Review

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Stem cell-derived extracellular vesicles for rare diseases: A clinical -bottleneck-to-mechanism framework for translation

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SUMMARY: Rare diseases impose a disproportionate clinical burden, and yet therapeutic progress is hindered by small cohorts, biological heterogeneity, and limited disease-specific options. Stem cell-derived extracellular vesicles (EVs), and especially exosome-enriched products, are emerging as adaptable cell-free therapeutics that preserve key paracrine activities of parent cells while offering improved controllability, engineering flexibility, and potentially lower acute immunogenicity than living-cell products. This review proposes a clinically driven bottleneck-to-mechanism framework for rare-disease translation, matching each disease class to its dominant pathological barrier, mechanism-relevant EV function, route-aware delivery strategy, and measurable potency endpoint. Using this framework, EVs may enable immune circuit rewiring in autoimmune disorders, neuroprotection and toxic-protein clearance in neurodegeneration, osteogenic and matrix-supportive repair in skeletal/connective tissue diseases, and metabolic rescue in lysosomal or mitochondrial disorders. We further highlight a key conceptual distinction between EVs as active biologics and EVs as engineered delivery vehicles. Successful translation will depend on integrating cargo design, surface targeting, biodistribution-aware administration, scalable manufacturing, and quality-by-design control, while anticipating repeat-dose pharmacokinetics/pharmacodynamics (PK/PD), immunogenicity, complement activation, procoagulant risk, impurity control, and off-target organ-accumulation challenges. Multi-omics and artificial intelligence may further refine target selection and precision engineering. Overall, stem cell-derived EVs constitute a versatile platform for treating rare diseases, but clinical success requires closer alignment among mechanism, disease specificity, product definition, and translational endpoints.

Keywords: extracellular vesicles, exosomes, rare diseases, mesenchymal stem cells, cargo engineering, surface targeting, artificial intelligence

1. Introduction

Rare diseases, despite their low individual prevalence, collectively impose a major medical and societal burden. Their marked genetic heterogeneity, limited number of patients, and often severe phenotypes complicate mechanistic studies, biomarker discovery, and therapeutic development (*1-3*). Conventional drug-development pipelines are particularly strained in this setting because small trial populations restrict statistical power, lengthen recruitment, and lessen commercial incentives. As a result, many rare disorders still lack disease-modifying therapies, highlighting the need for therapeutic platforms that can be adapted across mechanistically distinct indications (*4,5*).

Stem cell-based interventions have emerged as one such platform, with mesenchymal stem cells (MSCs) receiving particular attention because of their immunomodulatory activity, trophic secretome, and relative ease of procurement from bone marrow, adipose tissue, the umbilical cord, or related sources (*6-8*). However, direct cell transplantation faces persistent barriers, including variable engraftment, potential immunogenicity, theoretical tumorigenicity, ethical and manufacturing concerns, and inconsistent *in vivo* behavior across donors and culture conditions (*9,10*). These limitations have motivated interest in cell-free strategies that retain biological activity while improving safety, controllability, and scalability.

Among these strategies, stem cell-derived extracellular vesicles (EVs), and particularly exosome-enriched EV preparations, have become especially

attractive. EVs are lipid bilayer membrane-delimited particles released by cells; exosomes refer to an endosome-derived subtype, but most studies cannot prove biogenesis and should therefore report EV subtypes operationally and transparently. This terminology and reporting rigor are codified in minimal information for studies of extracellular vesicles (MISEV) guidelines, most recently updated as MISEV2023 (*11*). Importantly, EVs can recapitulate many beneficial paracrine effects of their parent cells while reducing risks associated with living-cell administration, such as ectopic engraftment or uncontrolled proliferation (*12,13*). Their relative stability in biological fluids, low immunogenicity, and ability to traverse selected biological barriers further enhance their appeal as rare–disease therapeutics (*14,15*).

The therapeutic logic of mesenchymal stem cell-derived EVs (MSC-EVs) is increasingly being defined at the level of mechanism rather than broad claims of regeneration. Here, regeneration is treated as a phenotypic outcome category; our emphasis is on mechanism-linked, testable endpoints that can serve as potency readouts and guide translation under small-cohort constraints. Their cargo can reshape inflammatory signaling, attenuate apoptosis, promote angiogenesis and tissue repair, and support metabolic homeostasis in recipient cells (*16-19*). In immune-mediated settings, MSC-EVs can reprogram macrophages, T-cell subsets, and cytokine networks; in neurological disease, they can provide trophic support and modulate neuroinflammation; and in tissue-fragility or metabolic disorders, they can be engineered to enhance pathway-specific correction (*20-23*).

Nonetheless, clinical translation remains constrained by unresolved issues in product definition, large-scale manufacturing, biodistribution, route selection, and potency testing. EV composition varies with cell source, donor state, priming conditions, and purification workflow, making batch comparability a central challenge rather than a secondary technical detail (*24-27*). Accordingly, progress in treating rare diseases will depend on integrating mechanistic precision, rare-disease clinical bottlenecks, engineering design, and regulatory–grade manufacturing into a single development framework.

At the same time, the pathways discussed in EV studies are not inherently novel therapeutic targets. Cytokine networks, AKT/ERK-linked survival signaling, Wnt-associated repair programs, and phagocyte responses are already addressed to varying degrees by existing biologics or small molecules. The justification for developing EV therapeutics is therefore not simply that they can affect these pathways but that they may combine multi-cargo biologic signaling, intracellular or nucleic-acid delivery, and route- or barrier-aware tissue access within a single platform, which may be especially relevant when rare-disease pathology involves several coupled bottlenecks that are not easily addressed by a single conventional agent. Conversely, when an approved drug already provides robust pathway control with acceptable safety, EV development should be justified by a clear added value such as improved tissue access, lower systemic exposure, combination-cargo capability, or the ability to support mutation-directed delivery rather than pathway modulation alone.

Compared to enzyme replacement therapy (ERT), antisense oligonucleotides (ASOs), AAV gene therapy, small-molecule chaperones, and immune biologics, EVs should not be considered to be intrinsically superior in treating every rare disease. Their realistic value is context-dependent: multi-cargo delivery when inflammatory, metabolic, and repair pathways must be modulated together; route-aware tissue access through local, intranasal, or matrix-retained delivery; lower systemic exposure when pathology is regional; surface/cargo engineering that combines targeting ligands with RNA, protein, enzyme, or small-molecule payloads; and adjunctive use with ERT, ASO, AAV, or biologics to improve tissue residence, reduce local inflammation, or broaden pathway coverage. Thus, EV development should be justified indication by indication by a measurable added value over available modalities.

As shown in Figure 1, the development pathway of stem cell-derived exosome therapy for rare diseases extends from fundamental exosome features and core therapeutic mechanisms to disease-specific preclinical advances in genetic metabolic, neurodegenerative, and skeletal or connective-tissue rare disorders, and then onward to clinical translation. In this review, we intentionally adopt a clinical-bottleneck-to-mechanism framework and focus on representative rare-disease classes where *i*) a dominant bottleneck can be identified, *ii*) EV mechanisms can be linked to measurable endpoints, and *iii*) translational constraints can be discussed in specific terms. We do not aim for exhaustive coverage of all rare diseases; instead, we emphasize design logic that can be re-used across indications. We emphasize four themes that are especially important for rare-disease translation: disease-matched mechanism selection, engineering logic for cargo and surface modification, clinically relevant delivery-route design, and manufacturing/regulatory strategies that can facilitate scalable and reproducible products (*28,29*).

2. Biological characteristics of stem cell-derived exosomes and their potential mechanisms in the treatment of rare diseases

2.1. Biogenesis, composition, and functions of exosomes

Exosomes are small EVs generated through the endosomal pathway, in which inward budding of endosomal membranes forms multivesicular bodies that subsequently fuse with the plasma membrane to release vesicles into the extracellular space. This biogenesis route shapes both membrane composition and cargo architecture, yielding vesicles enriched in canonical

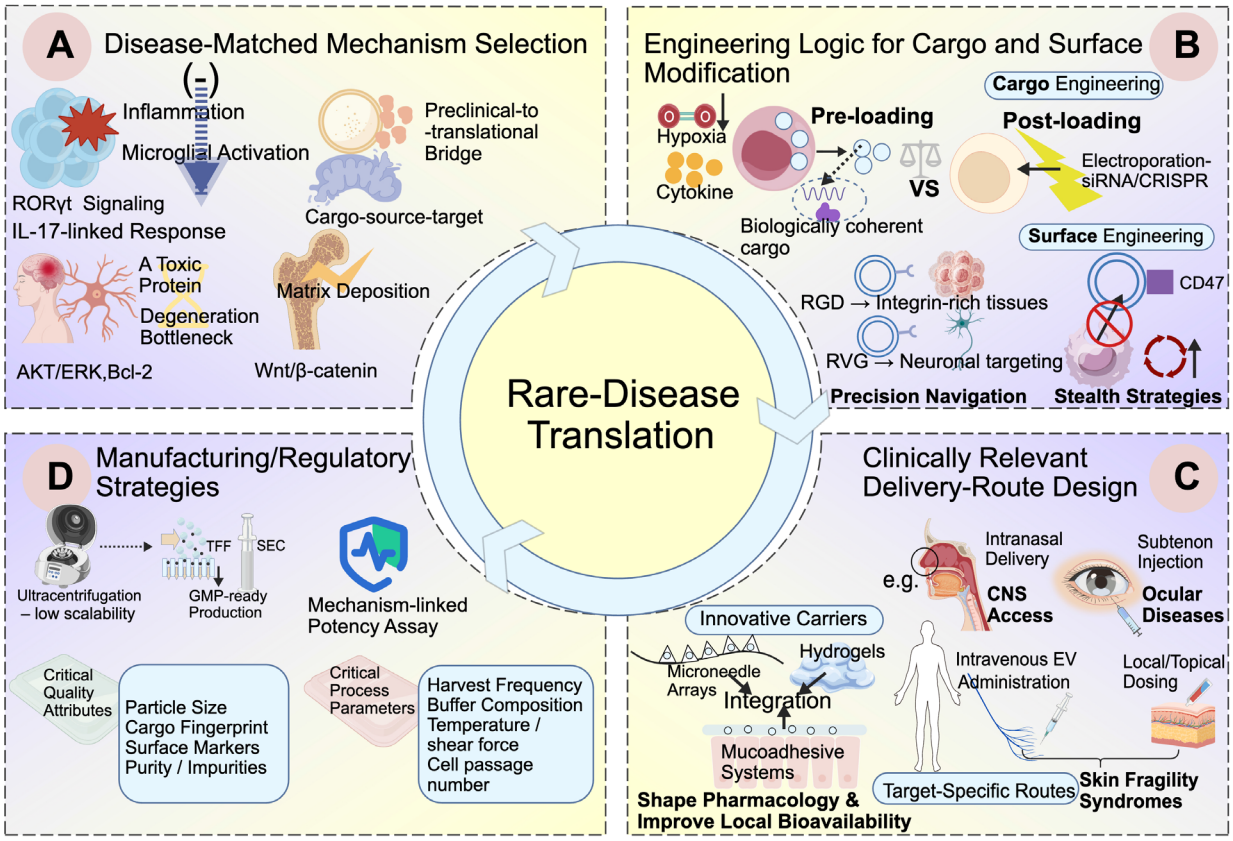

Figure 1. Clinically aligned translational workflow for stem cell-derived EV therapy in rare diseases. (A) Disease-matched mechanism selection: dominant disease bottlenecks are first defined and paired with EV functions, including suppression of inflammation and microglial activation, inhibition of RORγt/IL-17-linked immune responses, AKT/ERK–Bcl-2-associated neuroprotection, and Wnt/β-catenin-mediated matrix or bone repair. **(B)** Cargo and surface engineering: EVs may be modified through pre-loading strategies such as hypoxia or cytokine priming to enrich biologically coherent cargo or through post-loading methods such as electroporation to deliver siRNA, CRISPR-related components, or other defined payloads. Surface ligands, including RGD- or RVG-like motifs and CD47-based stealth modules, can improve tissue navigation and reduce phagocytic clearance. **(C)** Delivery-route design: intranasal, subtenon, intravenous, local, or topical administration is selected according to target tissue, while hydrogels, microneedle arrays, and mucoadhesive systems improve retention and local bioavailability. **(D)** Manufacturing and regulatory strategies: scalable purification, GMP-compatible production, critical quality attributes, critical process parameters, and mechanism-linked potency assays support reproducibility. **(E)** Integrated rare-disease translation: mechanism, engineering, route, manufacturing, and clinical endpoints are iteratively aligned.

markers such as CD9, CD63, and CD81, together with lipids, miRNAs, mRNAs, circular RNAs, enzymes, cytokines, and growth-related proteins (*30,31*). Because definitive assignment to "exosomes" requires evidence of biogenesis, we refer to "EVs" or "exosome-enriched EVs" when appropriate, and emphasize transparent reporting consistent with MISEV guidelines.

From a translational perspective, EV product information can be organized into three interacting layers (Figure 2): a payload layer consisting of miRNA, mRNA, proteins, lipids, enzymes, and any engineered therapeutic cargo; a navigation layer consisting of integrins, tetraspanins, adhesion molecules, targeting ligands, and anti-phagocytic or stealth modules that influence tissue tropism and uptake; and a manufacturing layer consisting of the source cell type, donor variability, passage number, culture and priming conditions, the method of purification, storage, and release testing. This layered framework explains why two products labeled simply as MSC-exosome may display markedly different *in vivo* behavior even when their operational identity-marker profiles appear similar.

MSC-EVs are particularly relevant to rare diseases because they often carry pro-repair and immunoregulatory cargo, including miR-21, miR-146a, members of the let-7 family, HGF, and VEGF, while also retaining membrane features that can influence uptake in target tissues (*17,31,32*). Together, these properties position EVs as natural nanocarriers that couple biological signaling with delivery potential. For rare-disease applications, however, descriptive marker panels are insufficient; functional interpretation must be linked to the disease bottleneck the vesicles are intended to overcome.

2.2. Core mechanisms underlying the therapeutic effects of EVs on rare diseases

A more clinically useful framework is to map a dominant EV mechanism to a dominant disease bottleneck (Figure 3). Importantly, rare-disease relevance is established by

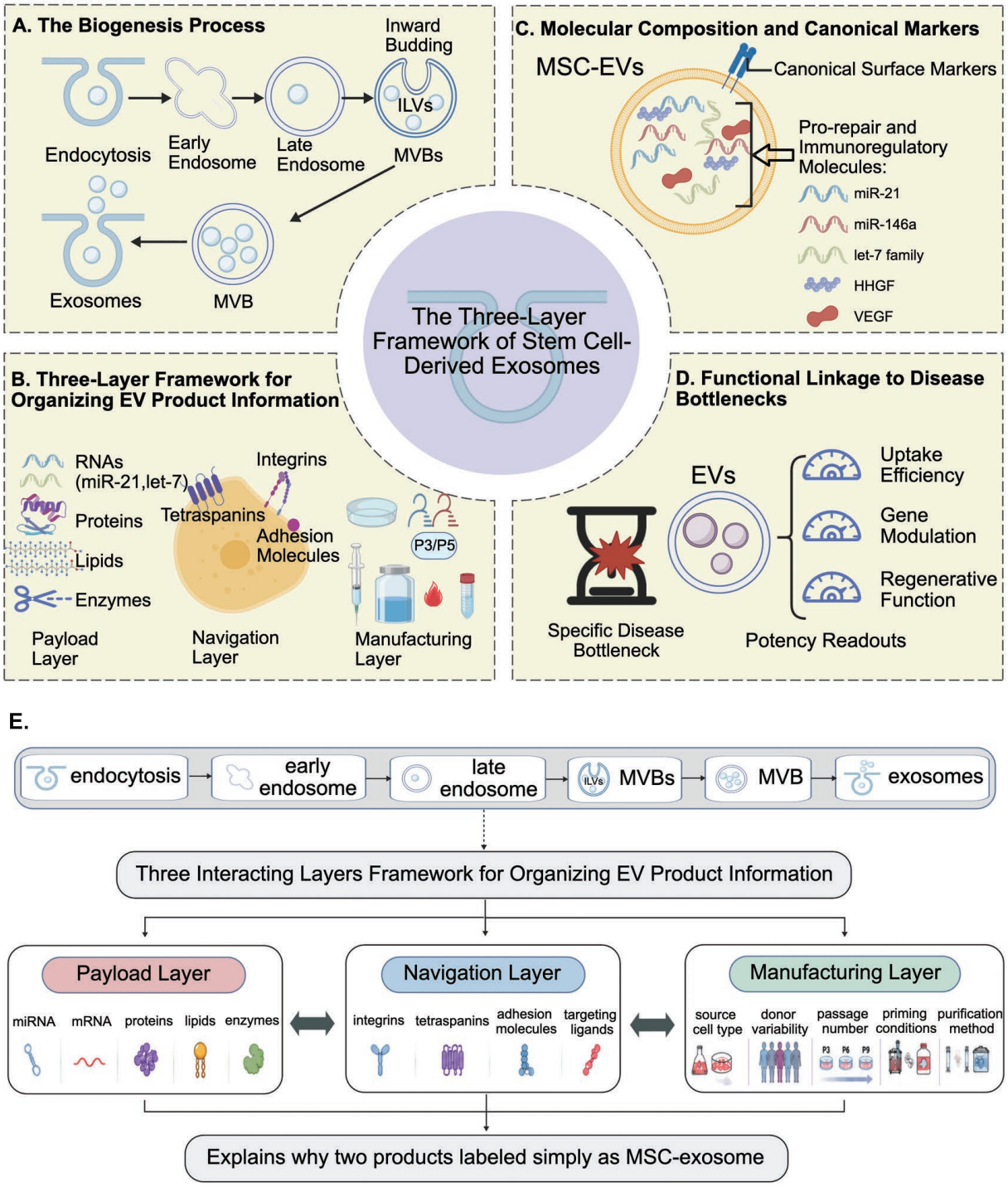

Figure 2. Biological characteristics of stem cell-derived exosomes. (A) Biogenesis process: exosomes arise from the endosomal pathway, in which endocytosis forms early endosomes, late endosomes, and multivesicular bodies; fusion of multivesicular bodies with the plasma membrane releases intraluminal vesicles as exosomes. **(B)** Three-layer framework: EV product information is organized into a payload layer, navigation layer, and manufacturing layer. The payload layer includes miRNAs, mRNAs, proteins, lipids, enzymes, cytokines, and engineered cargo; the navigation layer includes tetraspanins, integrins, adhesion molecules, targeting ligands, and anti-phagocytic modules; the manufacturing layer includes cell source, donor variability, passage number, culture conditions, purification, storage, and release testing. **(C)** Molecular composition and canonical markers: MSC-EVs commonly express CD9, CD63, and CD81 and carry regulatory cargo such as miR-21, miR-146a, let-7 family members, HGF, and VEGF. **(D)** Functional linkage to disease bottlenecks: after uptake, EVs may modulate inflammation, gene expression, angiogenesis, survival, metabolic stress, and repair pathways. **(E)** Product heterogeneity: differences in source and manufacturing explain why similarly named MSC-EV products may exhibit distinct potency and biodistribution.

bottleneck matching and measurable endpoints, not by claims that a pathway is unique to rare diseases.

In immune-mediated rare disorders, the relevant biology is not a generic anti-inflammatory effect, but selective rewiring of pathogenic immune circuits. EVs enriched in miR-146a can suppress inflammatory amplification, lessen microglial activation, and restrain Th17-skewed responses, while MSC-derived vesicles

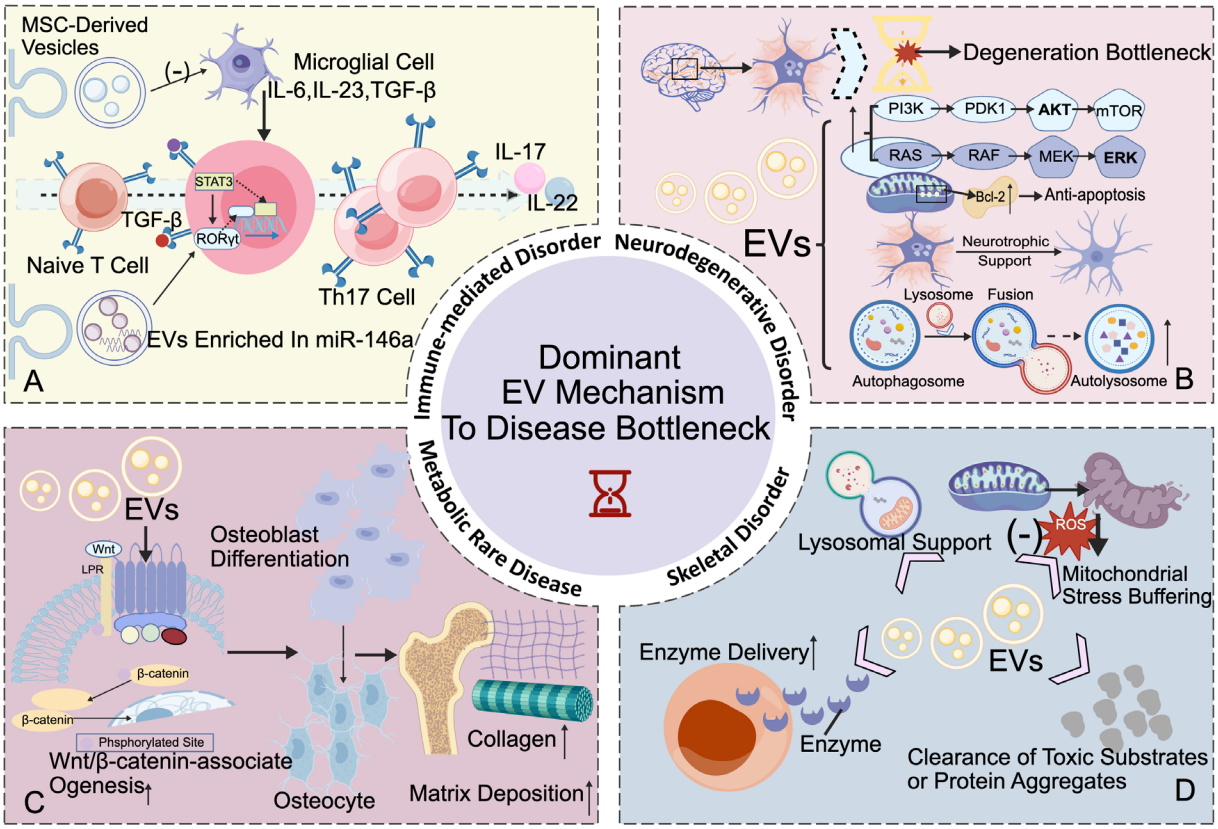

Figure 3. Mechanism-to-bottleneck matching of stem cell-derived EVs in representative rare-disease classes. (A) Immune-mediated rare diseases: MSC-EVs, and especially those enriched in miR-146a, may suppress microglial activation and inflammatory cytokines such as IL-6, IL-23, and TGF-β, thereby reducing STAT3/RORγt-driven Th17 differentiation and downstream IL-17/IL-22 effector responses. **(B)** Neurodegenerative rare diseases: EVs may protect vulnerable neurons by activating PI3K/AKT and RAS/RAF/MEK/ERK signaling, increasing Bcl-2-associated anti-apoptotic activity, reducing glial inflammatory stress, and facilitating autophagy-mediated clearance of toxic proteins through autophagosome–autolysosome flux. **(C)** Skeletal and connective-tissue rare diseases: EVs may enhance Wnt/β-catenin-associated osteogenesis, promote osteoblast differentiation, osteocyte maturation, mineralization, and extracellular-matrix deposition, which is particularly relevant to collagen-defective skeletal fragility. **(D)** Metabolic rare diseases: engineered or selected EVs may deliver enzymes, regulatory RNAs, or proteins that support lysosomal function, substrate clearance, mitochondrial stress buffering, mitophagy, and metabolic rescue. **(E)** Central principle: therapeutic EV development should match the key disease bottleneck with a measurable EV mechanism, including target-cell uptake, pathway engagement, biomarker modulation, and functional rescue.

have also been shown to destabilize RORγt signaling and reduce IL-17-linked effector programs (*33-35*). This logic is especially relevant to autoimmune encephalitis and related rare neuroimmune phenotypes.

In neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) and Huntington's disease, the central therapeutic priority shifts to preservation of vulnerable neurons and support cells in the presence of a toxic protein/degeneration bottleneck, where AKT/ERK activation, Bcl-2 upregulation, neurotrophic support, and autophagy enhancement are more informative mechanistic anchors than the broad claim of regeneration (*36-38*).

In skeletal disorders such as osteogenesis imperfecta, the key question becomes whether EVs can activate Wnt/β-catenin-associated osteogenesis, improve osteoblast differentiation, and support matrix deposition in a collagen-defective microenvironment (*39,40*).

The mechanistic emphasis changes again in metabolic rare diseases, where enzyme delivery, lysosomal support, mitochondrial stress buffering, and clearance of toxic substrates or protein aggregates may be more relevant than conventional regenerative endpoints (*41,42*). For these disorders, the most persuasive preclinical-to-translational bridge is a potency assay tied to enzyme activity, substrate burden, or organelle stress readouts. Representative cargo-source-target relationships that sharpen this logic are summarized in Table 1.

3. Preclinical advances in stem cell-derived exosomes to treat various rare diseases

Rare-disease preclinical models require critical appraisal. Although encouraging preclinical signals were summarized, the field faces a fundamental translational bottleneck: many monogenic rare-disease animal models only partially reproduce the full human phenotype, organ involvement, and time course, and they may compress or distort clinically relevant progression windows (*43*). For EV therapeutics, this matters because short-

Table 1. Representative cargo-source-target relationships that further define the mechanism-to-disease logic in exosome therapy for rare diseases

Stem cell source	Representative cargo	Rare-disease target/pathway	Example disease	Expected biological effect	Key caveat
Bone marrow MSCs	Osteogenic miRNAs, VEGF/HGF, pro-survival proteins	Wnt/β-catenin, AKT/ERK, COL1A1/2-related osteoblast program	Osteogenesis imperfecta	Osteoblast differentiation, vascular support, anti-apoptotic rescue	Requires osteogenesis-linked potency assay
Adipose MSCs	miR-146a, HGF, anti-inflammatory lipids	RORγt/Th17, NF-kB and NOTCH1 inflammatory circuits	Autoimmune encephalitis, rare immune phenotypes	Immune braking and cytokine reduction	May control phenotype without correcting a mutation
Umbilical cord MSCs	miR-124/miR-21, neurotrophic factors, low-immunogenic secretome	AKT/ERK-Bcl-2 axis, autophagy and CNS target engagement	ALS, Huntington's disease	Neuroprotection and support for BBB/intranasal delivery	Route optimization is decisive
Engineered parental MSCs	Loaded enzymes, siRNA/CRISPR cargo, VEGFC or other defined payloads	GBA/lysosome function, mutant HTT silencing, endothelial or lymphatic repair	Gaucher disease, Huntington's disease, rare lymphatic disorders	Metabolic rescue or precise pathway correction	Highest CMC and comparability burden

lived "phenotypic rescue" can be overestimated if the model's dominant failure mode differs from the human bottleneck. Accordingly, mechanistic endpoints should be treated as translatable only when they exist on a validated causal chain: EV exposure in the relevant tissue → target-cell uptake → pathway engagement → biomarker change → functional readout. We therefore recommend that rare-disease EV evidence be triangulated across complementary model layers (animal models, patient-derived iPSC/organoid systems, and disease-relevant *ex vivo* assays) and anchored to clinically interpretable biomarkers and natural-history knowledge where available (*44*).

3.1. Genetic metabolic rare diseases

In lysosomal and mitochondrial rare diseases, the bottleneck is often a defined metabolic deficit (enzyme deficiency, substrate accumulation, or organelle stress) for which phenotypic rescue may be achieved even without full genetic correction, provided a mechanism-linked axis is engaged.

In Gaucher disease and related lysosomal disorders, the appeal of EV therapy lies less in generalized regeneration than in whether vesicles can restore enzyme-dependent substrate handling in macrophages, glia, or neurons. Emerging evidence suggests that EVs can influence glucosylceramide trafficking, lysosomal function, and inflammatory amplification, but durable correction of glucosylceramidase β deficiency remains a preclinical objective rather than an achieved goal (*41*). Accordingly, translational claims in this area should carefully distinguish direct enzyme replacement from indirect lysosomal support or microenvironmental modulation.

In mitochondrial diseases, stem cell-derived exosomes are better viewed as tools for metabolic reprogramming than as complete genetic cures. Their cargo may modulate oxidative stress, mitophagy, mitochondrial biogenesis, and inflammatory crosstalk, thereby improving bioenergetic resilience in tissues with a high ATP demand (*42*). This distinction matters because partial restoration of stress-response pathways may still yield meaningful symptomatic benefit even when the causal mitochondrial or nuclear mutation is not directly corrected.

For metabolic rare diseases, the most informative preclinical readouts should therefore be mechanism-matched: enzyme activity, substrate burden, ATP production, reactive oxygen species levels, mitophagy markers, lysosome-autophagy flux, and tissue-specific functional rescue. These endpoints are more persuasive than broad histological improvement alone and are also more useful when translating a preclinical signal into a regulatory potency assay.

3.2. Neurodegenerative rare diseases

Central nervous system (CNS) rare diseases are dominated

by two coupled bottlenecks: tissue access and neuronal vulnerability/toxic protein burden, making route selection and target engagement inseparable from the mechanism.

In ALS, the rationale for MSC-EVs is strongest when neurotrophic support, control of neuroinflammation, and the route of administration are considered as a single therapeutic package. Exosomal miR-146a and trophic factors may modulate activated glia while *AKT/ERK-Bcl-2* signaling can protect stressed motor neurons, making the platform more than a passive substitute for the MSC secretome. CNS exposure remains a major bottleneck, so the route of delivery should be treated as part of the therapeutic hypothesis rather than as an aspect of the downstream formulation.

Huntington's disease illustrates why engineered exosomes may be more suitable than unmodified vesicles. Here, the dominant objective is to reduce the toxic huntingtin burden while preserving susceptible neurons, which makes gene-silencing cargo, CRISPR/Cas9-compatible loading strategies, and autophagy-enhancing signals especially relevant (*38,45*). In this context, exosomes function less as generalized anti-inflammatory vesicles and more as programmable nanocarriers for CNS target engagement.

Overall, neurodegenerative rare diseases indicate that the mechanism, tropism, route, dosing interval, and potency cannot be developed independently. Once the target tissue is the brain or spinal cord, surface engineering, intranasal design, blood-brain barrier models, and target-cell uptake assays become central translational variables rather than optional refinements.

3.3. Rare skeletal and connective tissue diseases

For skeletal/connective tissue rare diseases, the bottleneck often lies in matrix fragility and impaired cell-program execution, meaning that "repair" must be framed as program rescue rather than generic regeneration.

In osteogenesis imperfecta, the most persuasive therapeutic use of MSC-EVs is to drive osteoblast differentiation in a collagen-defective setting. Efficacy claims should therefore be linked explicitly to Wnt/β-catenin activation, Runx2/ALP-associated osteogenic programs, and matrix mineralization, rather than to broad regenerative language (*40*). This tighter framing better matches the biology of COL1A1/COL1A2-associated bone fragility and clarifies why osteogenesis-linked potency assays are essential.

For recessive dystrophic epidermolysis bullosa (RDEB) and related connective-tissue fragility syndromes, exosome therapy currently targets the wound-healing phenotype more than the germline defect itself. Near-term goals include local immune control, re-epithelialization, improved extracellular-matrix remodeling, and reduction of the chronic wound burden. That distinction is clinically important because a wound-directed extracellular-vesicle product may still provide meaningful benefit before direct mutation correction becomes feasible.

Evidence for treatment of Ehlers-Danlos syndrome remains limited and should be presented cautiously. At present, Ehlers-Danlos syndrome is best viewed as a hypothesis-generating indication in which exosomes may modulate fibroblast behavior and matrix homeostasis, but disease-specific efficacy data remain sparse. Stating this limitation explicitly strengthens the scientific rigor of this review.

4. Clinical translation status and challenges of stem cell-derived exosome therapy for rare diseases

4.1. Early clinical trials and safety evaluation

As summarized in Supplementary Table S1 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=306*), which consolidates the current rare-disease trial landscape from public registries, a consistent pattern emerges from this landscape: stem cell-derived EV/exosome-enriched studies of rare diseases remain in the early phase, have a small sample size, and are highly dependent on the route of administration. Ocular programs rely on subtenon injection, RDEB studies range from topical/local wound dosing to intravenous EV administration, and neuroimmune or neuromuscular programs increasingly favor intranasal delivery.

The congenital myasthenic syndrome study (NCT07226726) is especially instructive because intranasal delivery is treated as part of the therapeutic concept rather than as a secondary formulation choice. Intranasal administration may partly reduce systemic dilution and improve access to neuro-immune interfaces, but it also introduces additional variables, including nasal mucosal integrity, dose reproducibility, device compatibility, and residence time. The MESAE trial in autoimmune encephalitis (NCT07131683) likewise aligns the route, repeated dosing, and neurological outcome scales, illustrating how mechanism–aware trial design is beginning to emerge in the field.

At the same time, the public evidence base remains immature. Several registry entries still lack posted results, and record updates do not always keep pace with estimated completion dates or real-world study progress. At present, the most defensible clinical conclusion is feasibility and early safety rather than definitive disease modification.

4.2. Major challenges in clinical translation

The immediate barrier to clinical translation is not conceptual promise but product definition. For systemic and lifelong rare-disease indications, product definitions also need to anticipate exposure limits (PK/PD and biodistribution) and the possibility that repeat dosing will alter clearance and response. For rare-disease programs, an EV product should be described by a minimum

chemistry, manufacturing, and controls (CMC) package that includes the cell source, donor criteria, passage number, culture medium, conditioning strategy, harvest window, isolation train, storage conditions, and a potency assay explicitly linked to the intended mechanism. This aligns with the broader rare–disease development reality recognized by FDA guidance: small cohorts amplify the impact of manufacturing variability, making comparability and robust endpoints central rather than optional.

The EV dose is not self-evident, so nominal doses should not be compared across studies using the particle number, total protein, producer-cell equivalent, or cargo copy number alone. For rare-disease translation, we recommend a layered dose description: the particle number measured *via* validated single-particle analysis; the total protein and particle-to-protein ratio as purity context; the producer-cell equivalent for manufacturing comparability; and the disease-relevant cargo copy number, enzyme activity, or functional cargo activity when the payload is engineered or mechanism-defining. Release and comparability decisions should prioritize a mechanism-linked potency readout normalized to both the particle number and, when relevant, cargo dose—for example, substrate clearance per particle dose, cytokine suppression per protein-normalized dose, target-gene knockdown per cargo copy, or enzyme activity restored per defined EV dose.

In practical manufacturing terms, this is why scale-out workflows based on ultracentrifugation are increasingly inadequate: tangential flow filtration, usually followed by polishing steps such as size-exclusion chromatography, is better suited for closed, reproducible, and higher-throughput production (*46,47*).

A quality-by-design (QbD) framework is especially valuable for exosome therapeutics. Candidate critical quality attributes (CQAs) include the particle size distribution, particle-to-protein ratio, operational identity-marker panels, sterility/endotoxin, residual host-cell DNA and protein, culture-medium or serum-derived impurities, cargo fingerprints, leakage/stability of externally loaded payloads, and mechanism-linked potency. Critical process parameters (CPPs) include oxygen tension, serum-free adaptation, the cell density, passage number, harvest frequency, buffer composition, tangential flow filtration (TFF) concentration factor, and freeze-thaw history. Without prospectively defining these variables, batch comparability becomes extremely difficult, which is particularly problematic when rare-disease trials enroll only a few patients and every manufacturing lot has outsized clinical influence.

Regulatory strategy must also be rare-disease-specific. Orphan indications often rely on small cohorts, so developers may require biomarker-enriched enrollment, adaptive designs, external or intra-patient controls, and route-specific safety monitoring. And yet orphan-disease flexibility in trial design does not reduce the need for stringent comparability packages: any change in the upstream culture, purification sequence, or loading method can reshape cargo composition and therefore biological activity. In other words, rare-disease development may justify flexible trials, but it also requires stricter analytics.

4.3. PK/PD, biodistribution, and dose-interval design

EV translation in systemic rare diseases is frequently exposure-limited: after intravenous administration, many EV formulations distribute rapidly to mononuclear phagocyte system (MPS)-rich organs such as the liver and spleen, which can reduce bioavailability in harder-to-reach targets. *In vivo* studies show that EV biodistribution depends on the cell source, dose, and route, with prominent accumulation in the liver and spleen after systemic delivery (*48*), and MPS-dominated uptake can contribute to short circulation half-lives in some settings (*49*).

Protein corona and disease state can re-program EV kinetics and cellular uptake. Recent work has indicated that the serum-derived protein corona composition can retarget MSC-EVs away from hepatic macrophages and toward non-phagocytic liver cells, implying that "surface identity" is not fixed at release but is remodeled *in vivo* (*50*).

A practical implication for rare-disease translation is to treat route selection and PK/PD as part of the therapeutic hypothesis: which tissue needs what EV exposure for how long, and what PD biomarker will confirm mechanism engagement. In small-cohort settings, longitudinal, mechanism-linked PD readouts (*e.g.*, enzyme activity, substrate burden, and inflammatory network shifts) are often more informative than single time-point histology.

Dose-interval selection remains a major challenge. Rather than defaulting to weekly or monthly regimens, the interval should be derived from the duration of the PD effect in the target tissue, with the recognition that repeat dosing can also change PK through immune recognition or MPS adaptation. A minimal "PK/PD starter pack" for translation therefore includes: *i*) biodistribution mapping in relevant models, *ii*) target-tissue residence estimates, *iii*) a PD biomarker time course, and *iv*) a pre-specified rule for dose-interval adjustment if PD decay precedes clinical benefit.

4.4. Long-term repeat dosing, immune recognition, and accelerated clearance

Long-term repeat dosing is the default scenario for many rare diseases. Although EVs are often described as having lower immunogenicity than living-cell products, EVs can carry immunologically active surface molecules and can intersect with antigen processing/presentation and adaptive immunity (*51*). For allogeneic stem-cell-derived EVs, repeated administration may

therefore generate anti-product antibodies or functional neutralization, which can manifest as diminishing efficacy or faster clearance—outcomes that regulators and clinical pharmacology frameworks treat as clinically meaningful because it modifies PK, PD, and safety (*52*).

The risk is not merely theoretical: experience from nanomedicines shows that repeated dosing of polyethylene glycol-conjugated (PEGylated) carriers can trigger anti-PEG IgM and complement activation, leading to an accelerated blood clearance phenomenon and altered biodistribution. PEG immunogenicity and anti-PEG antibody prevalence are now recognized as clinically relevant to safety and efficacy for some nanomedicine platforms (*53,54*).

Risk-mitigation principles for rare-disease EV programs include treating immunogenicity as a longitudinal PK/PD modifier, monitoring for anti-EV/anti-excipient antibodies and complement activation signals in repeat-dose studies, minimizing immunogenic impurities (*e.g.*, serum-derived contaminants), and considering dosing-route strategies that reduce systemic exposure when the mechanism allows (*e.g.*, local delivery for wound-dominant phenotypes).

Safety evaluation should extend beyond immunogenicity. EV preparations may expose a phosphatidylserine or tissue factor and thereby increase procoagulant potential; activate a complement; retain host-cell DNA, host-cell proteins, culture-medium contaminants, serum-derived impurities, or adventitious agents; release an exogenously loaded cargo before target-tissue uptake; and accumulate in off-target organs, and especially the MPS-rich liver and spleen after systemic delivery. Repeat-dose studies should therefore include hemocompatibility and thrombin-generation assays, complement split products, residual DNA/protein and media/serum impurity testing, endotoxin/mycoplasma/adventitious-agent release criteria, loaded-cargo leakage and stability assays, and biodistribution panels that quantify off-target accumulation in route-relevant organs.

5. Engineering and functional modification to enhance the therapeutic efficacy of stem cell-derived exosomes in rare diseases

5.1. Cargo engineering modification

Cargo engineering is best discussed as a design trade-off rather than a binary technical choice. Pre-loading strategies, including hypoxia conditioning, cytokine priming, and parental-cell genetic engineering, benefit from biological coherence because the payload is packaged through endogenous vesicle biogenesis and the native membrane architecture is usually better preserved (*17,39,55*). Their main limitation is that the absolute loading dose is less controllable and may drift across donors or batches. This makes pre-loading attractive when the therapeutic goal is systems-level reprogramming, such as amplifying angiogenic or anti-inflammatory tone, but less ideal when a monogenic disorder requires a defined amount of small interfering RNA, messenger RNA, antisense oligonucleotide, or gene-editing cargo.

Post-loading strategies such as electroporation, sonication, extrusion, and chemical transfection offer greater control over the identity and nominal amount of an added therapeutic payload. These approaches are therefore attractive for precision interventions, including siRNA, antisense oligonucleotides, protein cargo, or CRISPR/Cas9-related components (*43,56*). However, higher loading efficiency may come at the cost of vesicle aggregation, RNA precipitation artefacts, membrane injury, altered uptake behavior, or reduced functional potency. For that reason, post-loading workflows should always be paired with assays that assess membrane integrity, retained tropism, and mechanism-linked activity, rather than marker panels alone.

EVs can function as active biologics or as delivery vehicles. A frequent source of conceptual drift in the literature is whether EVs are the active therapeutic entity or whether they are used primarily as delivery vehicles for a defined exogenous payload. This distinction has implications for CMC control, potency testing, and regulatory classification and should be stated explicitly for each rare-disease application.

In rare diseases, the most rational selection rule is mechanism-driven: use pre-loading when the desired product is a biologically enriched secretome, and use post-loading when the therapeutic objective is delivery of a quantifiable mutation-directed payload. Hybrid strategies may be especially relevant when a disorder requires both immune modulation and pathway-specific correction. A concise comparison of these approaches is provided in Table 2.

5.2. Membrane surface engineering modification

Surface engineering marks the transition from passive biodistribution to precision navigation. Classical ligand decoration with arginine-glycine-aspartic acid (RGD) or rabies virus glycoprotein (RVG) peptides remains useful because it operationalizes tissue selectivity-RGD-like motifs for integrin-rich diseased tissues and RVG-like ligands for neuronal targeting. And yet the field is moving beyond fixed peptide tags toward modular navigation systems that combine targeting with triggered release. Magnetic guidance, photo–responsive membranes, and thermos-responsive shells remain largely preclinical, but they are conceptually important because they separate two questions that are often conflated: how to reach the tissue and how to release cargo once the vesicle arrives.

Stealth engineering is equally important. In practice, "stealth" is the engineering response to the MPS-clearance bottleneck described above: without

Table 2. Critical comparison of pre-loading and post-loading strategies for cargo engineering in exosome therapeutics for rare diseases

Strategy Class	Representative methods	Strengths	Limitations	Best-fit rare-disease scenarios
Pre-loading	Hypoxia, IFN-γ or cytokine priming	Preserves endogenous membrane and coordinated biology	Payload dose less controllable, batch drift	Angiogenic or immunomodulatory indications
Pre-loading	Parental-cell gene engineering	Stable enrichment with biologically coherent cargo	Cell-engineering burden, added regulatory complexity	Repeated delivery of defined trophic or miRNA cargo
Post-loading	Electroporation of siRNA/ASO/CRISPR	Direct control of mutation-matched payload	Aggregation, membrane injury, variable encapsulation	Monogenic or toxic-gain-of-function disorders
Post-loading	Sonication, extrusion, chemical transfection	Greater loading for drugs or proteins	May alter native surface proteins and biodistribution	Local delivery or combination products

mitigating opsonization and phagocytic uptake, systemic EV exposure can be dominated by liver-and spleen-associated macrophages rather than the intended target tissue (*49*). Display of CD47 or CD47-mimetic "don't eat me" signals may reduce phagocytic clearance and extend circulation time, whereas PEG-like shielding can improve colloidal stability but may also mask uptake ligands if overused (*18,43*). However, any stealth strategy intended for chronic dosing should be evaluated under repeat-dose conditions for immune recognition and clearance drift rather than assuming a stable single-dose PK profile (*53,54*). A practical design principle is therefore to balance persistence against target-cell internalization rather than maximizing only one of them.

For rare diseases that target the CNS, the next frontier is multicomponent surface design: a brain-penetrating motif, an anti-phagocytic module, and a disease-receptor recognition signal on the same vesicle, ideally paired with a route-specific strategy such as intranasal delivery. At that stage, surface engineering becomes translationally decisive rather than simply elegant.

6. Future perspectives: Exploring the translational pathway from basic research to clinical application

6.1. Integrating multi-omics technologies and artificial intelligence (AI) to decipher mechanisms

AI should not be considered merely as a future accessory; it can define the exosome-discovery workflow itself. A practical pipeline begins with multi-omics characterization of producer cells and exosome batches-proteomics for membrane ligands and soluble effectors, small-RNA sequencing for regulatory cargo, lipidomics for membrane behavior, and single-cell atlases of recipient tissues for receptor availability (*57-59*). These layers can then be integrated using graph-based or multimodal models to predict which vesicle compositions are most likely to bind, enter, and reprogram disease-related target cells.

Rare-disease-specific AI/omics research presents both constraints and opportunities. Rare diseases are defined not only by prevalence but by small cohorts, high heterogeneity, and often a strong genotype-to-phenotype axis; AI methods must therefore address small-N learning and cross-center data barriers. Rare-disease knowledge bases and genome-phenome platforms (*e.g.*, Orphanet/Orphadata and RD-Connect GPAP) provide a structured substrate for standardized phenotypes and gene-disease mapping that can be linked to EV design variables. Methodologically, transfer learning and federated learning are particularly relevant because they help extract a signal from distributed, privacy-constrained datasets without requiring central pooling.

This enables a true in an *in vivo* target-selection paradigm. Rather than manufacturing a vesicle first and only later determining where it reaches, developers can computationally rank membrane-protein/receptor pairs, prioritize candidate targeting motifs, and then validate them experimentally with barcoded vesicle libraries, organ-on-chip blood-brain barrier models, or patient-derived organoids. For rare diseases, where patient material is scarce, this approach is particularly valuable because it reduces wet-lab iteration and concentrates resources on the most biologically plausible candidates.

The long-term objective should be an Exosome Atlas that links the source cell, manufacturing state, cargo composition, biodistribution, potency readouts, and patient phenotype. In rare-disease medicine, such an atlas could serve both diagnostic and therapeutic purposes by identifying disease-associated vesicle signatures while also guiding source-matched or engineering-matched exosome design.

6.2. Establishing regulatory science and industrialization frameworks

Industrial production will depend on whether exosome manufacturing can be made auditable, closed, and digitally traceable. Bioreactor-based upstream culture, TFF/size-exclusion chromatography-based downstream purification, in-line environmental monitoring, and electronic batch records are not simply engineering upgrades; they are prerequisites for lot comparability in geographically distributed rare-disease trials.

A mature regulatory-science package should therefore include reference materials, orthogonal identity assays, route-specific release criteria, and stability data linked to the final container-closure system. Many exosome products are biologically rich but analytically complex, so consensus potency assays—for example, suppression of a Th17 readout, induction of endothelial tube formation, restoration of enzyme activity, or rescue of a disease-relevant reporter cell line—are likely to become the bridge between mechanistic claims and regulatory acceptability.

6.3. Exploring combination therapies and innovative delivery systems

Combination therapy is especially attractive when exosomes cannot fully correct a monogenic defect on their own. In this setting, exosomes may serve as adjuncts to ERT, ASO, AAV gene therapy, small-molecule chaperones, immune biologics, or genome editing by improving tissue access, reducing local inflammation, or prolonging residence time at the target site (*27,39,60*). Biomaterial carriers such as hydrogels, adhesive wound dressings, microneedle arrays, or intranasal mucoadhesive systems should therefore be viewed not as passive containers but as pharmacology-shaping components that influence exposure kinetics and local bioavailability.

The strongest translational candidates are likely to be indication-specific platforms: wound-retentive matrices for RDEB, intranasal systems for neuroimmune rare diseases, and bone-targeting or integrin-targeting vesicles for skeletal disorders. Matching the carrier to the disease context may ultimately matter as much as matching the exosome to the cell type.

7. Conclusion

Stem cell-derived EVs remain one of the most promising cell-free platforms for rare-disease therapeutics, but their future depends on moving from proof-of-concept enthusiasm to matched product-disease design. The central message of this review is that exosome therapy will be far more persuasive when each disease section is anchored to a dominant rare-disease bottleneck, a route-aware delivery strategy, and a potency assay that can travel with the product into clinical development.

Viewed through this lens, the field is shifting from generic regenerative claims toward a more disciplined architecture: source-specific cargo mapping, explicit trade-offs between pre-loading and post-loading, precision surface navigation, TFF-compatible manufacturing, QbD-based control of CQAs and CPPs, and AI-enabled multi-omics pipelines for target selection. EV development for rare diseases is therefore not a smaller version of exosome development for common diseases; it is a stricter version, in which every lot, endpoint, and route decision carries greater weight because patient numbers are limited and heterogeneity is high.

In summary, the most credible path forward lies in integration: integration of the mechanism with disease specificity, engineering with biodistribution, manufacturing with regulatory science, and multi-omics with artificial intelligence. Only by linking these layers can stem cell-derived exosomes evolve from an appealing biological concept into scalable, targetable, and clinically credible therapeutics for patients with rare disorders. This integration should explicitly include PK/PD-aware dosing and proactive management of repeat-dose immunogenicity, procoagulant/complement safety, impurity control, cargo leakage, and off-target organ accumulation because rare-disease programs rarely succeed when assuming single administration.

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