International Journal of Nanomedicine

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

Effect Analysis of Extracellular Vesicles in the Treatment of Bronchopulmonary Dysplasia via Different Drug Delivery and Administration Routes

Wanting Xu[1–3], Siyu Chen[1–3], Ting Liang[1–3], Lan Kang[1–3], Qinxin Zheng[1–3], Yan Yang[1–3], Ling Guo[3], Jing Liu[3], Rong Zhang[1–3], Wenbin Dong[1–3]

[1]Division of Neonatology, Department of Pediatrics, the Affiliated Hospital of Southwest Medical University, Luzhou, People's Republic of China; [2]Department of Perinatology, The Affiliated Hospital of Southwest Medical University, Luzhou, People's Republic of China; [3]Sichuan Clinical Research Center for Birth Defects, Luzhou, People's Republic of China

Correspondence: Rong Zhang; Wenbin Dong, Division of Neonatology, Department of Pediatrics, the Affiliated Hospital of Southwest Medical University, Luzhou, China; Department of Perinatology, The Affiliated Hospital of Southwest Medical University, Luzhou, China; Sichuan Clinical Research Center for Birth Defects, Luzhou, People's Republic of China, Email zhangrong9604@163.com; dongwenbin2000@163.com

Abstract: Extracellular vesicles (EVs) are emerging as nanoscale, cell-free therapeutics for bronchopulmonary dysplasia (BPD), a chronic lung disease in premature infants characterized by underdeveloped alveoli and abnormal blood vessel formation. This review exhibits how different EV delivery methods influence therapeutic effects in BPD. Intra-tracheal administration of EVs enables localized pulmonary delivery, which may improve treatment efficiency via reducing inflammation and promoting lung development. Intravenous delivery provides systemic anti-inflammatory effects requiring higher doses because of lung's blood vessel barriers. Intraperitoneal administration requires higher dosages to produce comparable effects and shows lower drug accumulation in the lungs. Intragastric administration often results in poor absorption due to the digestive environment. The distribution of EVs in the body is largely dependent on delivery methods. Nebulized and tracheal administrated EVs primarily concentrate in the lungs, whereas intravenous EVs tend to distribute in the liver and spleen. Mechanistically, EVs reduce oxidative stress and cell damage by influencing important biological pathways like TGF-β1/Smad3 and PTEN/PI3K/Akt. Although previous studies in neonatal animal models demonstrated that EVs are safe and promising, clinical translation of EVs requires standardized production, optimized dosage, non-invasive administration method, and long-term safety verification. Future efforts are suggested to focus on neonate targeting, biomarker-guided clinical trials of EVs in treating BPD.

Plain Language Summary: Premature infants with bronchopulmonary dysplasia (BPD) experience chronic lung impairment, characterized by impaired lung development and persistent inflammation. Current therapeutic options for these infants are limited and may be associated with adverse effects. Extracellular vesicles are tiny particles released by cells, which have demonstrated potential in treating BPD in preclinical studies. This review compares different methods of delivering EVs, including intratracheal inhalation, intravenous injection, intraperitoneal injection, and oral-intragastric routes. Inhalation of EVs targets the lungs of affected infants, reducing inflammation and supporting alveolar development. Intraperitoneal delivery is less effective than intravenous injection, which requires larger doses but has anti-inflammatory effects throughout the body. Oral administration of EVs poses challenges for infants due to intestinal degradation. By transporting molecules (eg proteins, miRNAs) that guard against damaging pathways and restore lung tissue. In spite of promising preclinical results, further research is required to examine EV production, dosages, and safety before clinical use in premature infants. Future researches are suggested to focus on developing EVs for non-invasive targeted delivery, pharmacokinetic modeling specific to neonates, and biomarker-driven trials to treat BPD.

Keywords: extracellular vesicle, bronchopulmonary dysplasia, drug delivery, administration routes

Received: 4 April 2025
Accepted: 26 August 2025
Published: 22 September 2025

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease primarily affecting preterm infants, historically associated with mechanical ventilation and oxygen therapy, underlying a long process of definitions.[1–10] Current studies on the molecular mechanisms of BPD have proposed endotype-based classifications on account of transcriptomic information (Supplementary Figure 1).[11] Prematurity, small for gestational age (SGA), and low birth weight (LBW), mechanical ventilation and oxygen therapy, chorioamnionitis, pregnancy-induced hypertension (PIH), genetic susceptibility, inflammation and immune response, patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH)-III, inadequate nutrition, and maternal smoking are common risk factors for BPD.[12–19] However, the pathophysiology of BPD remains complex and not fully understood. Besides initial lung impairment, infants with BPD may face various clinical consequences, including pulmonary hypertension (PH), right ventricular hypertrophy (RVH), cerebral palsy, psychological disorders, and chronic pulmonary diseases like chronic obstructive pulmonary disease (COPD) (Figure 1).[20–23] Infants with BPD are often received pulmonary surfactant, caffeine, diuretics, bronchodilators, inhaled steroids to alleviate symptoms.[24,25] Thanks to current treatment of postnatal corticosteroids, less invasive surfactant administration, early non-invasive respiratory support in the delivery room, and early use of caffeine, BPD-related death or disability in preterm infants has been reduced significantly.[26–29] While vitamin A supplementation and early caffeine administration may reduce the incidence of BPD in preterm infants, therapeutic options remain limited, and further researches are needed to develop more effective and safer interventions.[30] The incidence of preterm infants with BPD resulting from differences in gestational age (GA) or birth weight varies from 2.4% to 71.6% in recent decades.[31–33] Preterm infants troubled by BPD often impose high financial burden on families, communities, and healthcare systems in addition to

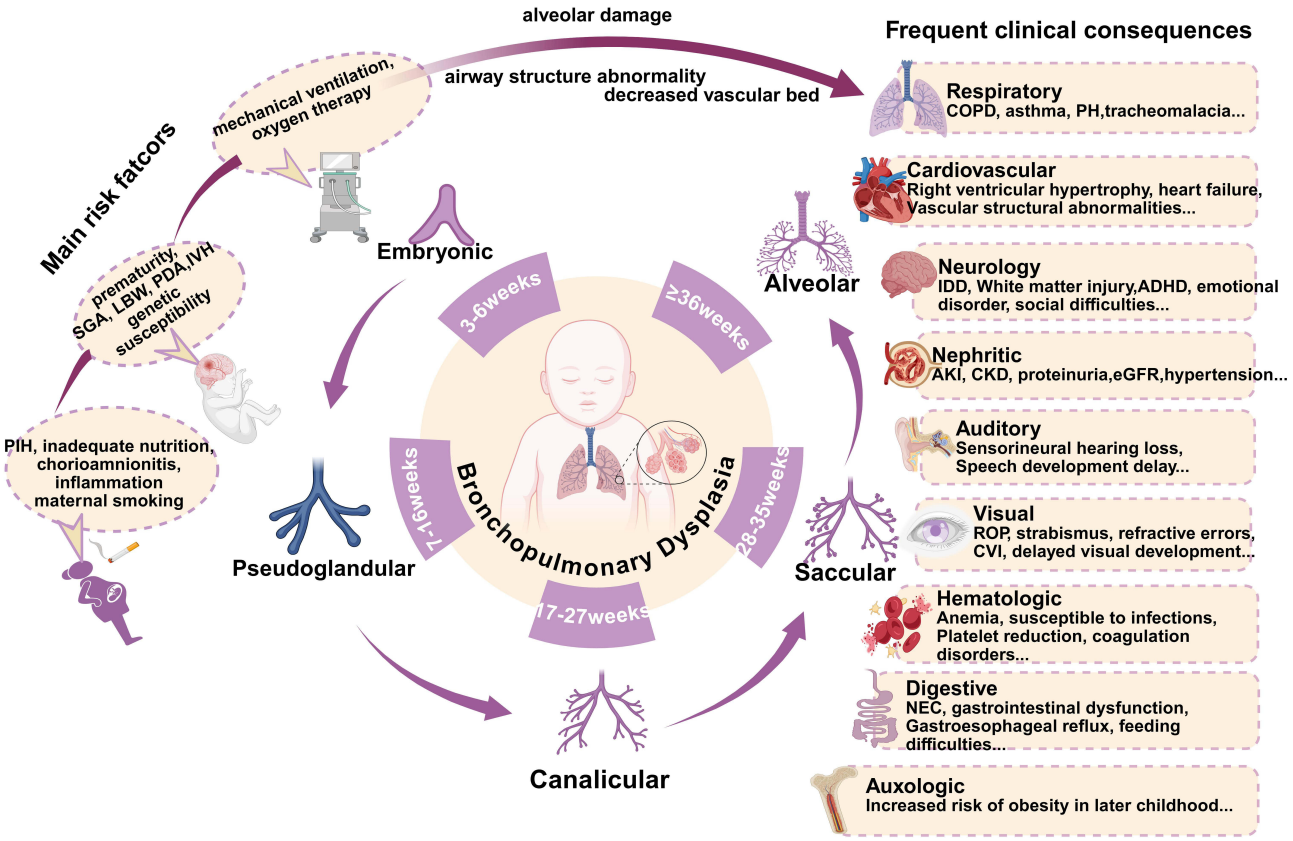

Figure 1 Overview of major risk factors, pathophysiological mechanisms, and frequent clinical consequences of BPD.

Abbreviations: PIH, pregnancy-induced hypertension; SGA, small gestational age; LBW, low birth weight; PDA, patent ductus arteriosus; IVH, intraventricular hemorrhage; COPD, chronic obstructive pulmonary disease; PH, pulmonary hypertension; IDD, intellectual developmental delay; ADHD, attention deficit and hyperactivity disorder; AKI, acute kidney injury; CKD, chronic kidney disease; eGFR, glomerular filtration rate; ROP, retinopathy of prematurity; CVI, cortical visual impairment; NEC, necrotizing enterocolitis of the newborn.

substantial morbidity. There is an urgent need for novel therapies to improve outcomes for infants with BPD.[34,35] Dini et al reported that a multiple strategy combining early non-invasive respiratory support, evidence-based pharmacologic interventions like caffeine and vitamin A, and cautious use of corticosteroids may be necessary for the effective prevention of BPD in extremely preterm infants. Stem cell-based therapy represents an emerging approach under BPD investigation.[36]

Mesenchymal stem cells (MSCs) indicate potentiality as a therapeutic option for infants with BPD due to their intrinsic capacity for self-renewal, high efficiency in cell generation, and ability to replace terminally differentiated cells.[37] MSCs, a broad spectrum of cells derived primarily from umbilical cord, bone marrow, and adipose tissue, have significant regenerative properties, including anti-inflammatory and immunomodulatory effects, enabling allogeneic transplantation. However, there are always challenges in the direct application of human umbilical cord mesenchymal stem cells (hUC-MSCs) therapy, including ethical debates regarding MSC origins, standardization, normalization of MSC culture, high dose intravenous microvascular occlusion, tumorigenicity, and pathogen transport.[38–40] Following these difficulties, MSC-derived EVs may offer a safer therapeutic alternative for infants with BPD.

EVs are nanoscale particles secreted by all types of cells, facilitating intercellular communication through the transfer of proteins, lipids, and RNA. They have been implicated in the pathophysiology of neonatal lung disease and are increasingly recognized as a novel mechanism of cell-to-cell signaling.[41,42] Recent study has demonstrated that macrophage-derived EVs transfer monocyte-activating polypeptide-II (EMAP-II) to neutrophils, activating the PI3K/AKT/mtROS pathway and causing mitochondrial oxidative stress, which in turn promotes the formation of neutrophil extracellular traps (NETs) in lung ischemia/reperfusion injury (LIRI) pathogenesis.[43] Nevertheless, EVs derived from septic acute lung injury (ALI) exacerbate pulmonary inflammation by delivering miR-128-3p to macrophages, which inhibits Rab20 to promote M1 polarization, proliferation, and the production of pro-inflammatory cytokines of TNF-α/IL-6.[44] Ruan et al confirmed that human menstrual blood-derived endometrial stem cells EVs protected alveolar barrier integrity by inhibiting MAPK-mediated necroptosis, thereby attenuating lung injury and restoring epithelial permeability in ALI/ARDS.[45] Additionally, EVs influence immunomodulation by transfering miR-223-3p to suppress circPWWP2A, through NLRP3 signaling pathway, via promoting barrier integrity, reducing inflammation, then alleviating PF.[46] Furthermore, proteomic analyses of EVs verified that, through distinct expressed gene enrichment, strong and intermittent hyperoxia induced different biological processes, including an inflammatory response.[47] Due to their lower risk of transplant-related side effects, EVs are a desirable alternative for clinical use in comparison to direct MSCs transplantation.[48–50] EVs obtained from MSCs maintain comparable biological properties, such as anti-inflammatory and regenerative capabilities, but raise minimal safety concerns.[51–53] One type of corticosteroid used to treat BPD, dexamethasone, only inhibits inflammation while posing a risk of neurotoxicity, in contrast, EVs simultaneously target antioxidant, inflammatory, lung development, and therapeutic intervention.[54] Intra-tracheal administration of EVs derived from amniotic fluid could not only achieved lung-specific delivery, but also promoted pulmonary alveolar development, reduced pulmonary hypertension, and inflammation, which exhibited potential translational therapeutics for BPD.[55] As for extended durability effects, Sharma et al found that EVs offered long-lasting protections for over three months with a single dose, while sildenafil required repeated doses due to a short-acting medication.[56] EVs also have the potential to address important pathological aspects of BPD, as evidenced by studies showing that they improved alveolarization, stimulated angiogenesis, reduced inflammation and oxidative stress damage.[57,58] Research on a variety of delivery techniques, including aerosol inhalation, intraperitoneal injection, intratracheal or intravenous administration, has shown that the best possible drug delivery is essential for optimizing therapeutic efficacy while reducing the likelihood of adverse effects.[59,60] Here we review the different methods of EVs delivery in the treatment of BPD and verify efficacy before these therapies being implemented clinically.

Characterizations of EVs

EVs are delimited by a lipid bilayer and secreted by various types of cells, that do not self-replicate. EVs typically carry transmembrane proteins such as CD63, CD81, and CD9, structures that may be co-isolated from the cytosol. It was optional to use secreted proteins with EVs or lipid-bound soluble proteins with intracellular compartments or other endosomes, without source-specific, co-isolates contaminants. Isolation, identification and mechanism analysis of EVs for BPD therapy experienced a complicated process for decades. EVs were initially isolated from fresh plasma after removal of intact platelets

via ultracentrifugation by Wolf P. in 1967, extruded on platelets storage, and identified as "platelet-dust".[61] Firstly named by Trams, E. in 1981, "exosomes (Exos)" were separated from exfoliated membrane vesicles and suggested to be carriers of information transfer between cells with a wide range of functions in vivo.[62] According to Lambertsen, R., hematopoietic stem cells (HSC) use cytoplasmic processes to interact with the mature macrophages' cell membrane. The coupling structure that results can be altered by ingesting or releasing small cell cytoplasmic vesicles.[63] Exos were also involved in forming during reticulocyte maturation and plasma membrane remodeling and are widely found in mammals and birds. The release of Exos was preceded by intracellular polyvesicles.[64] As for characterizations, Sokolova V. verified that nanoparticle tracking analysis (NTA) and scanning electron microscope (SEM) were effective complementary technique for evaluating Exos morphology and size. Ultracentrifugation does not alter the size of Exos, while storage conditions may largely affect their integrity and particle size.[65] Welsh et al updated MISEV 2023, giving a detailed explanation of the definition, origin, protein composition, analysis and topology of EVs (Table 1).[41] The essential milestones in EVs during the last 50 years were illustrated (Figure 2).

Therapeutic Mechanisms of EVs in BPD

Willis G. suggested that extracellular vesicles may protect against hyperoxia-induced neonatal BPD by delivering miRNA, or proteins that regulate the expression of target genes. These proteins can lower inflammatory factors tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) secretion, lower inflammation and pulmonary fibrosis (PF), enhance alveolar development, and control macrophage planning to the anti-inflammatory M2 state.[66] Small extracellular vesicles (sEVs) obtained from MSCs improved experimental BPD in vivo via partial restoration of pulmonary structure and improving motor function.[67] Willis G. additionally confirmed that mesenchymal stem cell-derived small extracellular vesicles altered monocytes' immunophenotype through epigenetic reprogramming and enhanced their immunosuppressive capacity. Additionally, it improved lung structure, reduces PF, and improves vascular remodeling by acting on bone marrow-derived myeloid cells (BMMC).[68] In recent years, EVs from mesenchymal stem cells have been extensively studied in treating BPD. Researches also demonstrated that EVs were crucial for both the positive and negative control of pulmonary injury induced by hyperoxia.[69,70]

Sources, Isolations, Purifications, Identifications and Pathophysiology of EVs

Exos, microvesicles, and apoptotic bodies are the three primary subtypes of EVs that are typically distinguished by their size, contents, transmembrane proteins, and segregation parameter. Exos, microvesicles, and apoptotic bodies were compared in detail, with each type's benefits highlighted (Table 2).[41,71–75] Studies have successfully isolated EVs from various sources,

Table 1 Recommendations of MISEV 2023 for EVs Characterization

Characterization of EVs as MISEV 2023 recommendations	
Definition	Particles released from cells, delimited by a lipid bilayer, and cannot replicate on their own
Sources and the preparations	Source of EVs: All types of cell cultured, including eukaryotic cells from multi- and unicellular organisms and prokaryotic cells. EV preparations: quality control measures through the sample collection, pre-pocessing and EV separation.
Protein composition	At least one positive protein of transmembrane, cytosolic and non-EV co-isolated structures, Lipid-bound soluble proteins with intracellular compartments other endosomes, or secreted proteins with EVs was optional. At least one negative protein component. No source-specific or co-isolates contaminants.
Single vesicle analysis with at least two different techniques	Particle number concentration, size; total protein, lipids, and RNA quantification, morphology, fluorescent and light scatter parameters.
Topology of EV components (luminal, membrane or external)	Important for understanding the biology.

Notes: Adapted from Welsh JA, Goberdhan DCI, O'Driscoll L et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J Extracell Vesicles* 2024, 13 (2), e12404. © 2024 The Authors. *Journal of Extracellular Vesicles* published by Wiley Periodicals, LLC on behalf of the International Society for Extracellular Vesicles.[41]

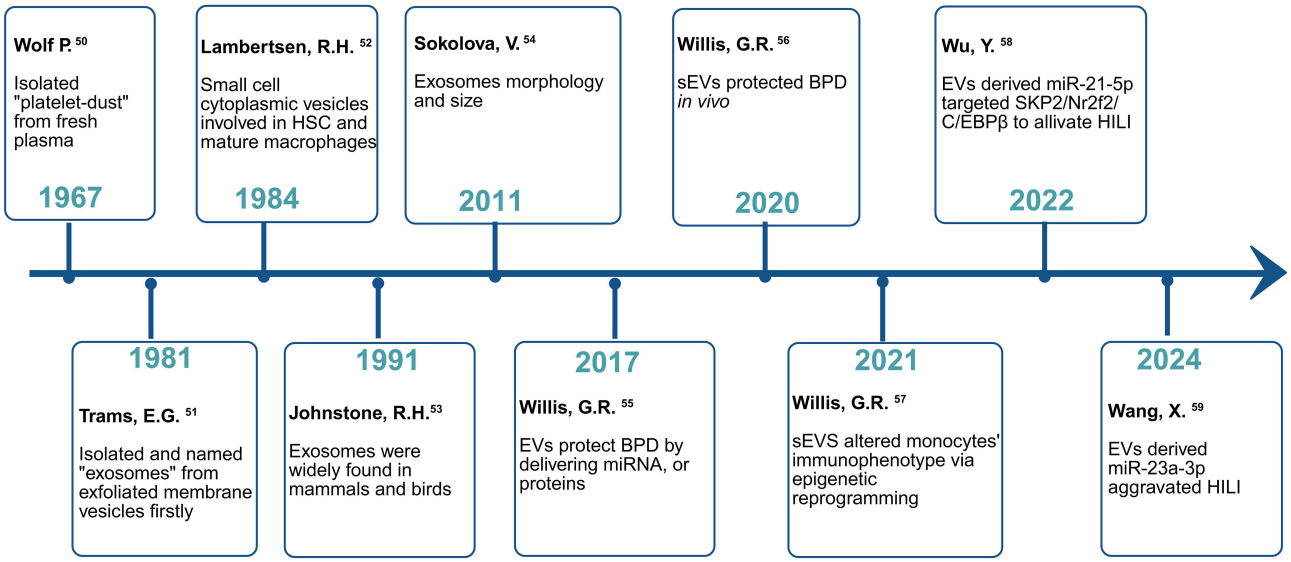

Figure 2 Timeline of EVs milestone studies.
Abbreviations: HSC, hematopoietic stem cell; sEVs, small extracellular vesicles; HILI, hyperoxygen-induced lung injury; MISEV, minimum information for studies of extracellular vesicles.

including MSCs derived from human umbilical cords, placenta, bone marrow, adipose tissue, induced pluripotent stem cells (iPSCs),[76–80] and body fluids such as amniotic fluid, cerebrospinal fluid, blood, plasma, lymphatic fluid, urine, saliva, breast milk, and vitreous body.[81–87] Additionally, EVs have been identified in fungi, bacteria, and plants.[88–90] EVs play important roles in intercellular communication, immune regulation and pathological processes. EVs derived miRNAs have signaling functions and can regulate gene expression in target cells, and specific proteins (such as SUMOylated hnRNPA2B1 and YBX-1) regulate the selective encapsulation of RNA. In addition, non-coding RNA may influence immune responses through cross-species regulations.[91] Bioinformatic analyses and nanopore sequencing revealed that the transcriptome sorting mechanisms of microvesicles and Exos differed and that different EV subtypes from the same origin have different physiological functions, suggesting different clinical translation opportunities.[92] Originated in the endosomal system, Exos are released by multi-vesicular bodies that range in size from 30 to 150 nm.[93] Heterogeneous Exos are secreted by a large range of cell types, and classified into different subtypes due to locations and functions.[94] MSCs are known for their characterizations of improving alveolar structure and angiogenesis, and suppressing inflammatory responses for treating BPD.[95] Although the effective therapy of MSCs in BPD are proven in several trials, safety concerns such as malignant transformation, aberrant osteochondral differentiation, vascular occlusion via intravenous injection, or the restricted rates of survival and differentiation occurred,

Table 2 Comparison Characteristics of Extracellular Vesicles Such as Exosomes, Microvesicles and Apoptotic Bodies

	Exosomes	Micro Vesicles	Apoptotic Bodies
Biogenesis	Endosomal system, released via multivesicular body	Plasma membrane, cell surface	Apoptotic cells
Size	30nm to 150nm	100 nm to 1000 nm	1000 nm to 5000 nm
Segmentation parameter	70,000g up to 200,000g	20,000g	16,000g
Contents	Protein, lipid, mRNA, miRNA, lncRNA, DNA, RNA, metabolites	Protein, lipid, mRNA, miRNA	Protein, lipid, nucleic acids, miRNA, other biomolecules,
Transmembrane protein	CD81, CD63, CD9, CD82, TSG101, Rab5, annexin and Alix, MHC	Matrix metalloproteinase, Integrin selectin A	Tissue factor
Advantages	Longer drug duration, lower clearance, and less toxicity	Cross-talk between cells	Ease of purification, enhanced targeting specificity, large size to load more drugs
References	39,60,61	60,62	63,64

https://doi.org/10.2147/IJN.S530819

which also limit their direct application in clinical trials.[96–99] Compared to MSCs, EVs have aroused huge attention for treating BPD by paracrine, non-cellular methods. The primary pathophysiological effects of EVs include reducing inflammation, apoptosis, and PF, as well as promoting the immune response, pulmonary repair, and vascular generation.

Isolation and purification of EVs is essential for clinical translations in vivo. However, the small size of Exos has led to challenges in isolation and purification. An ideal approach has all the characteristics of high yield, high specificity, high purity, low costing, as well as preservation of natural biological functions. Multiple methods have been used to isolate and purify EVs from various biological samples. Studies have indicated heterogeneity based on technologies of EVs' concentration, purification, size distribution, proteomic content, and source composition.[100,101] Each purification and isolation technique has benefits and drawbacks. Technologies that are widely used including precipitation techniques, density gradient ultracentrifugation, high-speed ultracentrifugation, ultrafiltration, sequential filtration, size-exclusion chromatography, polyethylene glycol (PEG) precipitation, size-exclusion chromatography, and affinity binding-based separation.[102,103] To date, differential ultracentrifugation is the most popular technique for isolating EVs, due to its simple protocol, relatively high yield, and specificity.[104] Nevertheless, ultracentrifugation may lead to mechanical or thermal damage to EVs, and spinning speeds are variable, which could alter their composition, structure, and cause instability of biological functions in the end.[105] As a consequence, advanced isolation techniques are used in EVs, such as microfluidic (chip) techniques, membrane-based separation, static water filtration dialysis, reagent kit extraction, heat shock protein binding, lectin, phosphatidylserine, and immune-affinity isolations.[106–111] Different techniques have their own pros and cons as for yield, specificity, purity, and costing. Origins of EVs, amount, costing of time and money, difficulty of operation, storage condition, and requirements of subsequent experiments are the critical reasons in choosing the most appropriate isolation and purification method. Unfortunately, no single method enables optimal isolation of EVs from all species. This could be resolved by combining two or more isolation techniques, such as combining traditional and advanced technologies, however, limitations still exist.

Identification of EVs is difficult by their varieties of heterogeneity and diversity, which can be attributed to differences in origin, size, morphology, transmembrane protein, and medicine cargo. At present, the ideal identification technology of EVs mainly based on morphology, particle size, protein contents, lipid contents, nucleic acid, biomarkers, purity and costing the least. At present, transmission electron microscopy (TEM) combined with immunogold labeling to verify double-stranded DNA presentation in the lumen has been recognized as a novel method to verify associated biomarkers of EVs.[112] Cryogenic transmission electron microscopy (cryo-TEM), and scanning electron microscopy (SEM) are both used for the morphological assessment for their advantages of maintaining the native state of EVs and high-resolution imaging.[113] Electrophoretic light scattering (ELC), NTA, and flow cytometry can be applied for primary screening EVs.[114] Nevertheless, more refined techniques such as resistive pulse sensing (RPS), tunable resistive pulse sensing (TRPS), tandem mass spectrometry (TMS), electrochemical sensing (ES), and interferonmetric reflectance imaging (IRI) might be helpful for a more comprehensive understanding of the morphological characteristics of EVs. Besides, Western blot is often used to verify typical transmembrane proteins with relatively low costing (Figure 3). In summary, combinations of these identification techniques might mitigate EVs heterogeneity to some extent, and recognize the presence of EVs further. This lay a crucial foundation for future research on functions and mechanisms of EVs in treating BPD.

Biogenesis, Release, and Cellular Uptake of EVs

Biogenesis and uptake of EVs is complicated, as a result of different cell origins, cell microenvironment homeostasis in physiological state, as well as external stimulus conditions.[115] The biogenesis of Exos is composed of three consecutive stages. Initially, endocytosis of the plasma membrane forms endocytotic vesicles. This process initiates early endosomes forming afterwards. In the second step, early endosomes are inward budding of late endosomes, then develop into multivesicular bodies. Finally, most of the multivesicular bodies degrade, and a small part of the multivesicular bodies fuse with the plasma membrane and release Exos in the extracellular region.[116] Microvesicles, which range in size from 100 to 1000 nm, are formulated by membrane buddings from plasma membrane of the donor cell into extracellular regions. Apoptotic bodies, whose size range from 1000 to 5000 nm, are mainly formed through membrane blebbing, starting from plasma membrane to extracellular regions.[117] Once released, EVs are able to impact on extracellular

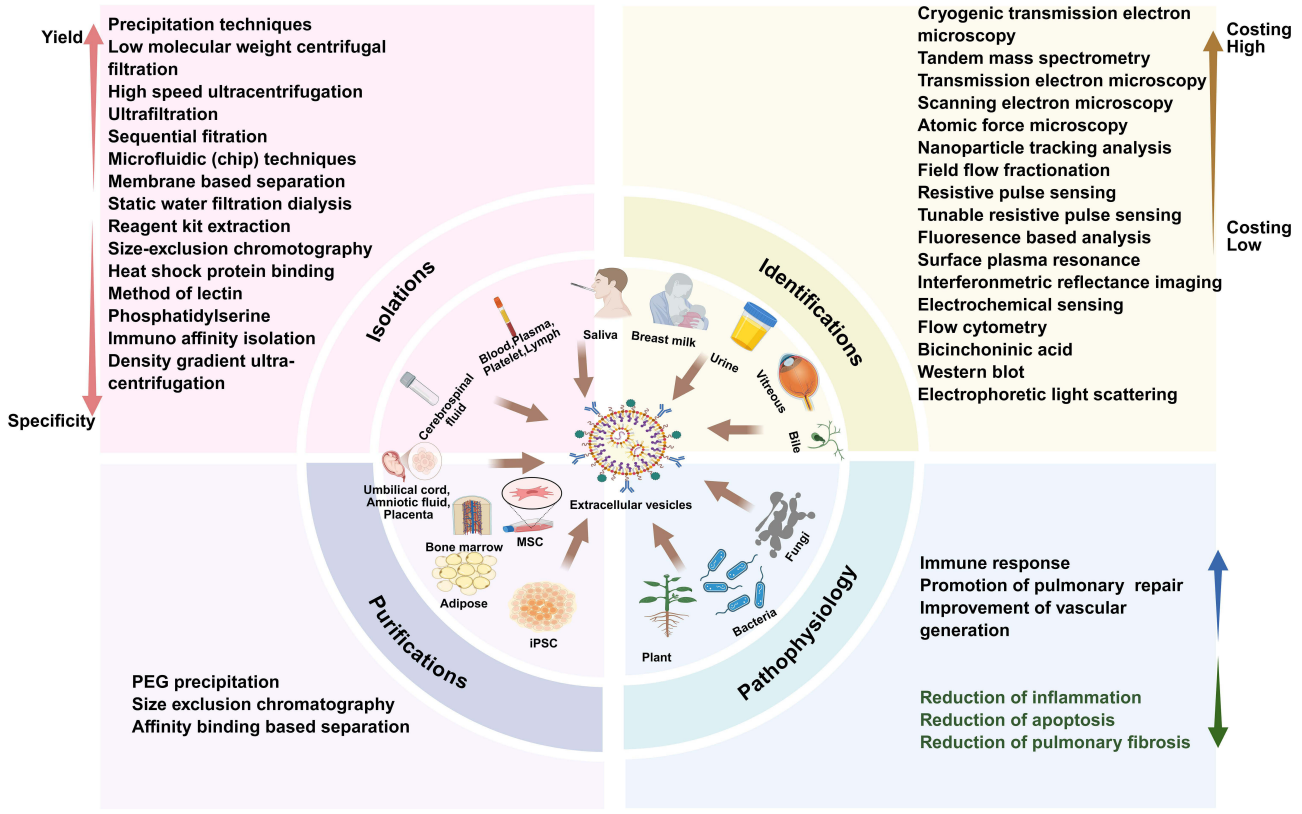

Figure 3 Sources, isolations, purifications, identifications and pathophysiology of EVs.
Abbreviations: EVs, extracellular vesicles; MSC, mesenchymal stem cell; iPSC, induced pluripotent stem cells.

environment to different extents, and act on target cells through four mechanisms. When reaching the receptor cells, EVs can either directly interact with the cells, be phagocytized by the plasma membrane, undergo macropinocytosis via plasma membrane invagination, or get internalized through receptor mediated endocytosis. Receptors involved in the internalization are mainly including clathrin-, caveolin-, lipid raft, and heparan sulfate proteoglycan (Figure 4). [118,119] As EVs play essential roles in transporting biological information and intercellular communication, a deep understanding of vesicular–cellular interactions is of great value to explore the pathophysiological mechanisms of targeted vesicular transport systems. For example, a progressive trajectory of relative enrichment and early increase of $CD24^+$ conveyed by EVs have been reported to be connected with developing BPD.[120] These EVs effectively transported $CD24^+$ proteins to target inhibiting immune overresponse induced by damage-from pathogen-associated molecular patterns (DAMPs), blocking toll-like receptors (TLRs) and Siglec-10-mediated NF-κB signaling pathways, thereby effectively inhibiting cytokine storms and reducing inflammation in lipopolysaccharide (LPS)-induced acute respiratory distress syndrome (ARDS) mouse model.[121]

Registered Clinical Trials Involving EVs for Respiratory Diseases

The transition of EV-based therapies from preclinical research to clinical translation necessitates rigorous evaluation through well-designed clinical trials. To compile relevant clinical trials, a systematic search was conducted using publicly available clinical trial registries, which was registered in the National Institutes of Health (NIH) database during the previous five years.[105,122–126] The search strategy incorporated keywords such as “extracellular vesicles”, “Exos”, “respiratory diseases”, and “clinical trials”. Inclusion criteria comprised trials that were actively recruiting, completed, and pending results, particularly in the field of respiratory diseases. Trials involving EVs derived from MSCs, human amniotic fluid (HAF), blood, urine, human embryonic kidney (HEK), alveolar lavage fluid (ALF), T-REx™-293 cells, human adipose derived mesenchymal progenitor cell (haMPC), corona virus disease-19

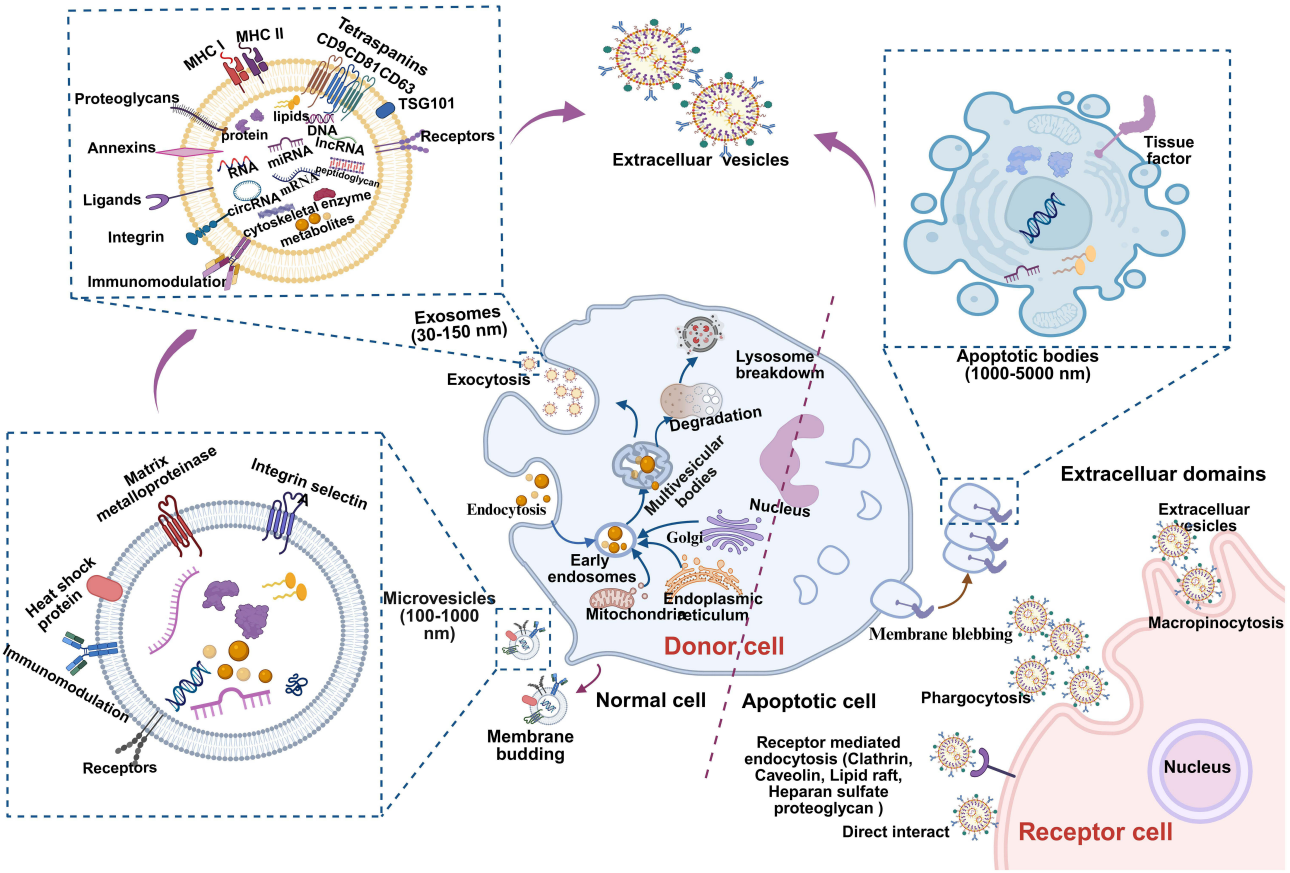

Figure 4 Biogenesis, release and cellular uptake of extracellular vesicles.

(COVID-19) Specific T cells and other sources were included, whereas studies focusing on unrelated conditions were excluded. Clinical trials were stratified according to respiratory disease types, EV sources, mode of administration, age, trial phases, study type, distribution countries, and start date. The collected information was systematically analyzed to assess the current landscape of EV-based clinical research in respiratory diseases. To date, 28 clinical studies have been registered using EVs to treat respiratory diseases. Analysis of registered clinical trials revealed a growing interest in EV-based therapies for respiratory diseases, and the majority of these trials focused on conditions including coronavirus disease 2019 (COVID-19), ARDS, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), pneumonia, and BPD (Table 3). Only one clinical study numbered NCT06279741, investigated the potential of MSC-derived EVs on BPD, while most of them are under the recruiting stage. Clinical trials have investigated diverse EV delivery methods to screen treatment for infants, including inhalation, intravenous injection, endotracheo-pulmonary instillation and diagnostic tests. While intravenous delivery facilitates systemic distribution, inhalation has been shown accumulated in lung tissues with optimizing therapeutic efficacy. Most extracellular vesicles are derived from mesenchymal stem cells and body fluids, including urine, blood, bronchoalveolar lavage fluid, and amniotic fluid. Geographically, clinical trials were predominantly concentrated in the United States, China, Israel, Russian Federation, and Europe (Figure 5). EVs therapeutics may offer distinct advantages over stem-cell therapies, such as lower risks of immune rejection and tumorigenicity, easier storage and administration. Compared to genetic therapy, EVs can provide a natural method of delivering therapeutic biomolecules without genetic modification, and may minimize ethical and safety concerns. However, there are still difficulties in determining the best dosage schedules, guaranteeing EV stability, and reducing possible immunogenicity.

Table 3 Registered Clinical Trials Involving EVs for Respiratory Diseases

NCT Number	Disease Type	EV Sources	Mode of Administration	Age	Trial Phases	Study Type	Distribution Countries	Start Date
NCT06279741	BPD	UCMSC-EVs	EI	Child	Phase I and II	Interventional	Italy	2023
NCT05387278	COVID-19, ARDS, RDS	UCMSC-Exos	IV	Adult	Phase I	Interventional	United States	2025
NCT05116761	COVID-19, Dyspnea	BMSC-EVs	IV	Adult	Phase I and II	Interventional	United States	2024
NCT04657458	COVID-19, ARDS, Hypoxia	BMSC-EVs	IV	Adult	Phase III	Expanded access	United States	2024
NCT06002841	SARS, ARDS, Pneumonia	MSC-EVs	IV	Adult	Phase I and II	Interventional	Brazil	2024
NCT04798716	COVID-19, Novel corona virus pneumonia, ARDS	MSC-Exos	IV	Adult	Phase I and II	Interventional	United States	2023
NCT04657406	COVID-19, SARS, ARDS	HAF-EVs	IV	Adult	NA	Expanded access	United States	2023
NCT05787288	Corona virus pneumonia	MSC-EVs	Inhalation	Adult	Early Phase I	Interventional	China	2023
NCT05808400	Long COVID-19 Syndrome	UCMSC-Exos	Inhalation	Adult	Early Phase I	Interventional	China	2023
NCT05855317	ARDS	Blood	Diagnostic test	Adult	NA	Observational	France	2023
NCT05947747	ARDS	HEKs	Inhalation	Adult	Phase II	Interventional	Israel	2023
NCT05451342	ARDS, ARF	Blood, BALD	Diagnostic test	Adult	NA	Observational	China	2022
NCT05476029	Sepsis complicated with ARDS	Blood, ALF	Diagnostic test	Adult	NA	Observational	China	2022
NCT05228899	COVID-19	HAF-EVs	IV	Adult	Phase I and II	Interventional	United States	2022
NCT05354141	ARDS	BMSC-EVs	IV	Adult	Phase III	Interventional	United States	2022
NCT05058768	ALI	Urine, blood, and ALF	Diagnostic test	Adult	NA	Observational	China	2022
NCT04902183	COVID-19	Genetically engineered exosomes-CD24	Inhalation	Adult	Phase II	Interventional	Greece	2021
NCT04969172	COVID-19	T-REx™-293 cells-Exos	Inhalation	Adult	Phase II	Interventional	Israel	2021
NCT05216562	SARS-CoV2 Infection	MSC-Exos	IV	Adult	Phase II and III	Interventional	Indonesia	2021
NCT04602104	ARDS	MSC-Exos	Inhalation	Adult	Phase I and II	Interventional	China	2020
NCT04602442	COVID-19, SARS-CoV-2 Pneumonia	MSC-Exos	Inhalation	Adult	Phase II	Interventional	Russian Federation	2020
NCT04747574	SARS-CoV-2	T-REx™-293 cells-Exos	Inhalation	Adult	Phase I	Interventional	Israel	2020
NCT04276987	Corona Virus	MSC-Exos	Inhalation	Adult	Phase I	Interventional	China	2020
NCT04384445	Corona Virus Infection, COVID-19, SARS, ARDS	HAF-EVs	IV	Adult	Phase I and II	Interventional	United States	2020
NCT04389385	Corona Virus Infection, Pneumonia	CSTC-Exos	Inhalation	Adult	Phase I	Interventional	Turkey	2020
NCT04491240	COVID-19, SARS-CoV-2 Pneumonia	MSC-Exos	Inhalation	Adult	Phase I and II	Interventional	Russian Federation	2020
NCT04493242	COVID-19, ARDS	BMSC-Exos	IV	Adult	Phase II	Interventional	United States	2020
NCT04544215	Drug-resistant	haMPC-Exos	Inhalation	Adult	Phase I and II	Interventional	China	2020

Note: Only those registered in the National Institutes of Health (NIH) database are involved.

Mechanisms Analysis of Different Administration Methods, Dosages and Frequencies of Extracellular Vesicles in Treating BPD Models in Vivo

Animal experiments have revealed that EVs in BPD treatment is strongly influenced by administration method, dosage, and frequency. EVs used in BPD treatment are derived from various sources, including hUC-MSCs, BM-MSCs, AD-MSCs, as well as iPSCs. These EVs contain bioactive molecules such as microRNAs, proteins, and lipids that play crucial roles in modulating lung inflammation, apoptosis, and vascular remodeling. EVs can be delivered through multiple routes, including intratracheal mode (IT), intravenous mode (IV), intra-amniotic mode (IA), and intra-oral mode (IO). A detailed comparison of IT, IV, IA, and IO administration route is provided, alongside an evaluation of the molecular mechanisms modulated by EV therapy in BPD (Figure 6). Each method presents unique advantages and limitations in terms of targeting efficiency, systemic effects, and cellular uptake. Different administration routes are

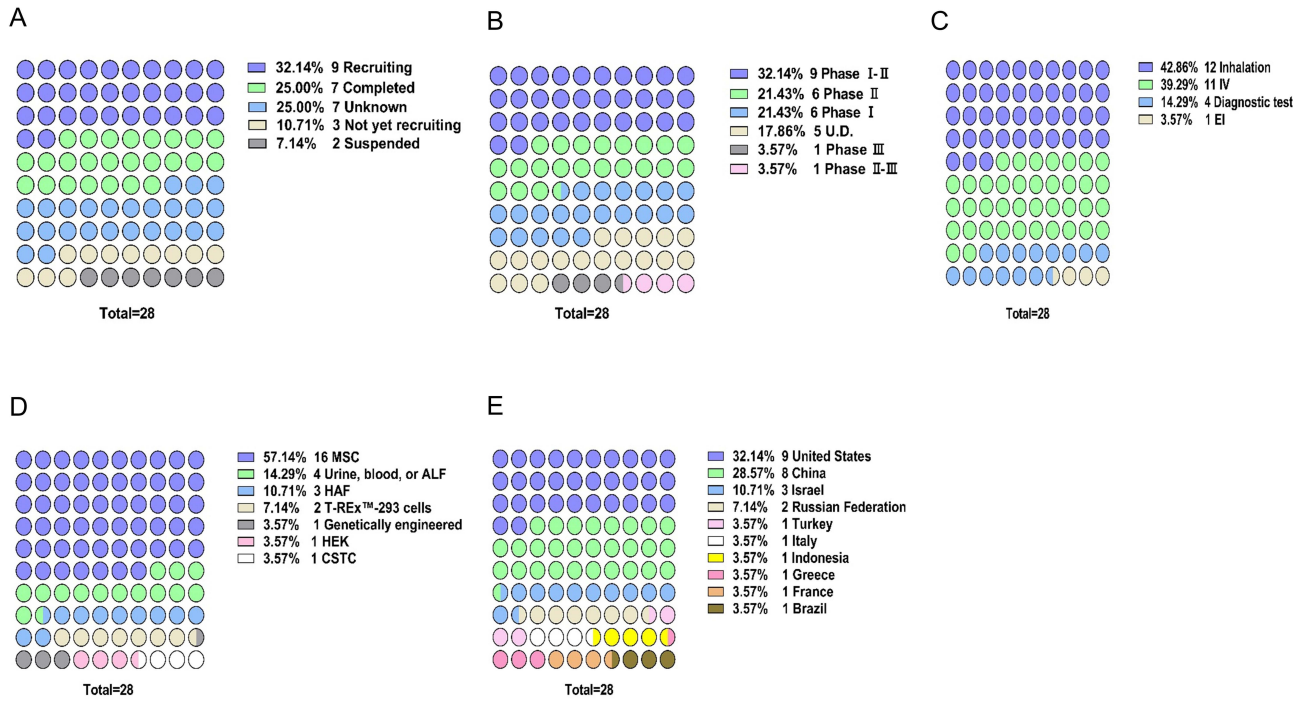

Figure 5 The whole 28 ongoing and completed clinical trials using EVs for respiratory diseases. The number of clinical trials related to each section is exhibited inside the boxes. (**A**) Clinical trial status introduced by clinicaltrials website. (**B**) Clinical trial phase introduced by clinicaltrials website. (**C**) Route of administration of EVs by clinicaltrials website. (**D**) Source of EVs applied in each clinical trial by clinicaltrials website. (**D**) Countries of EVs involved in clinical trial by clinicaltrials website.
Abbreviations: Recr., recruiting; N.D., not determined; EI, endotracheopulmonary instillation; ALF, alveolar lavage fluid; HAF, human amniotic fluid; HEK, human embryonic kidney cells, CSTC, COVID-19 specific T cells.

probably related to different mechanisms in BPD treatment. IT injection is widely used in preclinical BPD models primarily considering it rapid delivery into lungs, local therapeutic effects, and quick onset effects. IT administrated EVs promote vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1-alpha (HIF-1α) activation, enhancing pulmonary capillary formation and alveolarization. Meanwhile, NF-κB and transforming growth factor beta (TGF-β) pathway modulation decreases pro-inflammatory cytokines and inhibits apoptosis, thereby protecting alveolar epithelial cells.[115] IT administrated EVs attenuate fibrosis and protect against oxidative stress by reducing collagen deposition, enhancing mitochondrial integrity, and decreasing DNA oxidative damage.[127] Certain EV-derived microRNA, like miR-21-5p, target S-phase kinase-associated protein 2 (SKP2) and mediate cytoprotection by reducing inflammation, oxidative stress, and apoptosis through the SKP2/Nr2f2/CEBPα axis via intratracheal administration.[69] IT-administrated EVs increase anti-inflammatory cytokines, such as IL-4, IL-10, IL-13, improving lung structure, enhancing neural progenitor cell (NPC) self-renewal, and modulating of Wnt/Crabp2 signaling.[128] EVs delivered via the IT shift immune cells toward an anti-inflammatory state by reprogramming gene activity.[129] Additionally, IT-administrated EVs also decrease right RVH, promote angiogenesis, alter pro-angiogenic genes such as *enos*, c*xcr4*, and restore alveolar structure.[56] Apart from these advantages, IT administration also have some disadvantages, such as invasive procedure, potential airway irritation, and risks of uneven distribution in the lung, which has impeded its clinical translation to some extent. Preclinical models demonstrated the therapeutic effects of IV-administrated EVs are preponderant as non-invasive compared to IT, systematic effects beneficial for multiple organs, and compatible with neonatal intravenous therapy. IV-administrated EVs activate VEGF-R2 signaling, reduce pulmonary inflammation, and improve lung mechanics and respiratory gas exchange.[50] IV-administrated EVs restore alveolar architecture, reduce fibrosis, and alleviate pulmonary vascular remodeling by promoting an immunosuppressive bone marrow-derived myeloid cells (BMDMy) phenotype and initiating epigenetic and phenotypic reprogramming of myeloid cells.[68] EVs derived miR-425, target phosphatase and tensin homolog (PTEN) to activate phosphoinositide 3-kinase (P_{I3K})/Akt pathway, reducing in apoptosis and oxidative stress, enhancing proliferation of alveolar type II epithelial cells (TIIAECs) and pulmonary vascular endothelial cells

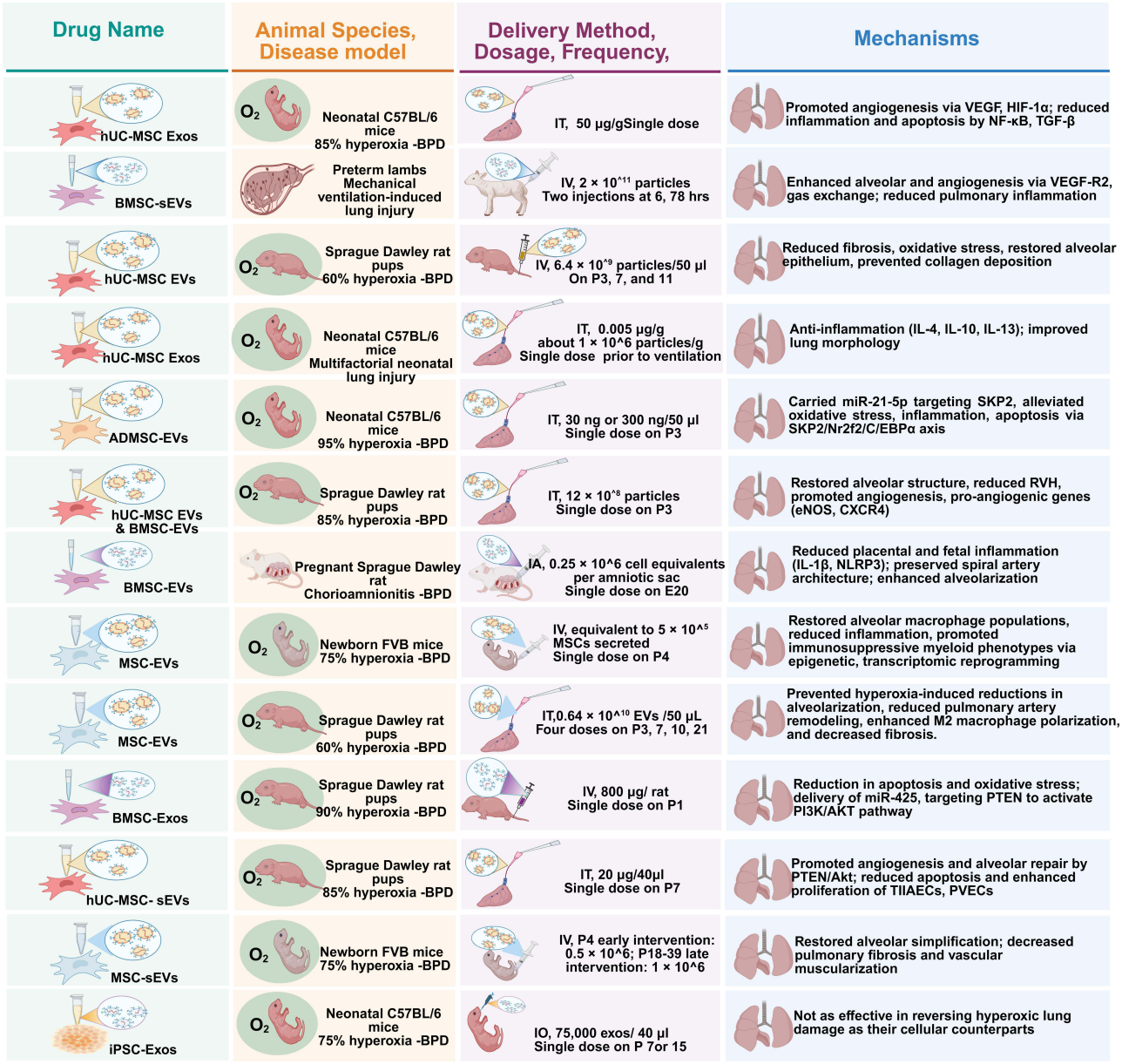

Figure 6 Mechanisms analysis of different administration methods, dosages and frequencies of EVs in treating BPD.
Abbreviations: IO, intraoral instillation; IT, intratracheal injection; IP, intraperitoneal injection; IA, intra-amniotic injection; IV, injection of vein; MS, media supplementation; FVB, friend leukemia virus B; TIIAECs, alveolar type II epithelial cells; PVECs, pulmonary vascular endothelial cells.

(PVECs) also by IV administration.[130,131] IV-administrated EVs reprogram alveolar macrophages into an M2 anti-inflammatory phenotype, reduce PF and vascular muscularization and increase peripheral blood vessel count in hyperoxia-induced lung injury.[67] IV administration also has some disadvantages, such as potential clearance by the immune system before reaching the lung, and risks of off-target effects in pulmonary tissues, which has hindered its clinical translation. IA administrated EVs is noticed in preclinical BPD model because of its prenatal intervention targeting early lung injury and potentiality for preventing severe BPD in high-risk pregnancies. In addition to preserving spiral arteries and vascular architecture, IA-administrated EVs also improve alveolarization and pulmonary vascular growth, increase VEGF and surfactant protein C (SPC) expression, and decrease placental and fetal inflammation by downregulating expressions of IL-1β and nucleotide-binding oligomerization domain-containing protein-like receptor protein 3 (NLRP3).[132] Due to its limited clinical translation, IA-administrated EVs as an invasive prenatal procedure currently lack sufficient data on efficacy and safety. The mechanism underlying the IO-administrated EVs in C57BL/6J

https://doi.org/10.2147/IJN.S530819

pups with BPD is a possible modulation of the inflammatory response.[133] IO-administrated EVs also have some disadvantages including poor bioavailability, unpredictable systemic absorption, and unclear local effective drug concentration in pulmonary vessels. Previous studies conducted by our team showed that intranasal administration of Exos was an effective non-invasive treatment for BPD. This treatment repaired alveolar damage without causing any toxicity or tumorigenic risks, and it prevented inflammation and the epithelial–mesenchymal transition in neonatal mice induced with BPD-like pathology.[134] Overall, EVs administration without invasive procedures has shown encouraging in vivo outcomes. Further work is demanded to investigate the safety and effectiveness of non-invasive administration of EVs in vivo to discuss more suitable administrated mode to minimize the probable side effects.

Numerous studies have shown that the BPD therapeutic dose of EVs in vivo was influenced by a variety of factors, including animal species, frequencies, local or systemic administration mode, and sources. Therapeutic dosage of MSCs-EVs varied 400 times in animal models with BPD. These doses ranged from 0.25×10^6 to 1×10^{10} particles.[55,59,132,135] The isolation and characterization of EVs are highly heterogeneous, EVs units varied widely and included EVs released over time or dosed by animal weight, as well as absolute protein amount, particle number, or amount of EVs released by a specific number of MSCs. The majority of studies provided single-dose therapy. For multiple dosing, the median study was four doses, ranging two to fifteen doses.[136] Similar to the EVs field overall, there were few studies on dose response, frequency, and administration route in BPD disease. There are currently 15 different studies that examine the effects of EVs in animal models with BPD in vivo at different dosages, frequencies, and routes of administration (Table 4). Numerous studies have verified that different EVs administration routes can achieve different extents of biological distribution in lung tissue, thus affecting BPD progression. The establishment of almost all animal models with BPD involved keeping rodents in environments with hyperoxia, which ranged from 60–90% O_2. Intraperitoneal administrated MSC-EVs fourteen times from postnatal day (PND) 1 to 14 protected alveolar growth in hyperoxia-exposed rats on the P21 and P56. Exos multiple injection promoting alveolar and blood vessel growth, and preventing the development of right ventricular hypertrophy.[137] Intratracheal administrated hUCMSC-EVs three doses on the P3, P7, and P10 at the respective dosage of 8×10^8, 4.5×10^8, and 3×10^8 particles/g enhanced increasing in total alveoli number, and decreasing in mean alveolar volume. Furthermore, hUC-MSC EVs are being explored as cell-free alternative to MSCs as better effects of alveolarization, preventing small pulmonary vessels medial thickness under hyperoxia.[138] A late single bolus intervention at P18 with the dosage of 1×10^6 cell equivalents on newborn FVB mice under hyperoxia, partially restored alveolar simplification by alleviating mean liner intercept (MLI) induced by hyperoxia exposure. A over 4-week's intervention of late mesenchymal stem cell-derived exosomes (MSC-MEx) intravenously may contribute to MLI and alveolar thickness, though it may not as effective as early administration. Interestingly, both the early and the late MSC-MEx intervention promoted blood vessel count, alleviated right ventricular hypertrophy to the same extent via intravenous route.[67] Amniotic fluid derived EVs (AF-EVs), which were given intravenously at P3, decreased MLI, vascular density, macrophage infiltration, and the expression of inflammatory cytokines such as macrophage inflammatory protein-1 alpha (MIP-1α), monocyte chemoattractant protein-1 (MCP-1), and IL-1β, in comparison to the hyperoxia group. Nevertheless, the late administration of AF-EVs in the same model at P7 did not clearly improve pulmonary vascular density or alveolar structure, compared to the hyperoxia group.[55] Yahui Zhou et al investigated the mechanism and efficacy of human breast milk derived exosomes (HBM-Exos) in BPD treatment via intragastric administration from P1 to P7. Their findings indicated that intragastric administrated HBM-Exos promoted the suspension of cell proliferation, the expression of TIIAECs surface marker SPC, inhibited IL-17 signalling pathway and increased apoptosis induced by hyperoxia.[139] Alison N. Abele et al examined whether antenatal treatment of MEx obtained from human bone marrow MSCs (HBM-MSCs) protect pulmonary development in rat model with BPD. Their findings verified intra-amniotic administrated MEx with the dosage of 0.25×10^6 cell equivalents at embryonic day 20, provided one-time prenatal intervention sustaining improvements in reducing inflammatory cytokines, and preserving distal pulmonary growth in BPD.[132] Moreover, optimal dose and route of administration of good manufacturing practices (GMP)-grade EVs, its long-term effects and how it modifies the lung transcriptome was studied by Mayank Sharma et al. Their findings indicated that EVs obtained from BM-MSCs or WJ-MSCs have comparable efficacies in restoring pulmonary vascular density, preventing the arrest of alveolarization. Interestingly, three different WJ-MSC EVs doses, including low dose (2×10^8 particles/g), medium dose (12×10^8 particles/g), or high dose (60×10^8 particles/g) were administrated respectively

Table 4 Studies of Therapeutic Effects of EVs in BPD Animal Models in Vivo at Different Dosages, Frequencies, and Administration Routes

EVs Name	EVs Source	Recipient Animal	EVs Administration Route	Dosage	Frequency	Dose Difference on Lung	Mechanism of EVs	Therapeutic Effects	Year	References
MSC-EVs	Bone marrow-derived MSCs	Newborn C57/BL6 mice	Intraperitoneal (IP)	3.4×10^{9} particles	Fourteen dose (P1-P14)	Multiple injection protected alveolar growth at P21 and P56	Carries VEGF leading to improve vascular growth	Protects developing lungs from hyperoxygen-induced damage to alveolar and blood vessel growth, and the development of right ventricular hypertrophy.	2018	Braun RK[128]
hUCMSC-EVs	Human umbilical cord MSCs	Newborn Sprague Dawley rats	Intratracheal (IT)	8×10^{8} at P3, 4.5×10^{8} at P7, and 3×10^{8} at P10 particles/g	Three doses (P3, P7, P10)	No significant dose dependence	Enhances alveolarization, decreasing in mean alveolar volume	Reduced lung inflammation, improved lung architecture	2019	Porzionato A[129]
MSC-MEx	Mesenchymal stromal cell	Newborn mice (FVB strain)	Intravenous (IV)	0.5×10^{6} cell equivalents, or 1×10^{6} cell equivalents	Single dose at P18, or repeated dose P18-39	No significant dose dependence	Reduces pulmonary fibrosis, enhancing lung function	Late MEx intervention was just as effective at restoring pulmonary blood vessel number and ameliorating RVH as the early MEx treatment.	2020	Willis GR[56]
AF-EVs	Amniotic fluid	Newborn Sprague Dawley rats	Intratracheal (IV)	1×10^{10} cell equivalents	Single dose (P3, or P7)	No significant dose dependence	Decreased MLI, vascular density, macrophage infiltration, the expression of inflammatory cytokines such as MIP-1α, IL-1β, and MCP-1 as a preventative agent.	Early administration reduced pulmonary hypertension, preserving alveolar structure, reducing vascular remodeling, and lung inflammation.	2021	Bellio MA[125]
HBM-Exos	Human breast milk	Newborn Sprague Dawley rats	Intragastric (IG)	200 μg/mL	Seven doses (P1-P7)	No significant dose dependence	Prevents AT II apoptosis, modulating IL-17 pathway, inhibiting downstream target gene FADD	Protected lung tissue, and reduced apoptosis	2022	Zhou Y[130]
MEx	Human bone marrow MSCs	Pregnant Sprague–Dawley rats	Intra-amniotic (IA)	0.25×10^{6} cell equivalents	Single dose (E20)	No significant dose dependence	Preserves alveolar structure, restoring vessel density, arterial wall thickness, and right ventricular hypertrophy	One-time prenatal intervention sustains improvements in alveolarization, pulmonary vasculature, RVH, and lung function at P14	2022	Abele AN[122]
GMP-grade EVs	Wharton's Jelly MSCs and bone marrow-derived MSCs	Newborn Sprague Dawley rats	Intratracheal (IT) or Intravenous (IV)	2×10^{8} (low), 12×10^{8} (medium), 60×10^{8} (high) particles/g	Single dose (P3)	No significant dose dependence	Reduces inflammation and pulmonary vascular remodeling	Prevented BPD-PH, Reduced lung injury and pulmonary hypertension	2022	Sharma M[119]
MSC-EVs	Human umbilical cord MSCs	Newborn Sprague Dawley rats	Intraperitoneal (IP)	5 μg (low), 10 μg (medium), 15μg (high)	Single dose (P4)	Ameliorating hyperoxia-induced lung injury in a dose-dependent manner	Decreases WNT5a expression and the transdifferentiation of primary fetal rat AT2 cells induced by hyperoxia in vitro, improving pulmonary vascular growth	Restored lung structure and function	2022	Ai D[131]

(*Continued*)

Table 4 (Continued).

EVs Name	EVs Source	Recipient Animal	EVs Administration Route	Dosage	Frequency	Dose Difference on Lung	Mechanism of EVs	Therapeutic Effects	Year	References
hAEC-EVs	Human amniotic epithelial cell MSCs	Newborn C57/BL6 mice	Intravenous (IV)	10 μg	Single dose (P4)	Only term hAEC-EVs were beneficial in the BPD mice model	Term hAEC-EVs ameliorates alveolar simplification, improving secondary septal crest density, reducing airway hyper-responsiveness, mitigating pulmonary hypertension	Term hAEC-EVs reduced inflammation and vascular remodeling in the lung	2022	Zhu D[132]
hUCMSC-MVs	Human umbilical cord MSCs	Newborn Sprague Dawley rats	Intratracheal (IT)	20 μg	Single dose (P7)	No significant dose dependence	Reverses protein levels of PTEN and p-AKT, promoting AT2 cell proliferation, reducing lung inflammation	Restored lung structure and function	2022	Zhou O[134]
hUCMSC-EVs	Human umbilical cord MSCs	Newborn C57/BL6 mice	Intratracheal (IT)	0.005 μg/g, approximately 1×10^{6} particles/g	Single dose (P9, or P10)	No significant dose dependence	Improves lung architecture, vessel formation, and inflammatory modulation	Pulmonary alterations were mitigated.	2022	Lithopoulos MA[117]
UCB-Exos	Umbilical cord blood	Newborn FVB/NJ mice	Intraperitoneal (IP)	5×10^{5} particles	Three doses (P4, P5 and P10)	No significant dose dependence	Restoring CD31 and VEGFA levels, elevating miR-185-5p, inhibiting CDK6 in pulmonary endothelium and apoptosis	Improved alveolar structure, reduced collagen in hyperoxic lung injury, promoting pulmonary vascular development.	2023	Zhong XQ[41]
BMSC-sEVs	Bone marrow-derived MSCs	Preterm lambs	Intravenous (IV)	2×10^{11} particles	Two doses (Hour 6 and hour 78 post-delivery)	No significant dose dependence	Promotes alveolar and vascular development	Improved lung function in preterm lambs	2024	Albertine KH [47]
AMSC-Exos	Adipose MSCs	Newborn Sprague Dawley rats	Intratracheal (IT)	15 μg	Single dose (P4)	No significant dose dependence	Inhibits NF-κB pathway, reducing inflammation, improving alveolarization and pulmonary vascularization, facilitated proliferation, reduced apoptosis in AECIIs	Alleviated hyperoxia-induced lung injury	2024	Chen C[45]
ASC-EVs	Plasma	Newborn C57/BL6 mice	Intravenous (IV)	5 mg/kg	Single dose (P3, or P10)	No significant dose dependence	Activates inflammasome cascade; inducing pyroptosis, apoptosis, and necroptosis in alveolar macrophages cells	Induced hallmark BPD features: reduces alveolarization, pulmonary vascular density, infiltrates inflammatory macrophage	2024	Starke N[135]
hUCMSC-Exos	Human umbilical cord MSCs	Premature C57/BL6 mice	Intravenous (IV)	2×10^{10}/ particles/kg	Single dose (E10.5, or E15.5)	No significant dose dependence	Term-Exos enrich with Wnt5a, activating Wnt5a/ROCK1 signaling axis, promoting ROCK1 phosphorylation, upregulating RhoA expression, and inducing autophagy in AT2 cells.	Term-Exos facilitated lamellar body development and alveolar maturation, enhanced alveolarization, reduced BPD incidence.	2025	Li X[133]

via a single intra-tracheal injection on P3, showed no dose-dependent relationship involving pulmonary vascular density, VEGF expression, vascular remodeling, and lung structure. After long-term observation of three months, a single intra-tracheal dose of WJ-MSC EVs on P3 was reported prolonged effects in reducing RVH, PH, and vascular remodeling in BPD models. On P3, there were no discernible differences between systemically administrated MSC-EVs via intravenous administration and locally administrated MSC-EVs via intratracheal administration in terms of pulmonary vascular angiogenesis, remodeling, and structure as measured by hemodynamic or morphometric measures.[56] However, Danyang Ai et al took a different view on the route of administration and the dose-dependent relationship about MSC-EVs in treating BPD. Three different doses of MSC-EVs were administrated intraperitoneally on P4 respectively, including low (5 μg), medium (10 μg), and high (15 μg). They ameliorated hyperoxia-induced lung injury in a dose-dependent manner, and high-dose MSC-EVs attenuated alveolar simplification. MSC-EVs with high dosage of 15 μg enhanced vascular growth and mitigated pulmonary hypertension, decreased *pdpn* and *wnt5α* expression, inhibited primary fatal rat TIIAECs transdifferentiation.[140] Dandan Zhu et al reported that intravenously administrated term human amniotic epithelial cell MSCs derived EVs (hAEC-EVs) at a single of 10 μg on P4 effectively improved tissue-to-airspace ratio and septal crest density, increased TIIAECs in lung injury, released airway hyper-responsiveness, alleviated pulmonary hypertension, and protected from RVH, compared to the preterm hAEC-EVs.[141] In an additional study, intravenous administrated term hUCMSC-Exos at single dose of 2×10^{10}/particles/kg at E10.5 or E15.5 facilitated lamellar body development and alveolar maturation, enhanced alveolarization via activating Wnt5a/ROCK1 signaling axis, promoting ROCK1 phosphorylation, upregulating RhoA expression, and inducing autophagy in AT2 cells.[142] Ou Zhou et al found that human umbilical cord mesenchymal stem cell-derived microvesicles (hUCMSC-MVs), which were given intratracheally at a single dose of 20 μg on P7 to newborn Sprague Dawley (SD) rats with BPD, showed a potential therapeutic effect for BPD by reversing the protein levels of PTEN and p-AKT, decreasing the level of lung homogenate of IL-6 and IL-10, and increasing the number of TIIAECs cells and SPC expression.[143] Marissa A. Lithopoulos et al investigated therapeutic effects of hUCMSC-EVs in newborn C57/BL6 mice with BPD. Their findings indicated that intratracheal administrated hUCMSC-EVs at a single dose of 0.005 μg/g, approximately 1×10^6 particles/g on the P9 or P10 showed a significantly lower MLI score, improved lung vessel density, lung architecture, as well as inflammatory modulation.[128] In the BPD model among newborn FVB/NJ mice, intraperitoneal administrated umbilical cord blood-derived exosomes (UCB-Exos) at 5×10^5 particles each time for three consecutive days on P4, P5 and P10, rescued the disrupted angiogenesis of lung tissues via increasing CD31 levels, and restoring VEGFA levels in hyperoxia-insulted mice.[51] MSC-sEVs administrated preterm lambs showed lower in respiratory severity score, distal airspace wall thickness, smooth muscle thickness about bronchioles and lung arterioles, higher in radial alveolar count, alveolar capillary surface density, and secondary septal volume density. Thus, in preterm lambs with BPD, intravenous administrated two doses of MSC-sEVs respectively at 6 hour and 78 hour post-delivery improved respiratory system physiology and pulmonary alveolarization in mechanically ventilated preterm lambs.[50] In the newborn SD rats with BPD, a single 15 μg dose of AMSC-Exos on P4 intratracheally alleviated hyperoxia-induced lung injury by inhibiting NF-κB pathway, inflammation reactions, improving alveolarization and proliferation, reducing apoptosis in AECIIs.[58] Natalie Starke et al investigated inflammasome cascade molecules of the hyperoxia exposuring preterm infants' plasma derived EVs. Their findings demonstrated that these EVs induced lung inflammation with increasing cargo of $CD11b^+/ASC^+$, $CD11c^+/ASC^+$, and $CD206^+/ASC^+$, inhibiting alveolarization and vascular development, and aggravating BPD.[144] These studies indicate that the ideal therapeutic dose of EVs in treating BPD is subject to comprehensive consideration of optimizing both local and systemic delivery routes, animal weight, frequencies, delivery efficiency and associated mechanisms. In the following part, we provide a thorough analysis and comparison of kinds of administration routes, their biodistribution, potential components of EVs for BPD treatments and potential mechanisms.

The Biodistribution, Potential Components, Efficacy Evaluations of Extracellular Vesicles of Diverse Administration Methods in Treating BPD Models in Vivo

Due to their inherent capacity for self-renewal, high efficiency in producing and replacing terminally differentiated cells, and function in pulmonary vascular regeneration, MSCs were considered as viable and promising treatment options for

BPD.[145] EVs obtained from MSCs remain biologically similar, such as having anti-inflammatory and regenerative properties, however, they decreased the risk of thrombosis, iatrogenic tumor formation, and immunogenicity.[146,147] To date, various types of cell-derived EVs have demonstrated the therapeutic potential in experimental BPD models, such as adipose-derived MSCs, bone marrow-derived MSCs, human umbilical cord-derived MSCs, human breast milk, and plasma. EVs normally carry their bioactive cargo, including mRNAs, microRNAs, proteins and growth factors, into the recipient cells, making them candidate therapeutic agents in BPD (Table 5).[105]

Intratracheal Administration

IT administration has been used as a widely used administrative route for EVs treatment in BPD. Previous study indicated that IT-administrated mice had the highest fluorescent signal in lung after 24h compared with in the liver and spleen, which was observed by IVIS analyses system.[152] IT-administered EVs appeared to be effective in rat pups with BPD pathology, which was more practical as a non-systemic IV injection. IT administrated MSC-EVs on P3, P7, P10 significantly interrupted CD68 +/αSMA+ macrophages in lung accumulation and decreased the expressions of Tgfβ1, Nrf2, αSMA, Col1a1, 8-oxo-dG. Additionally, by increasing Sftpc expression, MSC-EVs restored AT II cell function and decreased ROS production in mitochondria by enhancing Sod2 expression, preserving pulmonary epithelial function and delaying the onset of fibrosis.[127] The miR-34c-5p in BMSC-EVs targets ovarian tumor protease domain-containing protein (OTUD) 3 mRNA, leading to reduced OTUD3 protein expression. This subsequently decreases PTEN levels, due to loss of OTUD3-mediated stabilization, resulting in activation of the PI3K/Akt pathway which promotes angiogenesis and alveolar repair in BPD.[53] Upregulation of miR-21-5p in adipose-derived MSCs (ADMSCs) reduced SKP2 expression, which in turn decreased Nr2f2 ubiquitination, enhanced C/EBPα transcription, and ultimately attenuated hyperoxia-induced oxidative stress, inflammation, and apoptosis in the lungs.[69] Additionally, IT administrated MSC-EVs for 4 doses on P3, P7, P10, and P21 decreased collagen deposition and medical wall thickness, decreased fibrosis, and effectively promoted M2 polarization by increasing the density of alveolar M2 macrophages with CD163-positive and vascular endothelial cells with ki67-positive.[129] Interestingly, IT-administered EVs from hUC-MSCs also increased p-Akt and VEGF-A expressions in BPD models, decreased apoptosis, and promoted proliferation in pulmonary vascular endothelial cells that were Ki-67 positive.[131]

Intravenous Administration

IV administration remains the most widely used route for EVs in treating BPD in vivo. Through this pathway, EVs can enter the circulatory system directly, allowing for precise control over their dosage and concentration in the blood. EVs primarily build up in organs like the liver, spleen, lungs, and kidneys after IV.[153] Most studies investigating EV biodistribution have relied on nuclear imaging or fluorescence imaging in vivo, indicating lung accumulated concentration changes with the injection time.[154,155] Linchao Yu et al demonstrated that miR-139-3p carried by MVs of clinical plasma samples of BPD suppressed endothelial proliferation and angiogenesis through downregulating 4EBP1, which was elevated by IV administration. In mice induced with BPD-like pathology, the inhibitor of miR-139-3p reduced vascular and alveolar simplification, which served as a possible treatment strategy for MVs.[148] According to Gareth R. Willis et al, EVs made from human umbilical cord Wharton's Jelly-MSCs (MEx) inhibited pro-inflammatory factors like TNFα, IL4, IL6, IL17a, and IFNγ, increased *Arg1, Il-10*, and *CX3CR1* expressions, and changed the monocyte/macrophage phenotype toward an immunosuppressive state similar to that of MDSCs. MEx showed regenerative effects in improving exercise capacity, lung architecture, and function by restoring CD45 in a dose-dependent method.[68] Function of BMSCs-Exos in mitigating hyperoxia-induced RLE-6TN cell injury is diminished when miR-425 is silenced.[130] Xiaomeng Yi et al highlighted the possible prevention of acute lung injury (ALI) by MSC-Exos overexpressing miR-30b-3p. In the LPS-pretreated MLE-12 cells, overexpressing miR-30b-3p of MSC-Exos decreased the levels of phosphorylated NF-κB, IκB-α, ERK, MEK1/2, p38MARK, and JNK by binding to SAA3. This promoted cell proliferation by reducing inflammation and AEC apoptosis.[149] Gareth R. Willis et al showed that IV administration of MSC-Exos on P4 reduced inflammation, fibrosis, and vascular remodeling while suppressing the M1 phenotype, promoting M2 macrophages, and reprogramming the macrophage response.[150] IT administrated-Exos packaged circular RNA (circRNA)-circABPD1 targeted miR-330-3p, normalized pulmonary angiogenesis by inhibiting HIF-1α driven vascular leakage, reducing pulmonary edema by 60% in neonatal SD rats with BPD. This improved the viability of A549 cells, decreased ROS, increased GSH, SOD, and SPC expression, and lessened damage to lung epithelial cells.[151] However, a large

Table 5 Different Administered Routes of EVs Carrying Bioactive Cargos in Treating BPD

EVs Delivery Method	EVs Source	Major Components/ Drug	Upregulation/Downregulation	Mechanism Insights	Biodistribution	Efficacy Evaluation	Safety Analysis	Year	References
IV	Plasma of BPD model	miR-139-3p	miR-139-3p elevated in BPD MVs; ↓ 4EBP1	miR-139-3p suppresses endothelial proliferation and angiogenesis via downregulation of 4EBP1	Systemic circulation; affecting pulmonary vasculature	miR-139-3p inhibitor alleviates alveolar and vascular simplification in BPD mice	No safety evaluation performed	2023	Yu L[143]
IV	hUC-MSC	CD63+	↑ Il10, Arg1, CX3CR1; ↓ Ccr2, TNFα, IL6, IFNγ; shifts monocyte/macrophage phenotype toward MDSC-like immunosuppressive state	Interacts with lung myeloid cells, restores CD45+, promotes anti-inflammatory monocytes/macrophages	Detected in lung/ liver	Enhance lung architecture, function, and exercise capacity; effects confirmed dose-dependently	No adverse effects	2021	Willis GR[57]
IV	BMSCs (rats origin)	miR-425	↓PTEN → ↑PI3K/Akt → ↓apoptosis, ↓oxidative stress	Promotes epithelial survival, reduces Bax, increases Bcl-2, blocks ROS-mediated apoptosis	Localized in alveolar epithelial cells (RLE-6TN)	Improve RAC, reduces MLI and MAD, suppresses TUNEL+ cells, rescues alveolar structure	No adverse effects reported in vivo	2020	Wu Y[120]
IV	BMSCs (mice origin)	miR-30b-3p.	↓ SAA3→ ↓ NF-κB, IκB-α, ERK, MEK, JNK, and p38MAPK phosphorylation	Reduces AEC apoptosis and inflammation; promotes cell proliferation; inhibits ALI via miR-30b-3p/SAA3 axis	Confirmed pulmonary targeting via PKH26 fluorescence labeling	Improve lung histopathology, reduces lung W/D ratio, neutrophil count, and MPO activity	No safety evaluation performed	2020	Yi X[144]
IV	WJMSCs, BMSC, or HDF	CD9, CD63, FLOT1+	↓ M1 phenotype, ↑ M2 macrophages	Reprograms macrophage response, reduces fibrosis, vascular remodeling, inflammation	No adverse biodistribution; localized lung improvement	Decrease in fibrosis and pulmonary vascular remodeling, and amelioration of pulmonary hypertension	Safe; no toxicity or off-target effects reported;	2018	Willis GR[145]
IT	hUC-MSCs (clinical GMP-grade)	CD9, CD63, CD81	↓Tgfβ1, Nrf2, αSMA, Col1a1, 8-oxo-dG; ↑Sftpc, Sod2	EVs reduces ROS production, restoring ATII cell function, maintaining epithelial identity	Detected in lung since 3 hrs post injection	Improve survival, alveolar morphology, surfactant production, GAGs, and macrophage phenotypes	No safety evaluation performed	2024	Bisaccia P[116]
IT	BMSCs (mice origin)	miR-34c-5p	↓ OTUD3,↑ubiquitination →↓ PTEN stability	Suppresses OTUD3/PTEN axis, promoting angiogenesis, proliferation, and reducing inflammation	EVs confirmed internalized by lung	Reverse hyperoxia-induced damage in lung tissue and vascular	No safety evaluation performed	2023	He X[43]
IT	ADMSCs (mice origin)	miR-21-5p	↑ miR-21-5p → ↓ SKP2 → ↓ ubiquitination of Nr2f2 → ↑ Nr2f2 → ↑ C/EBPα transcription → ↓ apoptosis, oxidative stress, inflammation	Alleviates inflammation, apoptosis, oxidative stress via SKP2/Nr2f2/C/EBPα axis	Fluorescent EV tracking confirmed uptake in lung	Significant reduction in TNF-α, IL-6, MDA; enhanced SOD and miR-21-5p levels in lung tissues	High-dose EVs safe; no adverse outcomes reported in vivo	2022	Wu Y[58]

(*Continued*)

Table 5 (Continued).

EVs Delivery Method	EVs Source	Major Components/ Drug	Upregulation/Downregulation	Mechanism Insights	Biodistribution	Efficacy Evaluation	Safety Analysis	Year	References
IT	hUC-MSCs	CD9, CD63, CD81 +, annexin V (internal)	↑ $CD163^+$ (M2) macrophages, ↑ $Ki67^+$ cell proliferation, ↓ collagen deposition, ↓ medial wall thickness of <100 μm arteries	Ameliorates alveolar simplification, reduces fibrosis, promotes M2 polarization	Confirmed uptake; histological changes localized in lung	Significant reduction in MLI, fibrosis, and vessel wall thickness; increase in alveolar count and Ki67+ cells	No toxicity or weight loss; safe in neonatal administration	2021	Porzionato A[118]
IT	hUC-MSCs	CD63+, Alix+	↓ PTEN and cleaved caspase-3; ↑ p-Akt and VEGF-A	Protects TIIAECs and PVECs, enhances proliferation (Ki-67), suppresses apoptosis (TUNEL)	Confirmed lung uptake, retention for ≥72 h	Ameliorates pulmonary hypertension, fibrosis, apoptosis, and promotes angiogenesis	No safety evaluation performed	2020	You J[121]
IP	Cord blood derived monocytes or tracheal aspirates	miR-23a-3p	↑ miR-23a-3p in hyperoxic macrophage-derived EVs; leads to PTEN downregulation	miR-23a-3p targets PTEN, reducing EPC maintenance and angiogenesis	Localized lung uptaking	AntagomiR-23a-3p restores c-Kit+ EPCs, increases capillary density, reduces alveolar simplification	No safety evaluation performed	2024	Wang X[59]
IP	HBM	circDNAJB6 (hsa_circ_0083171)	↑ transcription of host gene DNAJB6	Nuclear circDNAJB6 activates DNAJB6 transcription; enhances ATII cell viability and reduces injury	Delivered to lung, confirmed via Ki67-labeling and imaging	Promotes alveolar epithelial proliferation and function, mitigates alveolar simplification in BPD	No safety evaluation performed	2024	Li Y[148]
IP	HBM (preterm colostrum origin)	miR-330-3p	circABPD1 targeted ↓ miR-330-3p, ↑HIF1α	circABPD1 functions as a ceRNA, sequestering miR-330-3p and preventing its repression of HIF1α.	Functional delivery to lung	Improved A549 cells viability, reduced ROS, increased GSH, SOD, SPC expression	No safety evaluation performed	2023	Li H[149]
IP	BMSCs (human origin)	Exosomes + Tempol (antioxidant)	↑ HIF-1α, VEGF, p-PI3K, p-Akt; ↓ IL-1β, IL-6, IL-17, IFN-γ	Improves alveolarization, vascular remodeling, and reduces oxidative stress	MSC-EXO was shown to reach lung	Reverses inflammation, enhances angiogenesis, and restores lung structure and function	No toxic effects observed	2022	Wang J[126]
IP	Early gestational hUC-MSCs (25 and 30 wks)	TSG-6	↓ IL-1β, TNF-α, IL-6	Systemic immunomodulation across lung, heart, brain; TSG-6 knockdown abrogates benefits	Detected in lung, heart, brain	Effective multi-organ protection; corrects lung/ heart/brain histopathology	Safe for neonatal mice; no toxicity observed	2018	Chaubey S[150]
IP	UCB from very preterm delivery of BPD	miR-183, miR-328, and miR-27a,	↓ Fgf9 and Cacna2d3, ↓MAPK and PPAR pathways, ↓VEGFA and CD31	Exacerbated alveolar simplification, increased fibrosis, and impaired angiogenesis.	No direct imaging data	Increased histopathology score, collagen deposition, apoptosis, and impaired angiogenesis.	BPD-EXO induced chronic and irreversible lung injury.	2023	Zhong XQ[151]

amount of research shows that EVs have a very short half-life and the amount of lung tissue that accumulates after IV administration is less than that of the liver and spleen.[156,157] Drug release extension has been indicated bioactive scaffolds loaded with EVs, with development of nanofiber hydrogels, nanocomposite hydrogels, and 3D printing.[158] For instance, CD47 has been inserted into EV membranes to evade phagocytosis, while Rltrkrglk (RLTR) peptide-functionalized Exos represent an emerging strategy for targeted delivery. However, their efficacy in BPD requires further preclinical validation, particularly for IV administration.[159] PEG-modified EVs exhibit improved stability by reducing immune recognition after IV injection.[160] Receptor-targeting peptides, including RGD, significantly increase EV retention in injured lungs versus untargeted EVs, which indicates peptide functionalization might be a valid strategy for pulmonary delivery.[161]

Intraperitoneal Administration

By utilizing the vast abdominal vasculature, IP avoids the gastrointestinal tract's first-pass metabolism and offers a large absorption area. This administration route has been the focus of a set of researches because it can also handle higher drug volumes.[162,163] For example, IP administration of EVs derived from cord blood-derived monocytes or tracheal aspirates transmitted *miR-23A-3p* targeted PTEN, reducing endothelial progenitor cells (EPC) maintenance and angiogenesis, which was a candidate therapeutic target for BPD.[70] Another study indicated that IP-administrated Exos derived from human breast milk (HBK) that contained circDNAJB6 enhanced AT II cell viability and decreased damage by targeting the downstream DNAJB6 gene and promoting DNAJB6 transcription.[164] According to the research conducted by Juanmei Wang et al, IP-administrated BMSC Exos for 14 consecutive doses enhanced the expression of VEGF, HIF-1α, p-PI3K, and p-Akt while decreasing the expression of inflammatory cytokines like IL-1β, IL-6, IL-17, and IFN-γ. They also improved alveolarization, vascular remodeling, and oxidative stress control.[135] TNF-stimulated gene 6 (TSG-6) of Exos extracted from healthy donors' hUC-MSCs' preterm deliveries at 25 wks decreased the expression of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β and reversed hyperoxia-induced morphometric changes in the lung. In addition, the therapeutic effects of Exos were disrupted by TSG-6 knockdown, making it a potential option for nano non-cell therapy for BPD.[165] However, not all Exos from preterm infants biological samples are benefit for BPD treatment. Xinfqi Zhong et al reported that the administration of Exos from very premature BPD in mice aggravated lung damage by reducing the expression of Fabp3, Fgf9 and Cacna2d3, inhibitor of MAPK and PPAR pathways and lowering of endothelial marker secretagogue, including VEGFA and CD31 epithelial markers.[166] According to the research conducted by Cristiane S. R. Fonteles et al, IP-administrated Exos showed comparable accumulation in the liver, lungs, uterus, kidneys, brain, heart, and spleen 24 hours after injection.[167] IP route has some advantages over IV administration, including preventing cell entrapment in the pulmonary vasculature, accumulating in the surrounding tissue, and having soluble factors that affect the recipient's immune system.[168] Further studies are demanded to investigate bioavailability of major components of EVs to reach pulmonary vessels by IP administration.

Intragastric Administration

The preferred method of treating BPD is still IG administration because it is affordable, simple to use, and allowing for repeated dosing. Numerous studies have shown that in simulated gastric environments, natural M-EVs lose about 85% of their protein content and face major obstacles from the mucus-epithelial barrier.[169] In order to improve the stability and intestinal epithelial absorption of M-EVs, researchers have used hydrophilic and zwitterionic peptides to modify their surfaces.[169] EVs from a variety of sources, including bacteria, plants, milk, and intestinal epithelial cells, have been shown to withstand the conditions of the gastrointestinal tract.[170] More research is demanded to fully understand MEVs' potential and mechanisms for overcoming gastrointestinal barriers. It has been demonstrated that EVs originated from human milk (M-EVs) can survive pancreatic and gastric digestion simulations.[171,172] For example, Yahui Zhou et al indicated that IG administrated HBM-Exos enhanced the expression of the AT II surface marker SPC, inhibited the IL-17 signaling pathway (FADD), which was a crucial mechanism in preventing BPD, and inhibited proliferation and apoptosis caused by hyperoxia. In addition to improving the inhibition of Ki67 and the elevation of cleaved-caspase 3 (C-Caspase3) brought on by exposure to hyperoxia, they verified that DiR-labeled HBM-Exo specifically targeted lung tissue in vivo 12 hours after administration.[139]

Future Directions

EVs have shown promise as nanoscale, cell-free treatments for BPD, that affects preterm infants and is characterized by vascular dysgenesis, persistent inflammation, and arrested alveolarization.[57,173] Present preclinical and early clinical research shows that EVs made from engineered sources, amniotic fluid, or MSCs provide a variety of therapeutic benefits by delivering bioactive cargo, including proteins, lipids, and miRNAs, that alter important pathways linked to the pathophysiology of BPD.[174,175] For example, amniotic fluid EVs-mediated inhibition of TGF-β1/small mother against decapentaplegic (Smad) 3 signaling reduces pulmonary fibrosis by decreasing collagen deposition and αSMA+ myofibroblast activation, thereby preserving lung elasticity and improving gas exchange. And MSC-EVs rich in miR-30b-5p inhibit hyperoxia-induced bronchial epithelial cell apoptosis through PTEN/P_{I3}K/Akt signaling pathway.[176] According to clinical trials (eg NCT04313647, NCT05624203), these results that assess EVs for respiratory conditions, which show promising safety profiles and initial effectiveness in promoting tissue repair and lowering inflammation.[177,178] Therefore, while EVs hold substantial therapeutic potential for BPD, more robust, large-scale, and well-controlled clinical trials are essential to determine their role in clinical practice. Nevertheless, there are still few BPD-specific EV trials, and the majority of research that is available focuses on proof-of-concept rodent models, leaving gaps in mechanistic specificity, dosage, and delivery optimization. However, safety dose of EVs obtained from a variety of origins remains not fully discussed. There is an urgent need for a large body of research to clarify the safety dosage of EVs from various sources and administered using different routes.

Safe dosage of EVs in the treatment of animal models with BPD is affected by many factors, such as the source of EVs, animal species, and administration routes. EV-based preclinical studies suggest that different dosages need to be considered in both animal and cell models of BPD, and the ideal therapeutic dosage is still under exploring.[179] IT delivery, the most researched route in preclinical models, achieves high pulmonary retention EVs localized to lung tissue within 24 hours and permits low-dose efficacy. In neonatal Sprague-Dawley rats with BPD, Exos treatment reduced pulmonary hypertension, increased survival in a non-dose-dependent way, and increased survival at a dose of 6×10^9 particles/g via IT administration. IT administration directly targets alveolar macrophages and epithelial cells, attenuating inflammation and promoting alveolarization. However, rapid mucociliary clearance and immune cell uptake may necessitate repeated dosing. In contrast, IV administration reduces inflammation throughout the body, but higher dosages are needed to overcome the pulmonary endothelial barriers and reach therapeutic lung concentrations. The biodistribution of EVs at 30 μg intravenously in a preclinical experiment appears that once and twice administrations were non-toxic, and primarily accumulated in the liver, spleen, and lung. Additionally, EVs obtained from hUC-MSCs may reduce inflammation and lung damage brought on by LPS.[180] An intravenous injection of Exos at a dose of 3.85×10^{12} particles did not cause any discernible adverse effects on Th1/Th2 cytokines or general clinical conditions, according to a safety dose study conducted in cynomolgus monkeys.[181] As for IV injection, Exos at a dose of 3.85×10^{12} particles did not cause any discernible adverse effects on Th1/Th2 cytokines or general clinical conditions, according to a safety dose study conducted in cynomolgus monkeys.[181] Although IV-administered EVs have a great deal of potential to treat BPD, care should be taken into account because they may cause thrombogenicity through the angiotensin II receptor type 1 (AT1R)/nicotinamide adenine dinucleotide phosphate (NADPH) oxidases/sodium-glucose cotransporter 2 (SGLT2) pro-oxidant pathway, which causes endothelial dysfunction, and off-target organ accumulation in the pulmonary vascular system.[161,182] IP injection of EVs is a systemic delivery method that may be useful in treating BPD. The timing of treatment and the method of IP administration produce different effects, but there were no differences in efficacy when comparing the tissue sources of MSCs or animal species.[136] IP-administered Exos at a dose of 3.4×10^9 can reduce lung damage by regulating systemic inflammation and promoting alveolar repair, according to preclinical research in hyperoxia-induced BPD models. Mechanistically, IP-administered EVs achieve partial lung bio-distribution and engage immune cells by crossing the peritoneal lining and entering the systemic circulation, and hUC-MSCs mediate this effect in part through the secretion of TSG-6, which is an anti-inflammatory factor.[183] Dosage of 10 μg sEVs have indicated acceptable safety of temporary peritoneal irritation, ascites arousing, but no long-term organ-toxicity observed.[184] IG route is a potential non-invasive method of treating BPD, though harsh gastrointestinal environment and the low bioavailability. According to available data, in the severe acute pancreatitis (SAP)-associated ALI model, IG-administered Exos derived from bronchoalveolar lavage fluid (BALF) at a dose of 0.25 mg/kg contained the NOVEL-

rno-miR-29a-3p, which has anti-inflammatory properties and may lessen SAP-ALI.[185] However, EV degradation by gastric acids and proteases necessitates encapsulation in pH-resistant carriers, such as chitosan nanoparticles or lyophilization to preserve cargo integrity.[186] Despite the fact that intragastric delivery eliminates the dangers of invasive routes, biodistribution studies showed that most EVs localized to the liver and intestinal epithelium, with minimal accumulation in lungs.[187]

Additionally, we are still in the exploratory phase of the technology, considerably more needs to be learned about the biogenesis, uptake, administration, and mechanism of EVs in treating BPD, considering their heterogeneity. The variability of EVs yield, purity and cargo is due to the variation of cell sources, culture conditions including serum-free or serum-rich media, and isolation methods such as ultracentrifugation or size-exclusion chromatography.[188] Recently, EVs that originate from an environment that complies with GMP and has a trustworthy and traceable source are subject to quality control procedures that analyze homogeneity and purity, according to the most recent literature.[189] A research verified that the synergistic effects of tryptophan and trehalose allowed milk-derived extracellular vesicles (mEVs) to be stable as a powder at room temperature for up to six months without substantially altering their structure and function in vitro. This paves the way for GMP separation and end-user application of mEV-fundamental treatment.[190] Furthermore, inhalable dry powder development of stabilized EVs has been used in treating respiratory diseases, including pneumonia,[191] COPD,[192] PF,[193] severe COVID-19.[194] Nebulized EVs, which are mostly derived from MSCs or engineered sources, have been shown in recent studies to achieve localized pulmonary enrichment. In female BALB/c mice models induced by hypoxia, 60–80% of administered EVs are retained in lung tissue within 24 hours after inhalation. These EVs exhibited delivering caveolin-1 (CAV-1) to suppress expression of phosphorylated signal transducer and activator of transcription 6 (p-STAT6), and promoting airway barrier.[195] Nevertheless, EVs integrity during nebulization remains a critical concern, as shear stress and temperature fluctuations may compromise cargo stability. Thus, lyophilization or encapsulation in biocompatible carriers, for example, chitosan nanoparticles used for nebulization has shown great potential in preserving EVs functionality during delivery.[196] In a rat model of paraquat inhalation toxicity, herbicide loading on pectin (PEC)/chitosan (CS) /tripolyphosphate (TPP) nanoparticles decreased fibrosis and acute lung injury. Expressions of α-SMA, oxidative stress, and apoptosis were all decreased in the lung tissue as a result of the encapsulation.[197] Safety assessments in more than 180 patients with ARDS indicated that Exo-mCD24 inhalation at a dose of 5×10^8 or 1×10^9 for five consecutive days did not result in drug-related adverse events, which were observed up to 443–575 days.[121,198] Moreover, EXO-CD24 improved survival in an animal model of pulmonary sepsis and also showed promise in a model of bleomycin-induced pulmonary fibrosis and ovalbumin type I-induced allergic asthma.[199] Future studies may help investigate therapy and safety of EVs in treating BPD with non-invasive, cell-free administrations, for example, inhalation of EVs or chitosan nanoparticles. In spite of many challenges of EVs in treating BPD, it may offer great potential in cell-free nano-medicine treatment in clinical trials (Figure 7).

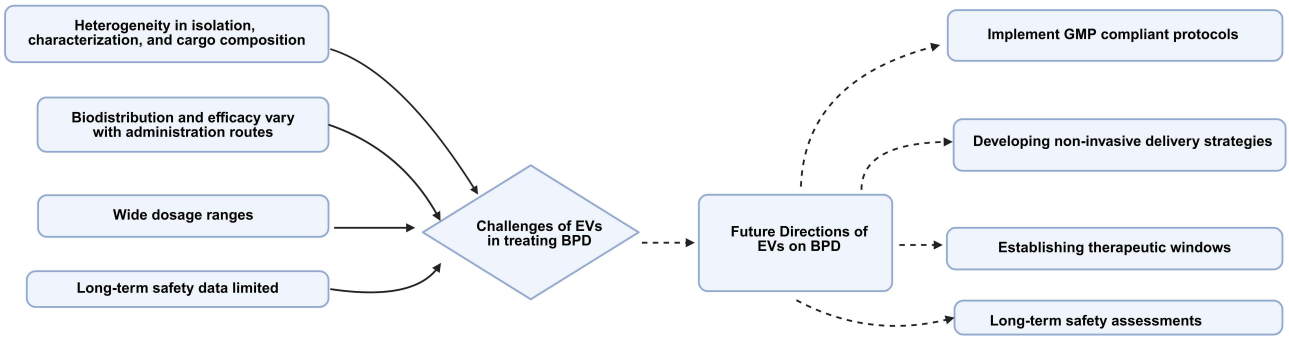

Figure 7 Roadmap for clinical translation of EVs in treating BPD.
Abbreviation: GMP, good manufacturing practice.

https://doi.org/10.2147/IJN.S530819

Conclusions

EVs have emerged as a potential tool for treating BPD. With their ability to transport bioactive cargos, for example, miRNAs, and proteins, EVs mitigate oxidative stress, inflammation injury, and promote pulmonary alveolarization. Intratracheal administration targets directly to lungs, while intravenous delivery offers systematic advantages, in spite of higher dosage requirements. Future researches are aimed to develop inhalable lyophilized EVs to address the practical and ethical limitations of invasive routes. Despite prospective preclinical outcomes, clinical translation primarily depends on resolving EVs heterogeneity, optimizing delivery methods, as well as validating safety dose in neonates. Future researches are suggested to prioritize GMP-compliant production, non-invasive delivery strategies, and clinical trials based on biomarkers to completely realize the therapeutic potential of EVs.

Data Sharing Statement

All the original data presented in this research are illustrated in the manuscript and supplementary materials. Further inquiries can be contacted directly to the corresponding author named Wenbin Dong.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by grants from projects of Wu Jieping Medical Foundation Clinical Research (No. 320.6750.2023-24-10) and National Natural Science Foundation of China (No. 82371710).

Disclosure

All authors in this research do not have any commercial or related interest in the work submitted.

References

1. Northway WH Jr, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med*. 1967;276(7):357–368. doi:10.1056/NEJM196702162760701
2. Tooley WH. Epidemiology of bronchopulmonary dysplasia. *J Pediatr*. 1979;95(5 Pt 2):851–858. doi:10.1016/s0022-3476(79)80451-5
3. Shennan AT, Dunn MS, Ohlsson A, et al. Abnormal pulmonary outcomes in premature infants: prediction from oxygen requirement in the neonatal period. *Pediatrics*. 1988;82(4):527–532. doi:10.1542/peds.82.4.527
4. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163(7):1723–1729. doi:10.1164/ajrccm.163.7.2011060
5. Quine D, Wong CM, Boyle EM, et al. Non-invasive measurement of reduced ventilation: perfusion ratio and shunt in infants with bronchopulmonary dysplasia: a physiological definition of the disease. *Arch Dis Child Fetal Neonatal Ed*. 2006;91(6):F409–414. doi:10.1136/adc.2006.095406
6. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD neonatal research network. *Pediatrics*. 2010;126(3):443–456. doi:10.1542/peds.2009-2959
7. Poindexter BB, Feng R, Schmidt B, et al. Comparisons and limitations of current definitions of bronchopulmonary dysplasia for the prematurity and respiratory outcomes program. *Ann Am Thorac Soc*. 2015;12(12):1822–1830. doi:10.1513/AnnalsATS.201504-218OC
8. Isayama T, Lee SK, Yang J, et al. Revisiting the definition of bronchopulmonary dysplasia: effect of changing panoply of respiratory support for preterm neonates. *JAMA Pediatr*. 2017;171(3):271–279. doi:10.1001/jamapediatrics.2016.4141
9. Chen Y, Chen Q, Zhu Q, et al. Small molecular theranostic assemblies functionalized by doxorubicin-hyaluronic acid-methotrexate prodrug for multiple tumor targeting and imaging-guided combined chemo-photothermal therapy. *Mol Pharm*. 2019;16(6):2470–2480. doi:10.1021/acs.molpharmaceut.9b00072
10. Allen J, Panitch H. Bronchopulmonary dysplasia-A historical perspective. *Pediatr Pulmonol*. 2021;56(11):3478–3489. doi:10.1002/ppul.25341
11. Moreira AG, Arora T, Arya S, et al. Leveraging transcriptomics to develop bronchopulmonary dysplasia endotypes: a concept paper. *Respir Res*. 2023;24(1):284. doi:10.1186/s12931-023-02596-y
12. Hwang JK, Kim DH, Na JY, et al. Two-stage learning-based prediction of bronchopulmonary dysplasia in very low birth weight infants: a nationwide cohort study. *Front Pediatr*. 2023;11:1155921. doi:10.3389/fped.2023.1155921
13. Duijts L, van Meel ER, Moschino L, et al. European respiratory society guideline on long-term management of children with bronchopulmonary dysplasia. *Eur Respir J*. 2020;55(1):1900788. doi:10.1183/13993003.00788-2019
14. Collaco JM, Eldredge LC, McGrath-Morrow SA. Long-term pulmonary outcomes in BPD throughout the life-course. *J Perinatol*. 2024. doi:10.1038/s41372-024-01957-9

15. Zhang Y, Ren X, Zhang L, et al. Hydrogen gas inhalation ameliorates LPS-induced BPD by inhibiting inflammation via regulating the TLR4-NFkappaB-IL6/NLRP3 signaling pathway in the placenta. *Eur J Med Res*. 2024;29(1):285. doi:10.1186/s40001-024-01874-9
16. Li Z, Mu X, Lv X, et al. Patent ductus arteriosus and the association between lung ultrasound score and bronchopulmonary dysplasia: a secondary analysis of a prospective study. *Eur J Pediatr*. 2024;183(10):4309–4317. doi:10.1007/s00431-024-05702-4
17. Wu TJ, Jing X, Teng M, et al. Role of myeloperoxidase, oxidative stress, and inflammation in bronchopulmonary dysplasia. *Antioxidants*. 2024;13(8):889. doi:10.3390/antiox13080889
18. Rumph JT, Stephens VR, Ameli S, et al. A paternal fish oil diet preconception reduces lung inflammation in a toxicant-driven murine model of new bronchopulmonary dysplasia. *Mar Drugs*. 2023;21(3):161. doi:10.3390/md21030161
19. Huang LY, Lin TI, Lin CH, et al. Comprehensive analysis of risk factors for bronchopulmonary dysplasia in preterm infants in Taiwan: a four-year study. *Children*. 2023;10(11):1822. doi:10.3390/children10111822
20. Gao J, Um-Bergstrom P, Pourbazargan M, et al. Large airway T cells in adults with former bronchopulmonary dysplasia. *Respir Res*. 2024;25(1):86. doi:10.1186/s12931-024-02717-1
21. Ding KL, Smith C, Seedorf G, et al. Nintedanib preserves lung growth and prevents pulmonary hypertension in a hyperoxia-induced lung injury model. *Pediatr Res*. 2024;97(5):1676–1683. doi:10.1038/s41390-024-03562-0
22. Darabi A, Faramarzi R, Boskabadi H, et al. Dataset on neonatal and maternal factors influencing neurodevelopmental outcomes in preterm infants: a study focused on the healthcare context of Mashhad, Iran. *Data Brief*. 2024;53:110058. doi:10.1016/j.dib.2024.110058
23. Bonadies L, Cavicchiolo ME, Priante E, et al. Prematurity and BPD: what general pediatricians should know. *Eur J Pediatr*. 2023;182(4):1505–1516. doi:10.1007/s00431-022-04797-x
24. Wang J, Yang G, Cai Z, et al. Point prevalence, characteristics and treatment variations for preterm infants with bronchopulmonary dysplasia in China: a 'snapshot' study. *BMJ Paediatr Open*. 2024;8(1):e002878. doi:10.1136/bmjpo-2024-002878
25. Enzer KG, Baker CD, Wisniewski BL. Bronchopulmonary dysplasia. *Clin Chest Med*. 2024;45(3):639–650. doi:10.1016/j.ccm.2024.03.007
26. Kuitunen I, Rasanen K. Less invasive surfactant administration compared to intubation, surfactant, rapid extubation method in preterm neonates: an umbrella review. *Neonatology*. 2024;121(4):485–493. doi:10.1159/000537903
27. van de Loo M, van Kaam A, Offringa M, et al. Corticosteroids for the prevention and treatment of bronchopulmonary dysplasia: an overview of systematic reviews. *Cochrane Database Syst Rev*. 2024;4(4):CD013271. doi:10.1002/14651858.CD013271.pub2
28. Cuevas Guaman M, Hagan J, Sabic D, et al. Volume-guarantee vs. pressure-limited ventilation in evolving bronchopulmonary dysplasia. *Front Pediatr*. 2022;10:952376. doi:10.3389/fped.2022.952376
29. Nissel J, Xu J, Wu L, et al. Why wearing a yellow hat is impossible: chinese and U.S. children's possibility judgments. *Cognition*. 2024;251(105856):105856. doi:10.1016/j.cognition.2024.105856
30. Teng M, Wu TJ, Jing X, et al. Temporal Dynamics of oxidative stress and inflammation in bronchopulmonary dysplasia. *Int J Mol Sci*. 2024;25(18):10145. doi:10.3390/ijms251810145
31. Durlak W, Thebaud B. BPD: latest strategies of prevention and treatment. *Neonatology*. 2024;121(5):596–607. doi:10.1159/000540002
32. He Y, Zhang M, Tang J, et al. A retrospective cohort study on mortality, morbidity, and care practices for 1750 very low birth weight infants, 2016-2021. *Chin Med J*. 2024;137(20):2452–2460. doi:10.1097/CM9.0000000000002923
33. Al Mandhari H, Khan A, Al Saadi A, et al. Prevalence, severity patterns and risk factors of bronchopulmonary dysplasia in preterm infants younger than 32 weeks of gestation in a tertiary centre in Oman. *Sultan Qaboos Univ Med J*. 2024;24(2):259–267. doi:10.18295/squmj.3.2024.017
34. Perveen S, Chen CM, Sobajima H, et al. Editorial: bronchopulmonary dysplasia: latest advances. *Front Pediatr*. 2023;11:1303761. doi:10.3389/fped.2023.1303761
35. Lai KC, Lorch SA. Healthcare costs of major morbidities associated with prematurity in US children's hospitals. *J Pediatr*. 2023;256:53–62e54. doi:10.1016/j.jpeds.2022.11.038
36. Dini G, Ceccarelli S, Celi F. Strategies for the prevention of bronchopulmonary dysplasia. *Front Pediatr*. 2024;12:1439265. doi:10.3389/fped.2024.1439265
37. Zhang S, Mulder C, Riddle S, et al. Mesenchymal stromal/stem cells and bronchopulmonary dysplasia. *Front Cell Dev Biol*. 2023;11:1247339. doi:10.3389/fcell.2023.1247339
38. Pan W, Gu L, Yang H, et al. Repeat-dose toxicity study of human umbilical cord mesenchymal stem cells in cynomolgus monkeys by intravenous and subcutaneous injection. *Front Cell Dev Biol*. 2023;11:1273723. doi:10.3389/fcell.2023.1273723
39. Akabane M, Imaoka Y, Kawashima J, et al. Innovative strategies for liver transplantation: the role of mesenchymal stem cells and their cell-free derivatives. *Cells*. 2024;13(19):1604. doi:10.3390/cells13191604
40. Ma CY, Zhai Y, Li CT, et al. Translating mesenchymal stem cell and their exosome research into GMP compliant advanced therapy products: promises, problems and prospects. *Med Res Rev*. 2024;44(3):919–938. doi:10.1002/med.22002
41. Welsh JA, Goberdhan DCI, O'Driscoll L, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J Extracell Vesicles*. 2024;13(2):e12404. doi:10.1002/jev2.12404
42. Schiller EA, Cohen K, Lin X, et al. Extracellular vesicle-microRNAs as diagnostic biomarkers in preterm neonates. *Int J Mol Sci*. 2023;24(3):2622. doi:10.3390/ijms24032622
43. Zhuang H, Song X, Li J, et al. EMAP-II from macrophage-derived extracellular vesicles drives neutrophil extracellular traps formation via PI3K/AKT/mtROS in lung ischemia/reperfusion injury. *Redox Biol*. 2025;85:103750. doi:10.1016/j.redox.2025.103750
44. Deng W, Zhu X, Li H, et al. Lung tissue extracellular vesicles-mediated delivery of miR-128-3p as a novel mechanism of acute lung inflammation. *Int J Nanomed*. 2025;20:4831–4848. doi:10.2147/IJN.S510241
45. Ruan T, Han J, Xue C, et al. Mesenchymal stem cells protect the integrity of the alveolar epithelial barrier through extracellular vesicles by inhibiting MAPK-mediated necroptosis. *Stem Cell Res Ther*. 2025;16(1):250. doi:10.1186/s13287-025-04388-1
46. Hou L, Zhu Z, Jiang F, et al. Human umbilical cord mesenchymal stem cell-derived extracellular vesicles alleviated silica induced lung inflammation and fibrosis in mice via circPWWP2A/miR-223-3p/NLRP3 axis. *Ecotoxicol Environ Saf*. 2023;251:114537. doi:10.1016/j.ecoenv.2023.114537
47. Schaubmayr W, Hochreiter B, Hunyadi-Gulyas E, et al. The proteome of extracellular vesicles released from pulmonary microvascular endothelium reveals impact of oxygen conditions on biotrauma. *Int J Mol Sci*. 2024;25(4):2415. doi:10.3390/ijms25042415

48. Bonadies L, De Vos B, Muraca M, et al. Extracellular Vesicles: a new promise for the prevention of bronchopulmonary dysplasia. *Am J Perinatol*. 2022;39(S 01):S23–S25. doi:10.1055/s-0042-1757351
49. Zayek AJ, Shivanna B. Promises and challenges of stem cell cargo therapy for bronchopulmonary dysplasia. *Pediatr Res*. 2024;97(2):471–472. doi:10.1038/s41390-024-03631-4
50. Albertine KH, Rebentisch A, Dawson E, et al. Mesenchymal stromal cell extracellular vesicles improve lung development in mechanically ventilated preterm lambs. *Am J Physiol Lung Cell Mol Physiol*. 2024;326(6):L770–L785. doi:10.1152/ajplung.00349.2023
51. Zhong XQ, Wang D, Chen S, et al. Umbilical cord blood-derived exosomes from healthy term pregnancies protect against hyperoxia-induced lung injury in mice. *Clin Transl Sci*. 2023;16(6):966–977. doi:10.1111/cts.13502
52. Tung S, Delavogia E, Fernandez-Gonzalez A, et al. Harnessing the therapeutic potential of the stem cell secretome in neonatal diseases. *Semin Perinatol*. 2023;47(3):151730. doi:10.1016/j.semperi.2023.151730
53. He X, Kuang J, Wang Y, et al. Bone marrow stromal cell-secreted extracellular vesicles containing miR-34c-5p alleviate lung injury and inflammation in bronchopulmonary dysplasia through promotion of PTEN degradation by targeting OTUD3. *Immunol Invest*. 2023;52 (6):681–702. doi:10.1080/08820139.2023.2217854
54. Luo L, Li Y, Long Z, et al. Exploring research trends and hotspots on oxidative stress and bronchopulmonary dysplasia: insights from bibliometric and visualized study. *Pediatr Pulmonol*. 2024;59(12):3610–3623. doi:10.1002/ppul.27268
55. Bellio MA, Young KC, Milberg J, et al. Amniotic fluid-derived extracellular vesicles: characterization and therapeutic efficacy in an experimental model of bronchopulmonary dysplasia. *Cytotherapy*. 2021;23(12):1097–1107. doi:10.1016/j.jcyt.2021.07.011
56. Sharma M, Bellio MA, Benny M, et al. Mesenchymal stem cell-derived extracellular vesicles prevent experimental bronchopulmonary dysplasia complicated by pulmonary hypertension. *Stem Cells Transl Med*. 2022;11(8):828–840. doi:10.1093/stcltm/szac041
57. Sun Y, Chen C, Liu Y, et al. Adipose stem cells derived exosomes alleviate bronchopulmonary dysplasia and regulate autophagy in neonatal rats. *Curr Stem Cell Res Ther*. 2024;19(6):919–932. doi:10.2174/011574888X260261230928094309
58. Chen C, Jin Y, Jin H, et al. Adipose mesenchymal stem cells-derived exosomes attenuated hyperoxia-induced lung injury in neonatal rats via inhibiting the NF-kappaB signaling pathway. *Pediatr Pulmonol*. 2024;59(10):2523–2534. doi:10.1002/ppul.27057
59. Goryunov K, Ivanov M, Kulikov A, et al. A review of the use of extracellular vesicles in the treatment of neonatal diseases: current state and problems with translation to the clinic. *Int J Mol Sci*. 2024;25(5):2879. doi:10.3390/ijms25052879
60. Gupta V, Singh S, Singh TG. Pervasive expostulation of p53 gene promoting the precipitation of neurogenic convulsions: a journey in therapeutic advancements. *Eur J Pharmacol*. 2024;983:176990. doi:10.1016/j.ejphar.2024.176990
61. Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol*. 1967;13(3):269–288. doi:10.1111/j.1365-2141.1967.tb08741.x
62. Trams EG, Lauter CJ, Salem N Jr, et al. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochim Biophys Acta*. 1981;645 (1):63–70. doi:10.1016/0005-2736(81)90512-5
63. Lambertsen RH. Interdigitative coupling of presumptive hematopoietic stem cells to macrophages in endocloned marrow colonies. *Blood*. 1984;63(5):1225–1229. doi:10.1182/blood.V63.5.1225.1225
64. Johnstone RM, Mathew A, Mason AB, et al. Exosome formation during maturation of mammalian and avian reticulocytes: evidence that exosome release is a major route for externalization of obsolete membrane proteins. *J Cell Physiol*. 1991;147(1):27–36. doi:10.1002/jcp.1041470105
65. Sokolova V, Ludwig AK, Hornung S, et al. Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy. *Colloids Surf B Biointerfaces*. 2011;87(1):146–150. doi:10.1016/j.colsurfb.2011.05.013
66. Willis GR, Kourembanas S, Mitsialis SA. Therapeutic applications of extracellular vesicles: perspectives from newborn medicine. *Methods Mol Biol*. 2017;1660:409–432. doi:10.1007/978-1-4939-7253-1_34
67. Willis GR, Fernandez-Gonzalez A, Reis M, et al. Mesenchymal stromal cell-derived small extracellular vesicles restore lung architecture and improve exercise capacity in a model of neonatal hyperoxia-induced lung injury. *J Extracell Vesicles*. 2020;9(1):1790874. doi:10.1080/20013078.2020.1790874
68. Willis GR, Reis M, Gheinani AH, et al. Extracellular vesicles protect the neonatal lung from hyperoxic injury through the epigenetic and transcriptomic reprogramming of myeloid cells. *Am J Respir Crit Care Med*. 2021;204(12):1418–1432. doi:10.1164/rccm.202102-0329OC
69. Wu Y, Zhang Z, Li J, et al. Mechanism of adipose-derived mesenchymal stem cell-derived extracellular vesicles carrying miR-21-5p in hyperoxia-induced lung injury. *Stem Cell Rev Rep*. 2022;18(3):1007–1024. doi:10.1007/s12015-021-10311-x
70. Wang X, Yao F, Yang L, et al. Macrophage extracellular vesicle-packaged miR-23a-3p impairs maintenance and angiogenic capacity of human endothelial progenitor cells in neonatal hyperoxia-induced lung injury. *Stem Cell Res Ther*. 2024;15(1):295. doi:10.1186/s13287-024-03920-z
71. Sani F, Shafiei F, Dehghani F, et al. Unveiling exosomes: cutting-edge isolation techniques and their therapeutic potential. *J Cell Mol Med*. 2024;28(20):e70139. doi:10.1111/jcmm.70139
72. Chavda VP, Luo G, Bezbaruah R, et al. Unveiling the promise: exosomes as game-changers in anti-infective therapy. *Exploration*. 2024;4 (5):20230139. doi:10.1002/EXP.20230139
73. Ashoub MH, Salavatipour MS, Kasgari FH, et al. Extracellular microvesicles: biologic properties, biogenesis, and applications in leukemia. *Mol Cell Biochem*. 2024;479(2):419–430. doi:10.1007/s11010-023-04734-y
74. Hu Z, Qian S, Zhao Q, et al. Engineering strategies for apoptotic bodies. *Smart Med*. 2024;3(3):e20240005. doi:10.1002/SMMD.20240005
75. Yu G, Ding J, Yang N, et al. Evaluating the pro-survival potential of apoptotic bodies derived from 2D- and 3D- cultured adipose stem cells in ischaemic flaps. *J Nanobiotechnology*. 2024;22(1):333. doi:10.1186/s12951-024-02533-1
76. Ekram S, Khalid S, Ramzan F, et al. Mesenchymal stem cell-derived extracellular vesicles protect rat nucleus pulposus cells from oxidative stress. *Cartilage*. 2024;15(3):328–344. doi:10.1177/19476035231172154
77. Zhang Y, Tang Y, Chen X, et al. Therapeutic potential of miRNAs in placental extracellular vesicles in ovarian and endometrial cancer. *Hum Cell*. 2024;37(1):285–296. doi:10.1007/s13577-023-00986-4
78. Sha Z, Liu W, Jiang T, et al. Astragaloside IV induces the protective effect of bone marrow mesenchymal stem cells derived exosomes in acute myocardial infarction by inducing angiogenesis and inhibiting apoptosis. *Biotechnol Genet Eng Rev*. 2024;40(3):1438–1455. doi:10.1080/02648725.2023.2194087

79. Shao Z, Xu J, Xu X, et al. Exosomes derived from human adipose mesenchymal stem cells inhibits fibrosis and treats oral submucous fibrosis via the miR-181a-5p/Smad2 axis. *Tissue Eng Regen Med*. 2024;21(1):123–135. doi:10.1007/s13770-023-00579-0
80. Bobis-Wozowicz S, Paw M, Sarna M, et al. Hypoxic extracellular vesicles from hiPSCs protect cardiomyocytes from oxidative damage by transferring antioxidant proteins and enhancing Akt/Erk/NRF2 signaling. *Cell Commun Signal*. 2024;22(1):356. doi:10.1186/s12964-024-01722-7
81. Chanda D, Del Rivero T, Ghimire R, et al. Acellular human amniotic fluid-derived extracellular vesicles as novel anti-inflammatory therapeutics against SARS-CoV-2 infection. *Viruses*. 2024;16(2):273. doi:10.3390/v16020273
82. Li H, Xia M, Zheng S, et al. Cerebrospinal fluid exosomal microRNAs as biomarkers for diagnosing or monitoring the progression of non-small cell lung cancer with leptomeningeal metastases. *Biotechnol Genet Eng Rev*. 2024;40(1):359–380. doi:10.1080/02648725.2023.2183613
83. Zhu L, Lou Y, Xiao Q, et al. Establishment and evaluation of exosomes-related gene risk model in hepatocellular carcinoma. *Biochem Genet*. 2024;62(2):698–717. doi:10.1007/s10528-023-10441-6
84. Wagner V, Kern F, Hahn O, et al. Characterizing expression changes in noncoding RNAs during aging and heterochronic parabiosis across mouse tissues. *Nat Biotechnol*. 2024;42(1):109–118. doi:10.1038/s41587-023-01751-6
85. Ramos-Zaldivar HM, Polakovicova I, Salas-Huenuleo E, et al. The cervical and meningeal lymphatic network as a pathway for retrograde nanoparticle transport to the brain. *Int J Nanomed*. 2024;19:10725–10743. doi:10.2147/IJN.S477159
86. Yu Z, Teng Y, Yang J, et al. The role of exosomes in adult neurogenesis: implications for neurodegenerative diseases. *Neural Regen Res*. 2024;19(2):282–288. doi:10.4103/1673-5374.379036
87. Rudraprasad D, V K, Nirmal J, et al. Complement Cascade 8 - Alpha and Calpain-2 in extracellular vesicles of human vitreous as biomarkers of infectious endophthalmitis. *Transl Vis Sci Technol*. 2024;13(5):14. doi:10.1167/tvst.13.5.14
88. Duan H, Meng F, Liu X, et al. Extracellular vesicles from Candida albicans modulate immune cells function and play a protective role in fungal keratitis. *Microb Pathog*. 2024;189:106606. doi:10.1016/j.micpath.2024.106606
89. Joseph A, Anton L, Guan Y, et al. Extracellular vesicles from vaginal Gardnerella vaginalis and Mobiluncus mulieris contain distinct proteomic cargo and induce inflammatory pathways. *NPJ Biofilms Microbiomes*. 2024;10(1):28. doi:10.1038/s41522-024-00502-y
90. Eldahshoury MK, Katsarou K, Farley JT, et al. Isolation of small extracellular vesicles (sEVs) from the apoplastic wash fluid of nicotiana benthamiana Leaves. *Curr Protoc*. 2024;4(11):e70026. doi:10.1002/cpz1.70026
91. Raposo G, Stahl PD. Extracellular vesicles, genetic programmers. *Nat Cell Biol*. 2024;26(1):22–23. doi:10.1038/s41556-023-01277-8
92. Yu D, Mei Y, Wang L, et al. Nano-seq analysis reveals different functional tendency between exosomes and microvesicles derived from hUMSC. *Stem Cell Res Ther*. 2023;14(1):272. doi:10.1186/s13287-023-03491-5
93. Glitscher M, Spannaus IM, Behr F, et al. The protease domain in HEV pORF1 mediates the replicase's localization to multivesicular bodies and its exosomal release. *Cell Mol Gastroenterol Hepatol*. 2024;17(4):589–605. doi:10.1016/j.jcmgh.2024.01.001
94. Sheykhhasan M, Heidari F, Farsani ME, et al. Dual role of exosome in neurodegenerative diseases: a review study. *Curr Stem Cell Res Ther*. 2024;19(6):852–864. doi:10.2174/1574888X18666230726161035
95. Ao M, Ma H, Guo M, et al. Research hotspots and emerging trends in mesenchymal stem/stromal cells in bronchopulmonary dysplasia. *Hum Cell*. 2024;37(2):381–393. doi:10.1007/s13577-023-01018-x
96. Kan C, Tan Z, Liu L, et al. Phase separation of SHP2E76K promotes malignant transformation of mesenchymal stem cells by activating mitochondrial complexes. *JCI Insight*. 2024;9(8). doi:10.1172/jci.insight.170340
97. Kaviani M, Soleimanian S, Keshtkar S, et al. Molecular prospective on malignant transformation of mesenchymal stem cells: an issue in cell therapy. *Cell Reprogram*. 2024;26(3):96–106. doi:10.1089/cell.2024.0026
98. Shan XQ, Luo YY, Chang J, et al. Immunomodulation: the next target of mesenchymal stem cell-derived exosomes in the context of ischemic stroke. *World J Stem Cells*. 2023;15(3):52–70. doi:10.4252/wjsc.v15.i3.52
99. Chen X, Qian W, Zhang Y, et al. Ginsenoside CK cooperates with bone mesenchymal stem cells to enhance angiogenesis post-stroke via GLUT1 and HIF-1alpha/VEGF pathway. *Phytother Res*. 2024;38(8):4321–4335. doi:10.1002/ptr.8235
100. Fei Z, Zheng J, Zheng X, et al. Engineering extracellular vesicles for diagnosis and therapy. *Trends Pharmacol Sci*. 2024;45(10):931–940. doi:10.1016/j.tips.2024.08.007
101. Dilsiz N. A comprehensive review on recent advances in exosome isolation and characterization: toward clinical applications. *Transl Oncol*. 2024;50:102121. doi:10.1016/j.tranon.2024.102121
102. Zeng YB, Deng X, Shen LS, et al. Advances in plant-derived extracellular vesicles: isolation, composition, and biological functions. *Food Funct*. 2024;15(23):11319–11341. doi:10.1039/d4fo04321a
103. Brezgin S, Danilik O, Yudaeva A, et al. Basic guide for approaching drug delivery with extracellular vesicles. *Int J Mol Sci*. 2024;25(19):10401. doi:10.3390/ijms251910401
104. Rivero-Pino F, Marquez-Paradas E, Montserrat-de la paz S. Food-derived vesicles as immunomodulatory drivers: current knowledge, gaps, and perspectives. *Food Chem*. 2024;457:140168. doi:10.1016/j.foodchem.2024.140168
105. Saneh H, Wanczyk H, Walker J, et al. Stem cell-derived extracellular vesicles: a potential intervention for bronchopulmonary dysplasia. *Pediatr Res*. 2024;97(2):497–509. doi:10.1038/s41390-024-03471-2
106. Ge K, Ren Y, Hong Z, et al. Microchip based isolation and drug delivery of patient-derived extracellular vesicles against their homologous tumor. *Adv Healthc Mater*. 2024;13(30):e2401990. doi:10.1002/adhm.202401990
107. Kumar S, Sinclair JA, Shi T, et al. Immunojanus particles for low-volume and isolation-free unlabeled characterization of small extracellular vesicle in biofluids: characterization of disease type by surface marker profiling. *bioRxiv*. 2024. doi:10.1101/2024.08.17.607528
108. Henriquez T, Santoro F, Medaglini D, et al. Analysis of the utility of a rapid vesicle isolation method for clinical strains of Pseudomonas aeruginosa. *Microbiol Spectr*. 2024;12(10):e0064924. doi:10.1128/spectrum.00649-24
109. Su N, Zhang J, Liu W, et al. Specific isolation and quantification of PD-L1 positive tumor derived exosomes for accurate breast cancer discrimination via aptamer-functionalized magnetic composites and SERS immunoassay. *Talanta*. 2025;281:126956. doi:10.1016/j.talanta.2024.126956
110. Gurriaran-Rodriguez U, De Repentigny Y, Kothary R, et al. Isolation of small extracellular vesicles from regenerating muscle tissue using tangential flow filtration and size exclusion chromatography. *Skelet Muscle*. 2024;14(1):22. doi:10.1186/s13395-024-00355-1

111. Wang L, Gong Z, Wang M, et al. Rapid and unbiased enrichment of extracellular vesicles via a meticulously engineered peptide. *Bioact Mater*. 2025;43:292–304. doi:10.1016/j.bioactmat.2024.09.023
112. Tsering T, Nadeau A, Rak J, et al. Analyzing extracellular vesicle-associated DNA using transmission electron microscopy at the single EV-level. *Curr Protoc*. 2024;4(11):e70047. doi:10.1002/cpz1.70047
113. Jeran M, Romolo A, Spasovski V, et al. Small cellular particles from european spruce needle homogenate. *Int J Mol Sci*. 2023;24(5):4349. doi:10.3390/ijms24054349
114. Zhang H, Xia J, Wang X, et al. Recent progress of exosomes in hematological malignancies: pathogenesis, diagnosis, and therapeutic strategies. *Int J Nanomed*. 2024;19:11611–11631. doi:10.2147/IJN.S479697
115. Cheng T, Mao M, Liu Y, et al. The potential therapeutic effect of human umbilical cord mesenchymal stem cell-derived exosomes in bronchopulmonary dysplasia. *Life Sci*. 2024;357:123047. doi:10.1016/j.lfs.2024.123047
116. Baruah H, Sarma A, Basak D, et al. Exosome: from biology to drug delivery. *Drug Deliv Transl Res*. 2024;14(6):1480–1516. doi:10.1007/s13346-024-01515-y
117. Fang X, Zhang Y, Zhang Y, et al. Endothelial extracellular vesicles: their possible function and clinical significance in diabetic vascular complications. *J Transl Med*. 2024;22(1):944. doi:10.1186/s12967-024-05760-0
118. Berdiaki A, Neagu M, Tzanakakis P, et al. Extracellular matrix components and mechanosensing pathways in health and disease. *Biomolecules*. 2024;14(9). doi:10.3390/biom14091186
119. Luo L, Wang Z. Negatively charged Cu(1.33)S nanochains: endocytic pathway, photothermal therapy and toxic effect in vivo. *Nanoscale Adv*. 2023;5(6):1706–1713. doi:10.1039/d2na00776b
120. Ransom MA, Bunn KE, Negretti NM, et al. Developmental trajectory of extracellular vesicle characteristics from the lungs of preterm infants. *Am J Physiol Lung Cell Mol Physiol*. 2023;324(3):L385–L392. doi:10.1152/ajplung.00389.2022
121. Shapira S, Ben Shimon M, Hay-Levi M, et al. A novel platform for attenuating immune hyperactivity using EXO-CD24 in COVID-19 and beyond. *EMBO Mol Med*. 2022;14(9):e15997. doi:10.15252/emmm.202215997
122. Yang Y, Zhu G, Yang L, et al. Targeting CD24 as a novel immunotherapy for solid cancers. *Cell Commun Signal*. 2023;21(1):312. doi:10.1186/s12964-023-01315-w
123. Grigoropoulos I, Tsioulos G, Kastrissianakis A, et al. The safety and potential efficacy of exosomes overexpressing CD24 (EXO-CD24) in mild-moderate COVID-19 related ARDS. *Respir Res*. 2024;25(1):151. doi:10.1186/s12931-024-02759-5
124. Lotfy A, AboQuella NM, Wang H. Mesenchymal stromal/stem cell (MSC)-derived exosomes in clinical trials. *Stem Cell Res Ther*. 2023;14(1):66. doi:10.1186/s13287-023-03287-7
125. Mazini L, Rochette L, Malka G. Exosomes contribution in COVID-19 patients' treatment. *J Transl Med*. 2021;19(1):234. doi:10.1186/s12967-021-02884-5
126. Zhu YG, Shi MM, Monsel A, et al. Nebulized exosomes derived from allogenic adipose tissue mesenchymal stromal cells in patients with severe COVID-19: a pilot study. *Stem Cell Res Ther*. 2022;13(1):220. doi:10.1186/s13287-022-02900-5
127. Bisaccia P, Magarotto F, D'Agostino S, et al. Extracellular vesicles from mesenchymal umbilical cord cells exert protection against oxidative stress and fibrosis in a rat model of bronchopulmonary dysplasia. *Stem Cells Transl Med*. 2024;13(1):43–59. doi:10.1093/stcltm/szad070
128. Lithopoulos MA, Strueby L, O'Reilly M, et al. Pulmonary and neurologic effects of mesenchymal stromal cell extracellular vesicles in a multifactorial lung injury model. *Am J Respir Crit Care Med*. 2022;205(10):1186–1201. doi:10.1164/rccm.202012-4520OC
129. Porzionato A, Zaramella P, Dedja A, et al. Intratracheal administration of mesenchymal stem cell-derived extracellular vesicles reduces lung injuries in a chronic rat model of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol*. 2021;320(5):L688–L704. doi:10.1152/ajplung.00148.2020
130. Wu Y, Li J, Yuan R, et al. Bone marrow mesenchymal stem cell-derived exosomes alleviate hyperoxia-induced lung injury via the manipulation of microRNA-425. *Arch Biochem Biophys*. 2021;697:108712. doi:10.1016/j.abb.2020.108712
131. You J, Zhou O, Liu J, et al. Human umbilical cord mesenchymal stem cell-derived small extracellular vesicles alleviate lung injury in rat model of bronchopulmonary dysplasia by affecting cell survival and angiogenesis. *Stem Cells Dev*. 2020;29(23):1520–1532. doi:10.1089/scd.2020.0156
132. Abele AN, Taglauer ES, Almeda M, et al. Antenatal mesenchymal stromal cell extracellular vesicle treatment preserves lung development in a model of bronchopulmonary dysplasia due to chorioamnionitis. *Am J Physiol Lung Cell Mol Physiol*. 2022;322(2):L179–L190. doi:10.1152/ajplung.00329.2021
133. Mitchell A, Wanczyk H, Jensen T, et al. Human induced pluripotent stem cells ameliorate hyperoxia-induced lung injury in a mouse model. *Am J Transl Res*. 2020;12(1):292–307.
134. Xu W, Jieda X, Wu Y, et al. Safety, efficacy and bio-distribution analysis of exosomes derived from human umbilical cord mesenchymal stem cells for effective treatment of bronchopulmonary dysplasia by intranasal administration in mice model. *Int J Nanomed*. 2025;20:2521–2553. doi:10.2147/IJN.S501843
135. Wang J, Zhang A, Huang F, et al. MSC-EXO and tempol ameliorate bronchopulmonary dysplasia in newborn rats by activating HIF-1alpha. *Pediatr Pulmonol*. 2023;58(5):1367–1379. doi:10.1002/ppul.26317
136. Tieu A, Hu K, Gnyra C, et al. Mesenchymal stromal cell extracellular vesicles as therapy for acute and chronic respiratory diseases: a meta-analysis. *J Extracell Vesicles*. 2021;10(12):e12141. doi:10.1002/jev2.12141
137. Braun RK, Chetty C, Balasubramaniam V, et al. Intraperitoneal injection of MSC-derived exosomes prevent experimental bronchopulmonary dysplasia. *Biochem Biophys Res Commun*. 2018;503(4):2653–2658. doi:10.1016/j.bbrc.2018.08.019
138. Porzionato A, Zaramella P, Dedja A, et al. Intratracheal administration of clinical-grade mesenchymal stem cell-derived extracellular vesicles reduces lung injury in a rat model of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol*. 2019;316(1):L6–L19. doi:10.1152/ajplung.00109.2018
139. Zhou Y, Liu Y, Xu G, et al. Human breast milk-derived exosomes through inhibiting AT II cell apoptosis to prevent bronchopulmonary dysplasia in rat lung. *J Cell Mol Med*. 2022;26(15):4169–4182. doi:10.1111/jcmm.17334
140. Ai D, Shen J, Sun J, et al. Mesenchymal stem cell-derived extracellular vesicles suppress hyperoxia-induced transdifferentiation of rat alveolar type 2 epithelial cells. *Stem Cells Dev*. 2022;31(3–4):53–66. doi:10.1089/scd.2021.0256

141. Zhu D, Krause M, Yawno T, et al. Assessing the impact of gestational age of donors on the efficacy of amniotic epithelial cell-derived extracellular vesicles in experimental bronchopulmonary dysplasia. *Stem Cell Res Ther*. 2022;13(1):196. doi:10.1186/s13287-022-02874-4
142. Li X, Huang L, Mao M, et al. HucMSCs-derived exosomes promote lung development in premature birth via Wnt5a/ROCK1 axis. *Stem Cell Rev Rep*. 2025;21(2):520–535. doi:10.1007/s12015-024-10824-1
143. Zhou O, You J, Xu X, et al. Microvesicles derived from human umbilical cord mesenchymal stem cells enhance alveolar type II cell proliferation and attenuate lung inflammation in a rat model of bronchopulmonary dysplasia. *Stem Cells Int*. 2022;2022:8465294. doi:10.1155/2022/8465294
144. Starke N, Challa NVD, Yuan H, et al. Extracellular vesicle ASC: a novel mediator for lung-brain axis in preterm brain injury. *Am J Respir Cell Mol Biol*. 2024;71(4):464–480. doi:10.1165/rcmb.2023-0402OC
145. Wu J, Huang QM, Liu Y, et al. Long-term hypoxic hUCMSCs-derived extracellular vesicles alleviates allergic rhinitis through triggering immunotolerance of their VEGF-mediated inhibition of dendritic cells maturation. *Int Immunopharmacol*. 2023;124(Pt B):110875. doi:10.1016/j.intimp.2023.110875
146. Sun M, Li M, Hu M, et al. Fully bioactive nanodrugs: stem cell-derived exosomes engineered with biomacromolecules to treat CCl(4)- and extreme hepatectomy-induced acute liver failure. *ACS Nano*. 2024;18(50):33907–33921. doi:10.1021/acsnano.4c07408
147. Qiu AW, Wang NY, Yin WJ, et al. Retinal muller cell-released exosomal MiR-92a-3p delivers Interleukin-17A signal by targeting notch-1 to promote diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2025;66(1):1. doi:10.1167/iovs.66.1.1
148. Yu L, He R, Liu C, et al. Circulating microvesicles miR139-3p from bronchopulmonary dysplasia aggravates pulmonary vascular simplification by targeting 4E binding protein 1. *J Gene Med*. 2024;26(2):e3675. doi:10.1002/jgm.3675
149. Yi X, Wei X, Lv H, et al. Exosomes derived from microRNA-30b-3p-overexpressing mesenchymal stem cells protect against lipopolysaccharide-induced acute lung injury by inhibiting SAA3. *Exp Cell Res*. 2019;383(2):111454. doi:10.1016/j.yexcr.2019.05.035
150. Willis GR, Fernandez-Gonzalez A, Anastas J, et al. Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. *Am J Respir Crit Care Med*. 2018;197(1):104–116. doi:10.1164/rccm.201705-0925OC
151. Li H, Ma K, Dou H, et al. CircABPD1 alleviates oxidative lung injury of bronchopulmonary dysplasia through regulating miR-330-3p/HIF1alpha axis. *Int J Biochem Cell Biol*. 2023;163:106464. doi:10.1016/j.biocel.2023.106464
152. Tolomeo AM, Zuccolotto G, Malvicini R, et al. Biodistribution of intratracheal, intranasal, and intravenous injections of human mesenchymal stromal cell-derived extracellular vesicles in a mouse model for drug delivery studies. *Pharmaceutics*. 2023;15(2):548. doi:10.3390/pharmaceutics15020548
153. Tieu A, Stewart DJ, Chwastek D, et al. Biodistribution of mesenchymal stromal cell-derived extracellular vesicles administered during acute lung injury. *Stem Cell Res Ther*. 2023;14(1):250. doi:10.1186/s13287-023-03472-8
154. Royo F, Cossio U, Ruiz de Angulo A, et al. Modification of the glycosylation of extracellular vesicles alters their biodistribution in mice. *Nanoscale*. 2019;11(4):1531–1537. doi:10.1039/c8nr03900c
155. Yang S, Liu P, Gao T, et al. Every road leads to Rome: therapeutic effect and mechanism of the extracellular vesicles of human embryonic stem cell-derived immune and matrix regulatory cells administered to mouse models of pulmonary fibrosis through different routes. *Stem Cell Res Ther*. 2022;13(1):163. doi:10.1186/s13287-022-02839-7
156. Guo Q, Zhao C, Gao X, et al. One-minute iodine isotope labeling technology enables noninvasive tracking and quantification of extracellular vesicles in tumor lesions and intact animals. *Mol Pharm*. 2023;20(7):3672–3682. doi:10.1021/acs.molpharmaceut.3c00299
157. Kang M, Jordan V, Blenkiron C, et al. Biodistribution of extracellular vesicles following administration into animals: a systematic review. *J Extracell Vesicles*. 2021;10(8):e12085. doi:10.1002/jev2.12085
158. Zou Z, Li H, Xu G, et al. Current knowledge and future perspectives of exosomes as nanocarriers in diagnosis and treatment of diseases. *Int J Nanomed*. 2023;18:4751–4778. doi:10.2147/IJN.S417422
159. Du W, Chen C, Liu Y, et al. A combined "eat me/don't eat me" strategy based on exosome for acute liver injury treatment. *Cell Rep Med*. 2025:102033. doi:10.1016/j.xcrm.2025.102033.
160. Xi Y, Ju R, Wang Y. Mesenchymal stem cell-derived extracellular vesicles for the treatment of bronchopulmonary dysplasia. *Front Pediatr*. 2022;10:852034. doi:10.3389/fped.2022.852034
161. Ren X, Wang S, Teng Y, et al. Engineered extracellular vesicles loaded in boronated cyclodextrin framework for pulmonary delivery. *Carbohydr Polym*. 2025;352:123160. doi:10.1016/j.carbpol.2024.123160
162. Zou T, Lu J, Zhu Y, et al. Mesenchymal stem cell-derived exosomes improved septic lung injury by reducing excessive NETs formation and alleviating inflammatory response. *Allergol Immunopathol*. 2025;53(1):63–68. doi:10.15586/aei.v53i1.1238
163. Lin YK, Pan YF, Jiang TY, et al. Blocking the SIRPalpha-CD47 axis promotes macrophage phagocytosis of exosomes derived from visceral adipose tissue and improves inflammation and metabolism in mice. *J Biomed Sci*. 2025;32(1):31. doi:10.1186/s12929-025-01124-y
164. Li Y, Yu B, Li H, et al. Human milk exosome-derived circDNAJB6 improves bronchopulmonary dysplasia model by promoting DNAJB6 gene transcription. *J Bioenerg Biomembr*. 2024;56(2):171–180. doi:10.1007/s10863-024-10002-5
165. Chaubey S, Thueson S, Ponnalagu D, et al. Early gestational mesenchymal stem cell secretome attenuates experimental bronchopulmonary dysplasia in part via exosome-associated factor TSG-6. *Stem Cell Res Ther*. 2018;9(1):173. doi:10.1186/s13287-018-0903-4
166. Zhong XQ, Hao TF, Zhu QJ, et al. Umbilical cord blood exosomes from very preterm infants with bronchopulmonary dysplasia aggravate lung injury in mice. *Sci Rep*. 2023;13(1):8648. doi:10.1038/s41598-023-35620-8
167. Fonteles CSR, Enterria-Rosales J, Lin Y, et al. Amniotic fluid-derived stem cells: potential factories of natural and mimetic strategies for congenital malformations. *Stem Cell Res Ther*. 2024;15(1):466. doi:10.1186/s13287-024-04082-8
168. Valiukevicius P, Maciulaitis J, Pangonyte D, et al. Human placental mesenchymal stem cells and derived extracellular vesicles ameliorate lung injury in acute respiratory distress syndrome murine model. *Cells*. 2023;12(23):2729. doi:10.3390/cells12232729
169. Zhang C, Zhang H, Millan Cotto HA, et al. Milk exosomes anchored with hydrophilic and zwitterionic motifs enhance mucus permeability for applications in oral gene delivery. *Biomater Sci*. 2024;12(3):634–649. doi:10.1039/d3bm01089a
170. Su X, Wang H, Li Q, et al. Extracellular vesicles: a review of their therapeutic potentials, sources, biodistribution, and administration routes. *Int J Nanomed*. 2025;20:3175–3199. doi:10.2147/IJN.S502591

171. Martin C, Patel M, Williams S, et al. Human breast milk-derived exosomes attenuate cell death in intestinal epithelial cells. *Innate Immun*. 2018;24(5):278–284. doi:10.1177/1753425918785715
172. Wang X, Yan X, Zhang L, et al. Identification and peptidomic profiling of exosomes in preterm human milk: insights into necrotizing enterocolitis prevention. *Mol Nutr Food Res*. 2019;63(13):e1801247. doi:10.1002/mnfr.201801247
173. Wang L, Xiao J, Zhang B, et al. Epigenetic modifications in the development of bronchopulmonary dysplasia: a review. *Pediatr Res*. 2024. doi:10.1038/s41390-024-03167-7
174. Zhong XQ, Yan Q, Chen ZG, et al. Umbilical cord blood-derived exosomes from very preterm infants with bronchopulmonary dysplasia impaired endothelial angiogenesis: roles of exosomal MicroRNAs. *Front Cell Dev Biol*. 2021;9:637248. doi:10.3389/fcell.2021.637248
175. Saneh H, Wanczyk H, Walker J, et al. Effectiveness of extracellular vesicles derived from hiPSCs in repairing hyperoxia-induced injury in a fetal murine lung explant model. *Stem Cell Res Ther*. 2024;15(1):80. doi:10.1186/s13287-024-03687-3
176. Wang W, Guo J, Wang Y. MicroRNA-30b-5p promotes the proliferation and migration of human airway smooth muscle cells induced by platelet-derived growth factor by targeting phosphatase and tensin homolog deleted on chromosome ten. *Bioengineered*. 2021;12(1):3662–3673. doi:10.1080/21655979.2021.1950401
177. Shi MM, Yang QY, Monsel A, et al. Preclinical efficacy and clinical safety of clinical-grade nebulized allogenic adipose mesenchymal stromal cells-derived extracellular vesicles. *J Extracell Vesicles*. 2021;10(10):e12134. doi:10.1002/jev2.12134
178. Mizenko RR, Feaver M, Bozkurt BT, et al. A critical systematic review of extracellular vesicle clinical trials. *J Extracell Vesicles*. 2024;13(10):e12510. doi:10.1002/jev2.12510
179. Lesage F, Thebaud B. Mesenchymal stromal cell-derived extracellular vesicles for neonatal lung disease: tiny particles, major promise, rigorous requirements for clinical translation. *Cells*. 2022;11(7):1176. doi:10.3390/cells11071176
180. Costa-Ferro ZSM, Rocha GV, da Silva KN, et al. GMP-compliant extracellular vesicles derived from umbilical cord mesenchymal stromal cells: manufacturing and pre-clinical evaluation in ARDS treatment. *Cytotherapy*. 2024;26(9):1013–1025. doi:10.1016/j.jcyt.2024.04.074
181. Hu XM, Wang CC, Xiao Y, et al. Non-clinical safety evaluation of exosomes derived from human umbilical cord mesenchymal stem cells in cynomolgus monkeys. *Int J Nanomed*. 2024;19:4923–4939. doi:10.2147/IJN.S454438
182. Hmadeh S, Trimaille A, Matsushita K, et al. Human aortic stenotic valve-derived extracellular vesicles induce endothelial dysfunction and thrombogenicity through AT1R/NADPH Oxidases/SGLT2 Pro-oxidant pathway. *JACC Basic Transl Sci*. 2024;9(7):845–864. doi:10.1016/j.jacbts.2024.02.012
183. Matei AC, Antounians L, Zani A. Extracellular vesicles as a potential therapy for neonatal conditions: state of the art and challenges in clinical translation. *Pharmaceutics*. 2019;11(8):404. doi:10.3390/pharmaceutics11080404
184. Duran-Jara E, Del Campo M, Gutierrez V, et al. Lactadherin immunoblockade in small extracellular vesicles inhibits sEV-mediated increase of pro-metastatic capacities. *Biol Res*. 2024;57(1):1. doi:10.1186/s40659-023-00477-8
185. Yang Q, Luo Y, Ge P, et al. Emodin ameliorates severe acute pancreatitis-associated acute lung injury in rats by modulating exosome-specific miRNA expression profiles. *Int J Nanomed*. 2023;18:6743–6761. doi:10.2147/IJN.S428924
186. Yang Q, Yang J, Sun S, et al. Rhodojaponin III-Loaded chitosan derivatives-modified solid lipid nanoparticles for multimodal antinociceptive effects in vivo. *Int J Nanomed*. 2022;17:3633–3653. doi:10.2147/IJN.S362443
187. Revilla G, Al Qtaish N, Caruana P, et al. Lenvatinib-Loaded Poly(lactic-co-glycolic acid) nanoparticles with epidermal growth factor receptor antibody conjugation as a preclinical approach to therapeutically improve thyroid cancer with aggressive behavior. *Biomolecules*. 2023;13(11):1647. doi:10.3390/biom13111647
188. Xiang B, Zhang S, Zhao IS, et al. Microenvironmental modulation for therapeutic efficacy of extracellular vesicles. *Adv Sci*. 2025;12(18):e2503027. doi:10.1002/advs.202503027
189. Thakur A, Rai D. Global requirements for manufacturing and validation of clinical grade extracellular vesicles. *J Liq Biopsy*. 2024;6:100278. doi:10.1016/j.jlb.2024.100278
190. Dogan AB, Marsh SR, Tschetter RJ, et al. Stabilizing milk-derived extracellular vesicles (mEVs) through lyophilization: a novel trehalose and tryptophan formulation for maintaining structure and Bioactivity during long-term storage. *J Biol Eng*. 2025;19(1):4. doi:10.1186/s13036-024-00470-z
191. Mehanny M, Boese A, Bornamehr B, et al. Spray-dried pneumococcal membrane vesicles are promising candidates for pulmonary immunization. *Int J Pharm*. 2022;621:121794. doi:10.1016/j.ijpharm.2022.121794
192. Jansen EM, van der Koog L, Elferink RAB, et al. Stabilized extracellular vesicle formulations for inhalable dry powder development. *Small*. 2025:e2411096. doi:10.1002/smll.202411096.
193. Zheng M, Zhu W, Gao F, et al. Novel inhalation therapy in pulmonary fibrosis: principles, applications and prospects. *J Nanobiotechnol*. 2024;22(1):136. doi:10.1186/s12951-024-02407-6
194. Gul F, Gonen ZB, Jones OY, et al. A pilot study for treatment of severe COVID-19 pneumonia by aerosolized formulation of convalescent human immune plasma exosomes (ChipEXO). *Front Immunol*. 2022;13:963309. doi:10.3389/fimmu.2022.963309
195. Luo X, Wang Y, Mao Y, et al. Nebulization of hypoxic hUCMSC-EVs attenuates airway epithelial barrier defects in chronic asthma mice by transferring CAV-1. *Int J Nanomed*. 2024;19:10941–10959. doi:10.2147/IJN.S476151
196. Changsan N, Atipairin A, Muenraya P, et al. In vitro evaluation of colistin conjugated with chitosan-capped gold nanoparticles as a possible formulation applied in a metered-dose inhaler. *Antibiotics*. 2024;13(7):630. doi:10.3390/antibiotics13070630
197. Rashidipour M, Rasoulian B, Maleki A, et al. Pectin/chitosan/tripolyphosphate encapsulation protects the rat lung from fibrosis and apoptosis induced by paraquat inhalation. *Pestic Biochem Physiol*. 2021;178:104919. doi:10.1016/j.pestbp.2021.104919
198. Green O, Shenberg G, Baruch R, et al. Inhaled exosomes genetically manipulated to overexpress CD24 (EXO-CD24) as a compassionate use in severe ARDS patients. *Biomedicines*. 2023;11(9):2523. doi:10.3390/biomedicines11092523
199. Shapira S, Schwartz R, Tsiodras S, et al. Inhaled CD24-enriched exosomes (EXO-CD24) as a novel immune modulator in respiratory disease. *Int J Mol Sci*. 2023;25(1):77. doi:10.3390/ijms25010077