold contributed. Ex vivo assays revealed improved mitochondrial respiration in glial cultures treated with sEV-SR3677, which was consistent with the restoration of defective mitophagy and energy metabolism [66].

7. Engineered sEVs and stem cell–derived sEVs as neuroprotective drug delivery systems)

Stem cell–derived small extracellular vesicles (sEVs) are being actively tested as **neuroprotective nanocarriers** that can cross the blood–brain barrier (BBB), modulate neuroinflammation, and deliver drugs or endogenous protective cargo in models of major neurodegenerative diseases. This original experimental work in Alzheimer's disease (AD), Parkinson's disease (PD), and neuroinflammation models illustrates both native and engineered sEV strategies. Small EVs from human Wharton's jelly mesenchymal stem cells (MSCs) were applied to hippocampal cultures derived from 5xFAD transgenic mice, an AD model carrying five familial AD mutations. These MSC-sEVs, which were isolated by asymmetric depth filtration, were non-toxic, were internalized by neurons and astrocytes, reduced the amount of extracellular Aβ peptide, and significantly increased the synaptic density in vitro [64]. These findings demonstrate that unmodified MSC-sEVs can restore synaptic marker expression and amyloid homeostasis, supporting their use as cell-free neuroprotective agents in AD.

In vivo, intranasal administration of sEVs from cytokine-preconditioned MSCs to 3xTg AD mice resulted in clear immunomodulatory and structural benefits. MSC-EVs reach the brain, dampen microglial activation, and increase dendritic spine density in hippocampal regions. Primary microglia treated with these EVs in vitro shift

toward an anti-inflammatory phenotype, indicating that microglial polarization is a key mechanism underlying in vivo neuroprotection [67]. Crucially, intranasal delivery offered a noninvasive route while still achieving central effects, underscoring the feasibility of repeated clinical dosing.-.

Neural stem cell–derived EVs (NSC-EVs) also show intrinsic neuroprotective properties. In two in vitro PD models, NSC-EVs significantly enhanced dopaminergic neuron survival and reduced apoptosis. These vesicles are naturally enriched in catalase, a strong antioxidant enzyme, which enables them to mitigate oxidative stress–mediated injury [68]. This work illustrates how the endogenous antioxidant cargo in stem cell-derived EVs can be leveraged without additional engineering.

In a preclinical AD mouse model, EVs from induced neural stem cells (iNSCs) derived from fibroblasts (iNSC-EVs) mitigated AD-like phenotypes, including deficits in learning and memory, suggesting that NSC-lineage EVs can reverse cognitive impairments and may be scalable via somatic cell reprogramming. [69]

In addition to their use as native cargo, stem cell–derived EVs have been used as vehicles for the delivery of small-molecule drugs against neuroinflammation. Human plasma-derived EVs (pEVs) and adipose-derived MSC-EVs (ADMSC-EVs) were loaded exogenously with donepezil (DNZ), a cholinesterase inhibitor with additional neuroprotective effects, to treat LPS-induced neuroinflammation. Both pEV-DNZ and ADMSC-EV-DNZ retain their nanoscale size (50–300 nm) and negative surface charge and display rapid, non-toxic uptake by human microglial HMC3 cells and BBB penetration in zebrafish [70].

In vitro, ADMSC-EVs alone significantly reduced inflammatory mediator release by HMC3 microglia, whereas DNZ-loaded pEVs and ADMSC-EVs decreased phagocytic activity and reactive oxygen species (ROS) levels. In zebrafish larvae, both formulations decreased microglial proliferation and exhibited antioxidant effects [70]. These data show that combining an intrinsically immunomodulatory carrier (ADMSC-EVs) with a neuroprotective drug (DNZ) can produce additive anti-inflammatory and antioxidant effects in vivo, highlighting engineered EVs as versatile CNS drug platforms.

Engineered EVs have also been explored for kinase inhibitor delivery in PD-related models, where macrophage-derived EVs loaded with a ROCK inhibitor improved mitochondrial function and dopaminergic signaling more effectively than free drugs did, illustrating how EV encapsulation can enhance CNS bioavailability and pathway modulation [68]. Although this work did not use stem cell sources, it provides a template for similar engineering of MSC- or NSC-EVs.

Across these studies, several mechanistic themes have emerged. First, BBB penetration and brain targeting have been consistently demonstrated: MSC– and ADMSC–derived EVs successfully crossed the BBB in zebrafish and reached the brain following intranasal administration in mice, supporting their feasibility as systemic or mucosal delivery vehicles. Second, microglial and astroglial reprogramming appears to be a shared mechanism, as MSC-EV- and ADMSC-EV-based formulations reliably attenuated pro-inflammatory microglial activation while promoting more reparative glial phenotypes, an effect central to their neuroprotective actions in AD and neuroinflammation models [67,70]. Third, synaptic and neuronal preservation is a recurring outcome: in 5xFAD cultures and 3xTg mice, stem cell–derived sEVs restored synaptic density and protected neurons from Aβ– and inflammation–induced toxicity, resulting in improved network integrity and, in vivo, enhanced cognitive performance [64,67,69]. Fourth, the studies highlight distinctions between endogenous and exogenous cargo: NSC-derived EVs enriched with catalase exert potent antioxidant effects without engineering [68], whereas DNZ-loaded ADMSC-derived EVs illustrate how classical drugs can be repurposed with improved delivery efficiency and synergize with intrinsic EV bioactivity [70]. Preclinical evidence indicates that both native and engineered stem cell–derived sEVs act as promising, minimally immunogenic neuroprotective drug-delivery systems capable of crossing the BBB, modulating neuroinflammatory pathways, and preserving neuronal and synaptic function across models of AD, PD, and neuroinflammation (Table 2).

8. Future directions and conclusions

Translating small extracellular vesicle (sEV) research into clinically useful biomarkers and therapeutics for neurodegenerative diseases (NDDs) will require coordinated advances in technology, trial design, and regulatory frameworks. Current evidence indicates that sEVs can both propagate pathology and serve as highly informative reporters of the cell state and drug-target engagement, positioning them uniquely at the interface of diagnosis and intervention [1,11,80–82].

Future biomarker development must progress beyond discovery toward rigorous clinical qualification. Although numerous EV and sEV signatures have been reported for Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and other NDDs, most remain insufficiently validated in large, diverse cohorts [82]. The key priorities include the following. First, standardized pre-analytical and analytical pipelines are essential, as heterogeneity in isolation methods (ultracentrifugation, precipitation, size exclusion, immunocapture), quantification, and normalization continues to undermine reproducibility [83–85]. Alignment with ISEV/MISEV-style minimal information standards, including reporting of yield, purity, and cell-of-origin markers, is critical. Second, high-throughput and in situ platforms are needed to overcome the labor-intensive nature of current isolation workflows; microfluidic, acoustic and immunosensor technologies that reduce or bypass bulk isolation will be central for clinical scalability. Third, multi-analyte, multi-omics panels are needed, as single markers are unlikely to capture NDD complexity. Integrating sEV proteins, lipids, and miRNAs with imaging and fluid biomarkers may increase the sensitivity for prodromal disease and enable more precise staging. Fourth, CNS-enriched vesicle capture through refined immunoaffinity approaches for neuron–, astrocyte–, and microglia–derived sEVs will improve disease specificity, particularly when peripheral EV noise from comorbid systemic conditions is high [86,87]. Finally, prospective, interventional studies are needed to elevate sEV readout from prognostic associations to regulatory-grade biomarkers; this will involve evaluating sEV measures as pharmacodynamic and surrogate end points in therapeutic trials, with rigorous comparison to established clinical and imaging standards. AI-driven analytics and single-vesicle profiling will likely be central for extracting robust signatures from high-dimensional sEV datasets and harmonizing measurements across centers [88].

On the therapeutic side, both the role of endogenous disease-modifying and exogenously engineered sEVs are under active investigation. Preclinical studies support sEVs and mesenchymal stem cell–derived EVs (MSC-EVs) as carriers of neuroprotective cargo and modulators of neuroinflammation, synaptic plasticity, and angiogenesis. A translational roadmap must address several domains. First, source selection and product definition require comparative studies of MSC-derived EVs, other stem-cell-derived EVs, and engineered donor cells to identify optimal sources for specific indications and to link cargo profiles to mechanisms of action [3,6,10]. Second, manufacturing at scale under GMP demands robust, scalable bioprocesses that ensure batch-to-batch consistency, with clearly defined critical quality attributes such as size distribution, potency assays, cargo identity, sterility, and stability aligned with regulatory expectations for biologicals and advanced therapies. Third, load engineering and targeting must balance improved loading of siRNAs, gene-editing tools, or small molecules (via parental-cell engineering or exogenous methods) with preservation of vesicle integrity and safety while optimizing surface engineering for CNS- and lesion–specific targeting. Fourth, safety, dosing and immunogenicity remain incompletely characterized; long-term biodistribution, off-target uptake and cumulative immune consequences of repeated systemic or intrathecal dosing require standardized toxicology and immunogenicity frameworks across NDD indications. Fifth, rational trial design and patient stratification should incorporate sEV biomarkers as companion diagnostics and pharmacodynamic readouts in early-

Table 2
Therapeutically Targeting Pathogenic sEV Pathways in Neurodegeneration.

Disease/model	Therapeutic sEV strategy (target/pathway)	Main mechanistic effect on pathogenic sEV pathway	Key therapeutic outcome in vivo	References
Sporadic Alzheimer's disease (STZ mouse)	iPSC-MSC-sEVs enriched in miR-223-3p targeting NLRP3/GSDMD inflammasome	sEV-miR-223-3p directly suppresses NLRP3, reducing pyroptosis-related neuroinflammation	↓ amyloid deposition, ↓ neuronal apoptosis, improved cognition	[71]
Optic nerve crush (RGC degeneration)	Müller glia-sEVs carrying miR-125b-5p/miR-16-5p targeting Cx3cl1–Cx3cr1	Downregulation of Cx3cl1 dampens microglial activation and inflammatory signaling	Preserved RGCs and visual function after injury	[45]
Traumatic spinal cord injury	Neural stem cell-sEVs activating autophagy	Induce LC3B/beclin-1, promoting autophagy and limiting apoptosis and inflammation	Reduced lesion size, improved locomotion, less neuroinflammation	[72]
Spinal cord injury	M2 BMDM-sEVs enriched in miR-421-3p targeting mTOR/autophagy	miR-421-3p inhibits mTOR, enhancing neuronal autophagy and survival	Better functional recovery, reduced neuronal apoptosis	[73]
Parkinson's disease (MPTP rat)	DPSC-sEVs as carrier for phloroglucinol (antioxidant)	sEV-encapsulation overcomes BBB limits, delivering antioxidant and anti-inflammatory cargo	Improved motor/non-motor deficits, ↑ TH neurons, ↓ TNF-α	[74]
Parkinson's disease	Inhibition of EV-tRF-02514 to restore ATG5/autophagy in microglia	Blocking tRF-02514 in PD-EVs rescues ATG5, increasing autophagy and reducing pyroptosis	Protected dopaminergic neurons, improved behavior, ↓ inflammatory factors	[34]
Parkinson's disease (Parkin mutant)	Macrophage-sEVs loaded with ROCK2 inhibitor SR3677 (mitophagy)	sEV-SR3677 enhances mitophagy gene expression and mitochondrial respiration	↑ brain dopamine, improved mitochondrial function vs free drug	[66]
ALS (SOD1G93A mice)	Intranasal MSC-sEVs modulating NF-κB & complement–coagulation	sEVs dampen NF-κB and complement cascade overactivation in spinal cord	Delayed disease progression, better motor function, prolonged survival	[41]
ALS (SOD1G93A)	In vivo self-assembled sEV-encapsulated SOD1-siRNA (RVG-tagged)	Liver-reprogrammed sEVs deliver SOD1-siRNA to CNS, silencing mutant SOD1	Ameliorated weight loss, motor decline, neuroinflammation, NMJ degeneration	[75]
Acute spinal cord injury (rat, monkey)	MSC-sEVs overexpressing BDNF (BDNF-sEVs)	Combines intrinsic sEV anti-inflammatory cargo with BDNF/TrkB activation	Reduced glial activation, ↑ axon rewiring and function in rats and monkeys	[76]
Cerebral ischemia (stroke mouse)	Intranasal BDNF-loaded MSC-sEVs	sEV-BDNF activates BDNF/TrkB and delivers neuroprotective miRNAs/proteins	Smaller infarcts, ↑ neurogenesis/angiogenesis, ↓ inflammatory cytokines	[77]
Diabetic peripheral neuropathy (neuroinflammatory neuropathy)	NSC-EVs loaded with sinomenine targeting WNT5a/TRPV1	Aptamer-targeted EVs shift microglia M1 → M2, suppress WNT5a/TRPV1 signaling	Improved neuropathic pain and nerve pathology in DPN mice	[78]
EAE/ALS models	IFN-γ-primed MSC-sEV miRNAs (miR-467f/466q) modulating p38 MAPK	sEV-miRNAs downregulate Map3k8/Mk2, reducing microglial pro-inflammatory phenotype	Lower spinal neuroinflammation markers in EAE mice	[32]
Acute brain injury (IL-1β striatal injection)	nSMase2 inhibitor PDDC to block EV biogenesis	Pharmacologic nSMase2 inhibition reduces neuron/oligodendrocyte/microglia EV release	Normalized EV levels; supports EV-biogenesis inhibition as neuroprotective strategy	[79]
Alzheimer's disease (5xFAD mice)	Targeting acid sphingomyelinase (ASM) to alter Aβ-EV interactions	ASM inhibitors disrupt Aβ–ceramide–EV complexes, reducing Aβ-laden, neurotoxic sEVs	Decreased EV-mediated neurotoxicity; ASM highlighted as AD EV target	[26]

phase trials, enabling dose selection, responder enrichment, and mechanism-based go/no-go decisions [2,4,89–91].

A practical roadmap for the clinical translation of sEV-based biomarkers and therapeutics in NDDs can be conceptualized across three intertwined tracks. The first is discovery and mechanistic anchoring, encompassing in vitro and animal models and deep molecular profiling of disease-associated and therapeutic sEVs. The second is translational optimization, including standardized platforms, scalable GMP manufacturing, validated potency and biomarker assays, and early human proof-of-concept studies. The third is regulatory and health-system integration, involving alignment with the FDA/EMA and ISEV guidelines, cost-effectiveness analyses, and incorporation into precision-medicine care pathways. sEV-based approaches are poised to reshape the diagnostic and therapeutic landscape of neurodegenerative diseases by coupling minimally invasive, CNS-informative biomarkers with targeted nanotherapeutics capable of traversing the blood–brain barrier. Realizing this potential will depend less on resolving fundamental biology, which already strongly supports their relevance, than on solving practical challenges of standardization, scalability, safety, and regulatory acceptance through coordinated, multidisciplinary efforts.

CRediT authorship contribution statement

Askarova Zebo Zafarjonovna: Writing – original draft, Project administration, Methodology, Investigation, Conceptualization. **Elmuratova Aysulu:** Writing – original draft, Visualization, Methodology, Investigation. **Sanoeva Matlyuba:** Writing – original draft, Methodology, Investigation. **Hamroyev Rashid:** Writing – original draft, Methodology, Investigation. **Jurakulov Bakhrom Azamatovich:** Writing – original draft, Methodology, Investigation. **Ahmadjonov Ahmadjon:** Writing – original draft, Methodology, Investigation. **Amirullayeva Barno:** Writing – original draft, Methodology, Investigation. **Azimova Mayram Kurbanovna:** Writing – original draft, Methodology, Investigation. **Mahsudali Rohataliyev Mahmudali ugli:** Writing – original draft, Methodology, Investigation. **Iskandarova Shaxodat:** Writing – original draft, Methodology, Investigation. **Turakulov Rustam:** Writing – original draft, Methodology, Investigation. **Matrizaeva Gulnara Jumaniyazovna:** Writing – original draft, Methodology, Investigation. **Alisher Ishankulov:** Writing – original draft, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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