Journal of Biomedical Research

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Cite this article as:
Wen Xi Goh, Chiew Yen Wong, Yih Yih Kok. Therapeutic small extracellular vesicles from key human stem and immune cellular sources: A review[J]. *Journal of Biomedical Research*, In press. doi: 10.7555/JBR.39.20250424

View online: https://doi.org/10.7555/JBR.39.20250424

Available online at www.jbr-pub.org.cn

Open Access at PubMed Central

Journal of Biomedical Research, 2026 40(0): 1–22

JBR

Review Article

Therapeutic small extracellular vesicles from key human stem and immune cellular sources: A review

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Abstract

Cell-to-cell communication is fundamental to life, with extracellular vesicles (EVs) playing a key role in intercellular signaling. Among them, small EVs (sEVs) have gained increasing attention as next-generation therapeutics. This review provides an overview of sEVs as therapeutic agents, comparing their advantages with cell-based and liposomal therapies, including unique benefits such as their enhanced ability to traverse biological barriers, ease of storage and administration, and their capability to utilize cellular machinery for fine-tuning therapeutic effects. Additionally, the therapeutic potential of sEVs from common human cell sources, including stem cells and immune cells, is discussed, highlighting their roles in modulating signaling pathways, cellular responses, and the microenvironment for treating cardiovascular, orthopedic, neurological, and autoimmune diseases, as well as cancer. Despite their promising outlook, challenges such as heterogeneity, extensive quality control requirements, systemic clearance, and scale-up limitations hinder clinical translation. Nonetheless, advancements in assay development, microfluidic models, computational databases, and bioengineering strategies continue to drive sEV-based therapies toward clinical and commercial viability.

Keywords: mesenchymal stem cells, dendritic cells, T cells, NK cells, exosomes

Introduction

It is well known that tightly regulated intercellular communication is fundamental to proper coordination of cell functions in all organisms, particularly multicellular ones. Often, such communication involves interactions between soluble factors (*e.g.*, cytokines, chemokines, hormones) secreted by one cell and specific receptors on another, or direct cell-to-cell contact[1]. However, the understanding of intercellular communication in mammalian cells has been reshaped during the last decade[1] with the introduction of novel signaling pathways that involve lesser-known players such as cytonemes[2], tunneling nanotubes[3], and bioelectrical potentials[4]. With accumulating evidence suggesting the biological significance of genetic material exchange in cellular communication[5], extracellular vesicles (EVs), a component of the secretome that harbours this genetic information (regulatory RNA and DNA) alongside various proteins, lipids, and metabolites, have become an intense area of study[6].

According to the latest guidelines set by the International Society for Extracellular Vesicles

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Received: 01 October 2025; Revised: 01 March 2026; Accepted: 02 March 2026;

CLC number: R329.2, Document code: A

The authors reported no conflict of interests.

https://doi.org/10.7555/JBR.39.20250424

(ISEV), 'EVs' is a term that describes non-replicating, phospholipid bilayer-enclosed vesicles generated by cells, which can be primarily classified into three major categories based on their biogenesis: apoptotic bodies, ectosomes, and exosomes[7]. Among these, apoptotic bodies (50–5 000 nm) are the largest EVs, originating from blebbing apoptotic cells[8]. In contrast, the most studied EV subtypes are ectosomes (50–1 000 nm) and exosomes (30–150 nm), which are generated through outward budding of the plasma membrane and inward budding of the endosomal membrane, respectively[8,9].

Over the years, several methods for isolating EVs have emerged for research purposes, as extensively described in the literature[10,11]. Briefly, these approaches separate EVs from biological fluids or culture media based on their biophysical attributes (*e.g.*, size, density, shape, acoustic, and electrical properties) and biochemical composition[11]. According to a recent systematic review analyzing EV publications from 2012 to 2020, although ultracentrifugation alone remains the dominant 'gold standard' method (32.6% of included studies), there is growing awareness within the scientific community of using a 'cocktail strategy' to address the shortcomings of individual methods and combine their advantages[12]. Among combinatorial approaches, one systematic review highlighted that differential ultracentrifugation coupled with size exclusion chromatography provides EV preparations with excellent purity at a reasonable yield[13]. Additionally, various promising EV isolation technologies are emerging. Notable examples include the EXODUS system[14], the size exclusion-fast protein liquid chromatography isolation system[15], and pre-treatments with chimeric nanocomposites[16] or copolymers[17], all of which offer improved isolation yield, purity, and efficiency while preserving EV integrity. However, there is no single best or one-size-fits-all method to date, and ISEV advises that the selection of the approach should depend on one's study objectives and design[7].

For characterization, there are no universal molecular markers shared among EVs regardless of their biological source[7]. Similarly, although tetraspanins (CD9, CD81, and CD63), ALIX, and TSG101 proteins are commonly considered specific markers for exosomes in the literature[10], they are present in all EVs[7]; there is generally consensus that there are no type-specific markers to distinguish different EV subtypes to date. Furthermore, EV subtypes such as exosomes and ectosomes share many overlapping attributes as mentioned in an earlier section[9]. Consequently, distinguishing EVs experimentally based on robust evidence of their subcellular origin is challenging. Despite recent improvements, biogenesis-related terms, particularly 'exosomes,' continue to be widely used in scientific journals as a generic descriptor for EVs[18]. In this review, the term 'small EVs (sEVs)' will be used to refer specifically to exosomes and subpopulations of ectosomes smaller than 200 nm[7]. These represent the main focus of our discussion, as they are the most intensively studied EV subtypes with potential for clinical applications[11].

sEVs can be produced by animals, plants, and microorganisms. However, plant-derived sEVs remain an emerging subfield that lacks established scalable purification and drug-loading strategies, and their clinical translation is further limited by the potential transmission of plant toxins and allergens[19,20]. Similarly, microbial sEVs face translational barriers due to their intrinsic immunotoxicity despite promising vaccine applications[21]. In comparison, human cell–derived sEVs present fewer safety concerns and are therefore better positioned for therapeutic development. Among them, mesenchymal stem cell-derived sEVs (MSC-sEVs) offer advantages such as broad source availability, potent immunomodulatory activity, and pro-regenerative properties[22]. Additionally, sEVs derived from immune cells are attractive as drug carriers and cancer vaccines because of their systemic compatibility, antigen-presenting capacity, and intrinsic chemotaxis[21]. These unique biological strengths form the basis for their selection as the key source categories discussed in this review.

Significance of small extracellular vesicles as a therapeutic

In this section, well-studied biotechnological approaches such as cell therapy and synthetic nanomedicine will first be discussed to provide an overview of their capabilities and limitations. Subsequently, a comparison with sEVs will be presented to highlight the advantages of sEVs as next-generation therapeutics (***Fig. 1***).

Cell therapy

Cell therapy is a medical approach that involves introducing living cells, as opposed to other common biological modalities (*e.g.*, antibodies, hormones, recombinant proteins), into a patient's body for therapeutic purposes. The earliest success in cell therapy occurred in 1906 when direct blood transfusion helped a patient who had experienced

Feature	Cells	Liposomes	sEVs
Risk of tumourigenecity	Yes (especially stem cells)	No (lack of nuclei)	No (lack of nuclei)
Blood-brain barrier transverse ability	Limited to some cells and disease conditions	Yes (with suitable surface modifications)	Yes
Ease of storage	Difficult (require cryopreservation)	Good	Good (can be lyophilised)
Native bioactive cargo	Yes	No (requires encapsulation)	Yes (inherent from parental cell)
Ease of scale-up	Moderate	High	Moderate
Need for functionalisation	Moderate	High	Moderate (inherent from parental cell)
Overall cost	High (cell culture, storage, and logistics)	Variable (may escalate with extensive functionalisation)	Lower (saves on storage and extensive functionalisation)

Favourable attribute
Satisfactory attribute
Unfavourable attribute

Fig. 1 **Comparative evaluation of cell-, liposome-, and small extracellular vesicle (sEV)-based therapeutics.** This figure summarizes some key considerations for the clinical translation of advanced therapeutics, integrating both biological functionality and practical utility. Attributes are categorized as favorable (green), satisfactory (yellow), or unfavorable (orange) based on current evidence. Among the three modalities, sEVs exhibit a highly favorable therapeutic profile, offering a balanced advantage over cell-based therapies and liposomes.

massive hemorrhage[23]. In recent years, despite stringent regulatory requirements for modern pharmaceuticals, several cellular therapeutics have been approved for commercial use in various areas, including oncology, dermatology, orthopedics, and ophthalmology[24,25]. Today, the field of cell therapy continues to grow exponentially, especially in cancer treatment[26]. This popularity of cell therapy compared with other established therapeutics stems from several characteristics of living cells and their *in vivo* responses post-administration.

As the third pillar of therapeutics, after small molecules and biologics, living cells provide unique solutions for therapeutic niches that demand dynamic yet precise control over the distribution and duration of therapeutic effects[27]. They achieve this by responding to molecular cues from the environment, making stimulus-directed decisions, and exhibiting complex behaviors, ultimately minimizing off-target effects[25,27]. For instance, stem cells in regenerative medicine first detect signals from injured tissues, then migrate to the target site, and subsequently secrete biomolecules that (i) promote the survival of damaged cells, (ii) stimulate proliferation of surviving cells, (iii) modulate immune cells to perform tissue repair-associated actions, and (iv) induce direct self-differentiation to repopulate injured tissue[28]. All of these functions are made possible by their naturally occurring cell surface receptors, intrinsic ability to express therapeutic transgenes, and capability to persist *in vivo*[25].

However, from a clinical perspective, a major concern of any form of cell therapy is the potential tumorigenicity of administered cells[25]. Although teratoma formation is inherent to pluripotent cells, the risk of tumor formation in other cell types still exists due to genetic instability and possible accidental activation of tumorigenic pathways during cell manufacturing processes[29]. Furthermore, despite ongoing efforts to elucidate tumorigenicity through innovative methods[30], current *in vivo* models present limitations for assessing the long-term effects of cell therapy; their short lifespan and practical challenges in administering clinically relevant cell dosages hinder the ability to draw meaningful conclusions about tumorigenicity[29,31]. From a commercialization perspective, scaling up production to generate cells identical in characteristics and therapeutic potency to those used in early clinical research, establishing distribution logistics to maintain maximum cell viability given their short shelf life at ambient temperatures, and keeping costs low, are all challenging issues that need to be addressed urgently to achieve commercial favorability[32].

Synthetic nanomedicine

In medicine, the therapeutic utility and administration routes of many potent drugs are limited by poor pharmacokinetic characteristics, such as low solubility, permeability, and bioavailability[33]. Meanwhile, conventional drug delivery systems (*e.g.*, tablets, capsules, syrups) offer little support to help pharmaceuticals overcome these problems, because they are characterized by a different set of issues, including uncontrolled immediate drug release, low drug solubility, and inadequate protection and

stabilization of drugs against physiological microenvironments. These limitations necessitate higher administration frequencies to achieve desired therapeutic effects, consequently increasing the risk of drug toxicity[34,35]. In this context, nanotechnology offers new opportunities to address these unmet medical needs by promoting the development of nanoparticles (NPs), which can serve both as therapeutics themselves or as drug carriers. In particular, lipid-based NPs such as liposomes represent the most prevalent category of NPs being studied in various translational investigations[36].

Like many NPs in general, liposomes can enhance the therapeutic efficacy and stability of drugs. Furthermore, they can traverse discontinuous microvasculature that is enriched in abnormal tissues, including tumor and inflammatory sites, enabling increased passive delivery of drugs to target sites with reduced return of fluids to the lymphatic circulation[37]. Additionally, compared to other advanced drug delivery systems, liposomes exhibit several outstanding properties, such as biocompatibility, biodegradability, colloidal stability, bilayer permeability, and ease of functionalization to (i) enable active targeting, (ii) improve drug circulation time in the blood, and (iii) aid in crossing difficult biological barriers such as the blood-brain barrier[38,39]. In terms of drug loading, both hydrophilic and hydrophobic drugs can be incorporated into liposomes due to their unique physiochemical characteristics, thus offering improved delivery of a diverse range of bioactive molecules[38].

However, the development of tools that can comprehensively assess the risks associated with the medical use of NPs is still in its infancy, lagging far behind the pace of novel NP development[40]. While safety risk assessment is important for any drug, this is especially critical for liposome-based formulations because liposomes possess innate immunogenicity, which can ultimately result in adverse effects including IgE-mediated allergies, complement activation-related pseudoallergies, and autoimmune diseases[41]. In addition, although numerous suitable methods for synthesizing liposomes at an industrial scale have been developed, the need for complex functionalization for optimal performance inevitably increases the steps and components required for complete formulation, resulting in increased fabrication cost[42].

Small extracellular vesicles

As a therapeutic, sEVs exhibit efficacies that closely resemble the cells from which they originate but provoke fewer unwanted effects alongside minimal immunogenicity[43]. One of the biggest benefits that sets sEVs apart from cells as therapeutics is that they lack nuclei, which is the basis of neoplastic transformation; hence, sEVs themselves are devoid of tumorigenicity risks[44]. In addition, sEVs demonstrate natural blood-brain barrier traversing ability under pathological conditions[45], making them valuable candidates for the treatment of neurodegenerative disorders. In contrast to immune cells, which can easily infiltrate injured or diseased brain[46], other therapeutic cells such as stem cells are much less efficient at transmigration through these barriers (requiring a 3–6-fold longer time than leukocytes)[47]. From a commercialization perspective, unlike cell therapy products that require robust cold chain transportation processes to create appropriate cryogenic conditions for optimal product storage during delivery to patients[48], sEVs can be lyophilized and stored at room temperature without compromising their structural and functional integrity[49]. Hence, they present a viable alternative that can circumvent the expensive costs of sophisticated cryopreservation facilities, reduce the cryopreservation-associated energy consumption and greenhouse gas emissions, and enable broader distribution and accessibility to consumers[50].

Similarly, sEVs possess many desirable properties that liposomes offer, such as the ability to improve the physicochemical characteristics, stability, and release profile of drugs; the tendency to migrate towards tumor and inflammatory sites *via* chemotaxis or tissue tropism; flexibility for modification to better suit *in vivo* microenvironments and facilitate drug delivery; as well as good inherent biocompatibility[45,51]. However, unlike synthetic nanomedicine, a major difference is that sEVs endogenously possess bioactive cargo, allowing them to naturally exert certain functions, including therapeutic benefits, without the need for drug encapsulation[52]. Moreover, if sEVs are to be used as nanocarriers, cellular machinery can be exploited for endogenous drug loading and surface modification, thereby minimizing degradation risks of RNA- and protein-based therapeutic components or targeting ligands, unlike those encountered during nanoparticle synthesis[53]. Furthermore, sEVs can employ endocytosis pathways or fusion events to facilitate intracellular delivery of compounds that have poor translocation spontaneity (*e.g.*, RNAs), enabling them to escape lysosomal pathways and reach targeted organelles[52,53]. In addition, recent studies have reported that sEVs can readily cross intact matrices despite being larger than

the mesh size through matrix mechanics-mediated EV transport and water channel-mediated EV deformability, a property that was not observed in liposomes of similar size[54]. Regarding industrial-scale applications of sEVs, their mass production is also easier than that of liposome-based therapeutics because the complex biological structure responsible for their favorable nanocarrier attributes is an inherent property of isolated EVs, eliminating the need for extensive functionalization processes that can significantly increase costs and disrupt compatibility with clinical-grade manufacturing[53].

Overall, given the aforementioned beneficial characteristics of sEVs as therapeutics compared to cell therapy and synthetic nanomedicine, it is evident that they combine the advantages of both approaches. Thus, sEVs and their potential therapeutic applications represent an exciting and worthy of exploration area, although many challenges remain to be addressed.

Important sources and therapeutic applications of human small extracellular vesicles

Stem cells

In September 2023, it was reported that of the 60 sEV-based interventional studies registered on ClinicalTrials.gov, 40 (66.7%) clinical trials utilized stem cell-derived sEVs[55]. Among them, the majority (95%) used MSC-sEVs, with the remainder using induced pluripotent stem cells (iPSCs) as the cellular source[55]. Human MSCs can be isolated from various sources, such as bone marrow (BM), adipose tissue (AT), blood, and breast milk in adults, as well as birth-associated tissues (*e.g.*, umbilical cord tissue [UC]) that are often treated as medical waste[56]. However, despite possessing many similarities in cellular characteristics, MSCs from different tissues exhibit different degrees of differentiation ability and stromal functions[57]. Given that many stromal functions, such as anti-inflammatory, immunomodulatory, and angiogenic properties of these MSCs, are attributed to their secretome, which encompasses sEVs, it is reasonable to hypothesize that sEVs from different tissues and cell origins may excel in different therapeutic applications. Some studies that directly compared properties and functions between human MSC-sEVs isolated from different sources are summarized in ***Table 1***.

Based on ***Table 1***, many studies have concluded that human AT-MSC-sEVs may have superior angiogenic effects compared to other sources, such as BM [58,60] and UC blood[66]. Proteomic profiling identified several proteins that were expressed only in AT-MSC-sEVs but not in those of BM origin, including Wnt proteins and growth factors such as FGF, PDGF, TGF-β[58]. Importantly, the Wnt signaling system plays a critical role in angiogenesis, whereby the canonical signaling pathway has been shown to expand vascular progenitor populations and promote the development of mature capillary-like tubes[69], whereas the non-canonical pathway coordinates mechanocoupling between endothelial cells, which is crucial for sprouting vascular morphogenesis[70]. Furthermore, interactions between canonical Wnt and TGF-β signaling pathways have been frequently reported, with the potential to activate one another[71], further enhancing angiogenesis, as TGF-β can promote its own expression while upregulating the most potent activators of angiogenesis, namely bFGF and VEGF[72,73]. In line with this, administration of AT-MSC-sEVs in myocardial infarction-induced rat models showed the highest microvascular density formation in heart tissue, and *in vitro* cardiomyocyte assays demonstrated the most significant increase in the expression of VEGF and bFGF compared to groups treated with other sEVs[66]. In this regard, AT-MSC-sEVs may hold greater promise than sEVs from alternative sources as therapeutics for wound healing as well as diseases that require extensive angiogenesis stimulation, such as ischemic heart disease and peripheral artery disease. Given their significant enrichment in ATP2B1 expression compared to BM- and UC-sourced MSC-sEVs[67], AT-MSC-sEVs may also aid in reducing susceptibility to ventricular arrhythmia, a common manifestation of ischemic heart disease, by regulating signaling pathways associated with cardiac rhythm[74].

On the other hand, BM-MSC-sEVs appear to demonstrate outstanding chondrogenic properties when compared with AT-MSC-sEVs[59,60]. In line with this, a donor-matched comparison of BM and AT MSCs showed that BM MSCs were not only superior in chondrogenic differentiation capacity but also in osteogenic differentiation capacity[75]. Proteomic analysis revealed that Notch2 was substantially expressed in BM-MSC-sEVs unlike in AT- and UC-MSC-sEVs[67]. Notably, the intracellular domain of Notch2 has been shown to play an important role in interacting with osteogenic-related gene promoters because its knockdown resulted in attenuated Jagged-1 activity[76], a potent bone-anabolic agent with reported therapeutic potential in healing traumatic or congenital bone defects[77]. Furthermore, BM-MSC-sEVs were

Table 1 **Comparative studies of human MSC-derived small extracellular vesicles from different sources as therapeutic interventions**

Sources being compared	Model organism/cells	Property being assessed	Therapeutic outcome(s)	Important finding(s)	Reference
BM *vs.* AT	*In vivo*: NSG mice; *In vitro*: Fibroblasts, keratinocytes, and endothelial cells	Wound healing ability on diabetic ulcers	*In vivo*: AT-MSC-sEVs promoted diabetic wound healing, whereas no difference was observed between control and groups treated with BM-MSC-sEVs. *In vitro*: AT-MSC-sEVs showed a higher effect on endothelial cell migration and formation of capillary-like structures. Only BM-MSC-sEVs were able to promote fibroblast, keratinocyte, and endothelial cell viability.	Bioinformatic analysis revealed that proteins highly enriched in AT-MSC-sEVs were highly correlated to angiogenesis pathways (Wnt, FGF, PDGF, TGF- β). Proteins enriched only in BM-MSC-sEVs were correlated to cell adhesion (integrin and cadherin) and metabolic processes (glycolysis and fructose galactose metabolism).	[58]
BM *vs.* AT	*In vivo*: BALB/c mice	Ability to ameliorate osteoarthritis	BM-MSC-sEVs treated group showed better improvement with significantly higher chondrogenesis process (grade 2) compared to AT-MSC-sEVs treated group (grade 3).	Both sEVs treatment groups increased expression of Sox9, collagen type II, and aggrecan genes to a similar extent and reduced expression of collagen type I. BM-MSC-sEVs may have higher chondrogenic potential than AT-MSC-sEVs.	[59]
BM *vs.* AT	*Ex vivo*: C57BL/6 mice fetus-derived metatarsal culture models	Endochondral ossification	AT-MSC-sEVs induced significantly higher increase in endothelial cord outgrowth. BM-MSC-sEVs prompted better growth plate organization.	AT-MSC-sEVs treated group exhibited higher number of $CD31^+$ cell outgrowth, representing sprouting angiogenesis. BM-MSC-sEVs contained a higher amount of pro-differentiation and chemotactic proteins to promote correct spatial organization of chondrocytes.	[60]
BM *vs.* AT *vs.* UC	*In vitro*: U87MG glioblastoma cell line	Ability to inhibit glioblastoma	BM- and UC-MSC-sEVs decreased proliferation and induced apoptosis in U87MG cells. AT-MSC-sEVs stimulated proliferation and did not affect viability of U87MG cells.	All sEVs were rapidly internalised by U87MG cells. AT-MSC-sEVs stimulated U87MG cells to enter S and G2/M phases, whereas BM- and UC-MSC-sEVs only induced sub-G1 phase.	[61]
BM *vs.* UC *vs.* Menstrual fluid *vs.* Chorion	*In vitro*: Embryonic Sprague Dawley rat-derived cortical neuron cultures and embryonic C57 mice-derived dorsal root ganglia cultures	Neuritic outgrowth	Menstrual- and BM-MSC-sEVs promoted neurite growth in both cortical and sensory neurons and were superior to other sources, which showed no changes compared to control.	Menstrual-MSC-sEVs treated groups had the longest cortical neurite length, whereas other sEVs did not alter neurite length. Only menstrual- and BM-MSC-sEVs increased rate of neuritic growth.	[62]
BM *vs.* Placenta	*In vitro*: human BM-MSCs	Osteogenic differentiation	BM- and Placental-MSC-sEVs treated groups showed overall similar osteogenic impact despite diversity in miRNA.	Placental MSCs grew at a significantly faster rate than BM MSCs and yielded more potent sEVs with much higher protein and lipid concentrations.	[63]
Adult BM *vs.* Foetal BM	*Ex vivo*: human UC blood $CD34^+$ cells	Expansion of hematopoietic stem and progenitor cells	Adult BM-MSC-sEVs showed better supportive expansion capacity of hematopoietic stem and progenitor cells than those of foetal origin.	Adult BM-MSC-sEVs were enriched in miR-125a and highly expressed proteins related to redox process, mitochondrial ATP synthesis coupled proton transport, or protein folding. Foetal BM-MSC-sEVs highly expressed proteins related to extracellular matrix organization, positive regulation of cell migration, or TGF-β receptor signaling pathway.	[64]

Table 1 **Comparative studies of human MSC-derived small extracellular vesicles from different sources as therapeutic interventions** (continued)

Sources being compared	Model organism/cells	Property being assessed	Therapeutic outcome(s)	Important finding(s)	Reference
BM *vs.* Dental pulp	*In vivo*: CAM Assay; *In vitro*: HUVECs	Angiogenic effects	*In vivo*: Only BM MSCs culture medium, not BM-MSC-sEVs treated groups, increased in ovo angiogenesis. *In vitro*: BM-MSC-sEVs had significantly stronger chemotactic effect than those of dental pulp origin.	No difference was observed in uptake rates of BM- and dental pulp-MSC-sEVs by HUVECs. Both BM- and dental pulp-MSC-sEVs contained several pro- and anti-angiogenic proteins.	[65]
BM *vs.* AT *vs.* UC blood	*In vivo*: Sprague Dawley rats; *In vitro*: Neonatal Sprague Dawley rat cardiomyocytes	Cardioprotection post-myocardial infarction	*In vivo*: All tested sEVs improved cardiac function and myocardium, but AT-MSC-sEVs had a superior effect. *In vitro*: All tested sEVs inhibited hypoxia-induced cardiomyocyte apoptosis and promoted angiogenesis, but AT-MSC-sEVs had a superior effect.	AT-MSC-sEVs treated groups showed the most significant reduction in cardiomyocyte apoptosis and infarction area while having the highest microvascular density in rat models. AT-MSC-sEVs treated groups had the highest expression of VEGF, bFGF, and HGF.	[66]
BM *vs.* AT *vs.* UC	None	Proteomic profiles	Shared proteins were concentrated in the extracellular matrix, anchoring junction, and adherent junction.	BM-MSC-sEVs were significantly enriched in ADAM9, ADAM10, CD81, CACNA2D1, NOTCH2, and HLA-A expression. AT-MSC-sEVs were significantly enriched in ATP2B1 and ATP1A1 expression and depleted in ITGA2 and LRP1 expression. UC-MSC-sEVs were significantly depleted in ITGB3 and SLC44A1 expression.	[67]
Adult BM *vs.* Foetal liver	None	Transcriptomic profiles	Both have similar transcriptomic profiles.	Foetal liver-MSC-sEVs contained significantly more proteins involved in pathways for collagen fibril organization, protein folding, and response to TGF-β compared to adult BM-MSC-sEVs.	[68]

Abbreviations: ADAM9, disintegrin and metalloproteinase domain-containing protein 9; ADAM10, disintegrin and metalloproteinase domain-containing protein 10; AT, adipose tissue; ATP, adenosine triphosphate; ATP1A1, sodium/potassium-transporting ATPase subunit alpha-1; ATP2B1, plasma membrane calcium-transporting ATPase 1; BALB/c, Bagg albino c; bFGF, basic fibroblast growth factor; BM, bone marrow; C57BL/6, C57 black 6; CACNA2D1, voltage-dependent calcium channel subunit alpha-2/delta-1; CAM, chicken chorioallantoic membrane; CD31, cluster of differentiation 31; CD34, cluster of differentiation 34; CD81, cluster of differentiation 81; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; HLA-A, human leukocyte antigen A; HUVECs, human umbilical vein endothelial cells; ITGA2, integrin alpha-2; ITGB3, integrin beta-3; LRP1, prolow-density lipoprotein receptor-related protein 1; NOTCH2, neurogenic locus notch homolog protein 2; NSG, non-obese diabetic severe combined immunodeficiency gamma; PDGF, platelet-derived growth factor; sEVs, small extracellular vesicles; SLC44A1, choline transporter-like protein 1; Sox9, SRY-box transcription factor 9; TGF-β, transforming growth factor-beta; U87MG, Uppsala 87 malignant glioma; UC, umbilical cord; VEGF, vascular endothelial growth factor; Wnt, wingless-related integration site.

also found to be enriched in ADAM10[67], a protein that has recently been acknowledged for its role in suppressing osteoclast differentiation[78]. Taken together, BM-MSC-sEVs might be potent in alleviating bone and joint diseases and bone loss-associated conditions like osteoporosis. In addition, BM-MSC-sEVs showed a chemotactic effect on chondrocytes and enabled a more defined organization of the growth plate in metatarsal bone explants, an observation that was not demonstrated by groups treated with AT-MSC-sEVs[60]. In the same study, urokinase-type plasminogen activator receptor (uPAR) was found to be significantly expressed in BM-MSC-sEVs. This may explain the coordination in chondrocyte orientation and position because uPAR plays a central role in extracellular matrix (ECM) remodelling and cells can respond to changes in the ECM *via* integrins, thereby activating interrelated cellular processes enabling cell survival, proliferation, differentiation, and cell migration[79,80]. Since progressive destruction of the pericellular matrix (a part of the ECM) and spatial chondrocyte rearrangement are two highly interconnected hallmarks of osteoarthritis[81], BM-MSC-sEVs might be a favorable choice for osteoarthritis treatment.

Beyond cartilage and bone diseases or disorders, BM-MSC-sEVs have shown superior effectiveness in promoting neuritic growth compared to MSC-sEVs sourced from UC and chorion[62]. It is worth mentioning that ADAM10, which is enriched in BM-MSC-sEVs, is one of two important components in α-secretase processing of amyloid precursor protein

(APP) to generate soluble APP alpha (sAPPα)[82], which in turn has the capacity to stimulate proliferation of adult neural progenitors, increase neurite outgrowth, and provide neuroprotection[83]. Notably, ADAM9, which is also abundant in BM-MSC-sEVs, is an indirect activator of ADAM10, thereby playing a role in the indirect modulation of sAPPα secretion[82]. In line with this, treatment of a clinically realistic swine model of traumatic brain injury and hemorrhagic shock with BM-MSC-sEVs has shown improved neurocognitive recovery[84]. Additionally, neuroprotective properties of BM-MSC-sEVs have also been reported in mice with middle cerebral artery occlusion *via* the reduction of immune infiltrates into the brain[85]. Another reported contribution of BM-MSC-sEVs to neuroprotection is *via* stimulation of the PI3K-Bcl-2 cell survival pathway in hippocampal cells and upregulation of host expression of neurotrophic factors under excitotoxicity conditions[86]. Based on these studies, BM-MSC-sEVs appear to be promising for treating various neurological diseases and injuries.

Overall, MSC-sEVs from different parts of the human body exhibit overlapping functionalities, including stimulating angiogenesis, promoting osteogenic and chondrogenic differentiation, coordinating cell spatial orientation, as well as supporting neurogenesis and providing neuroprotection. However, a deeper understanding of the distinct outstanding properties of MSC-sEVs isolated from different sources can help in making more well-informed decisions to optimize MSC-sEV combinations in cocktail prescriptions. For instance, AT-MSC-sEVs together with BM-MSC-sEVs at appropriate dosages may substantially enhance wound healing efficiency, as the former is more effective in acting on endothelial cells, thereby inducing angiogenesis, whereas the latter is better at promoting cell viability and proliferation in fibroblasts and keratinocytes[58,87]. In addition, enhanced knowledge of these differential effects can also aid in avoiding unexpected adverse events. As an example, since previous reports have observed that AT-MSC-sEVs stimulated glioblastoma cell proliferation without negatively affecting cell viability[61], their administration to patients with glioblastoma may be best avoided until the reason behind this phenomenon is elucidated. More comparative studies in the future, perhaps investigating the profiles of other sEV components (*e.g.*, miRNAs, tRNAs, and lipids), would undoubtedly be helpful in strengthening our knowledge of the unique advantages that each tissue-specific MSC-sEV offers and of their underlying mechanisms. Attention should also be given to less-studied sources of human MSC-sEVs, such as those of menstrual, placental, and dental pulp origin. These sources have demonstrated comparable or superior impact on neurite growth, osteogenic differentiation, and neuroprotection compared with the extensively researched BM-MSC-sEVs and are more accessible for harvesting from donors[62,63,86].

Immune cells

According to data from 2022, T cell therapy, with chimeric antigen receptor (CAR)-T therapies as the major representative (1 432 records), was leading the oncology cell therapy pipeline, followed by natural killer (NK) cell-based therapies (283 records), and finally, myeloid cell therapies (182 records) including dendritic cell (DC) and macrophage therapies[26]. However, in contrast to their success in treating hematological tumors, many applications in treating solid tumors were unsuccessful due to insufficient immune cell infiltration into tumor tissues and the influence of the immunosuppressive tumor microenvironment (TME)[88]. In this regard, immune cell-derived sEVs circumvent these challenges, as it has been reported that the acidic TME tends to facilitate sEV uptake by promoting their fusion with tumor cell membranes[89], and unlike their parental cells, sEVs are unresponsive to immunosuppressive cues in the TME[90]. In fact, the production and antitumor effects of sEVs derived from NK cells, for instance, have been shown to be enhanced under hypoxic conditions[91]. ***Table 2*** provides a glimpse into some studies that have investigated the role of human immune cell-derived sEVs in disease treatment.

Dendritic cell-derived small extracellular vesicles

Antigen presentation is an important process that bridges the innate and adaptive immune responses, enabling cancer vaccines to activate specific T cells for tumor regression. The main types of professional antigen-presenting cells include macrophages, B cells, and DCs, with DCs being the most potent among them. Beyond the well-described antigen presentation pathways that present endogenous and exogenous peptides to $CD8^+$ and $CD4^+$ T cells *via* MHC class I and II molecules, respectively, exogenous antigens can also be presented on MHC class I molecules through a process known as 'cross-presentation', which is most commonly observed in DCs *in vivo*[109,110].

Two decades ago, co-localization of both MHC class I and II molecules was observed on human DC-derived sEVs (DC-sEVs), and the authors speculated

Table 2 **Studies investigating human immune cell-derived small extracellular vesicles as therapeutic interventions**

Cell type	Source	Culture conditions	Model organism/cells	Property being assessed	Therapeutic outcome(s)	Important finding(s)	Reference
DC	Cervical cancer donors	DC isolation from PBMCs followed by maturation induction and transfection with catalase 2 (CAT2) siRNAs.	*In vitro*: M0 macrophage	Effect of CAT2-silenced DC-derived sEVs on macrophage migration and polarization.	CAT2-silenced DC-derived sEVs induced macrophage migration, M1 macrophage polarization, and reversed the pro-tumorigenic effects mediated by HPV16E7-pulsed DCs-derived sEVs.	CAT2-silenced DC-derived sEVs significantly lowered IL-10, TGF-β levels and Arg-1, and increased TNF-α, IL-12 and iNOS levels in macrophages.	[92]
DC	Commercial primary PBMCs	DC isolation from PBMCs followed by pulse education with tumor lysate and LPS stimulation.	*In vivo*: C57BL/6J mice and BALB/c mice (immune-cold tumor model)	Efficacy of DC-derived sEVs on stimulating the immune system against breast cancer.	DC-derived sEVs were significantly more effective than their parental cells in treating breast cancer by inducing lymphocyte infiltration and activation.	DC-derived sEVs had a greater enrichment of MHC molecules within the membrane proteins than parental cells and showed sustained efficacy in CD8 T cell priming over time.	[93]
DC	Healthy donors	DC isolation from PBMCs followed by transduction with α-foetoprotein-containing vector and LPS stimulation.	*In vitro*: HepG2 and SMMC-7 721 hepatocarcinoma cell lines	Antitumor effects of DCs sensitized with engineered DC-derived sEVs on hepatocellular carcinoma.	DCs sensitized with engineered DC-derived sEVs induced higher cytotoxicity on hepatocarcinoma cells than sEVs treatment alone.	DCs sensitized with engineered DC-derived sEVs induced the highest naïve T cell proliferation, immune effector molecule expression, and killing rate of hepatocarcinoma cells.	[94]
DC	Healthy donors	DC isolation from PBMCs followed by incubation with TSLP.	*In vitro*: activated $CD4^+$ T cells	Effect of TSLP-induced DC-derived sEVs on the differentiation of Th17 and Treg cells.	TSLP-induced DC-derived sEVs promote Th17 differentiation and inhibit Treg differentiation.	miR-21 is highly expressed in TSLP-induced DC-derived sEVs and regulates Th17/Treg differentiation by targeting Smad7.	[95]
DC	Healthy donors	DC isolation from PBMCs followed by incubation with TSLP.	*In vitro*: $CD4^+$ T cells	Effect of TSLP-induced DC-derived sEVs on the differentiation of Th2 cells.	TSLP-induced DC-derived sEVs promote Th2 differentiation.	OX40 ligand is highly expressed in TSLP-induced DC-derived sEVs and promotes Th2 differentiation.	[96]
DC	Healthy donors	Monocyte isolation from cord blood followed by immature DC induction and BGC823 tumor antigen-loading.	*In vitro*: $CD3^+$ T cells and BGC823 gastric adenocarcinoma cell line; *In vivo*: BALB/c (nu/nu) mice	Effect of sEVs derived from tumor total RNA-loaded DCs and tumor lytic antigen-loaded DCs on T cell proliferation and antitumor immunity.	*In vitro*: Both sEVs stimulated T cell proliferation, but sEVs from tumor RNA-transfected DCs were more potent in inducing T cell cytotoxic activity against BGC823 cells. *In vivo*: Co-administration of T cells and sEVs derived from tumor antigen-loaded DCs delayed tumor growth in mice.	Tumor antigen-loaded DCs produced sEVs with high expression levels of molecules related to DC maturation and immune response induction, including MHC-II, CD40, CD80, CD86, and CD54.	[97]
T cell	Healthy donors	$CD4^+$ T cell isolation from PBMCs followed by induction with IL-2.	*In vitro*: B16F10 melanoma cells; *In vivo*: C57BL/6J mice	Effect of IL-2-induced $CD4^+$ T cell-derived sEVs on antitumor activity of other T cell populations.	*In vitro*: IL-2-induced $CD4^+$ T cell-derived sEVs enhanced proliferation and cytotoxicity of $CD8^+$ T cells against B16F10 melanoma cells without influencing Tregs. *In vivo*: IL-2-induced $CD4^+$ T cell-derived sEVs displayed comparable antitumor activity to prominent antitumor agents used for melanoma patients.	Antitumor effect of IL-2-induced $CD4^+$ T cell-derived sEVs is mostly dependent on $CD8^+$ T cells. This enhanced antitumor function is attributed to the elevated levels of miR-155-5p, miR-215-5p, and miR-375 in sEVs.	[98]

Table 2 **Studies investigating human immune cell-derived small extracellular vesicles as therapeutic interventions** (continued)

Cell type	Source	Culture conditions	Model organism/cells	Property being assessed	Therapeutic outcome(s)	Important finding(s)	Reference
T cell	Healthy donors	$CD4^+$ T cell isolation from PBMCs followed by stimulation with anti - CD3/anti - CD28 antibody and IL - 2.	*In vitro*: THP-1 cells; *In vivo*: C57BL/6J mice	Effects of activated $CD4^+$ T cell-derived sEVs in modulation of macrophage function for tumor regression.	*In vitro*: Activated $CD4^+$ T cell-derived sEVs cause rapid and sustained sensitization of macrophages towards STING activation. *In vivo*: Activated $CD4^+$ T cell-derived sEVs combined with cGAMP stimulation promoted potent anti-tumor immunity against existing and re-challenging tumor.	Activated $CD4^+$ T cell-derived sEVs sensitized macrophages for enhanced STING signaling through surface-associated IFN-γ.	[99]
T cell	Healthy donors	$CD8^+$ T cell isolation from PBMCs followed by transfection with IL-2 and anti-EGFR antibody-containing vector.	*In vitro*: A549 lung cancer cells	Effects of engineering $CD8^+$ T cells on its ability to target and eliminate specific tumor cells.	Genetically modified $CD8^+$ T cell-derived sEVs possess enhanced targeting ability and cytotoxicity toward tumor cells.	Genetically modified $CD8^+$ T cell-derived sEVs inhibited tumor cell viability via reducing Rab27a expression and reinforced PBMC-mediated anti-tumor effects.	[100]
T cell	Healthy donors	T cell isolation from whole blood, activated with Human T-Activator CD3/CD28, transduced with vector encoding anti-EGFR CAR, followed by second stimulation using irradiated tumor cells.	*In vitro*: MDA-MB-231 breast cancer cells and HCC827 lung cancer cells; *In vivo*: BALB/c nude mice	Anti-tumor potency, target specificity, and safety of CAR T cell-derived sEVs.	*In vitro*: CAR T cell-derived sEVs exerted strong and specific cytotoxicity against high EGFR-expressing cells. *In vivo*: CAR T cell-derived sEVs achieved > 70% tumor growth inhibition compared to control.	CAR T cell-derived sEVs exerted tumor-specific cytotoxicity through expression of granzyme B and perforin.	[101]
T cell	Commercial primary PBMCs	T cell isolation from PBMCs followed by activation with Human T-Activator CD3/CD28 and IL-2.	*In vitro*: PY8119 breast cancer cells; *In vivo*: C57BL/6 mice	Role of T cell-derived PD-1-containing sEVs in anti-tumor immunity.	*In vitro*: T cell-derived PD-1-containing sEVs enhanced cytotoxic activity of effector T cells against tumor cells in the tumor immune microenvironment. *In vivo*: T cell-derived PD-1-containing sEVs increased the population of $CD8^+$ T cells in tumor-infiltrating immune cells.	T cell-derived PD-1-containing sEVs attenuates immunosuppression by binding to and inducing endocytosis of PD-L1 on tumor cells, and interacting with PD-L1-containing sEVs, together preventing PD-L1 binding to PD-1 on T cells.	[102]
T cell	Healthy donors	$CD45RO^-CD8^+$ T cell isolation from PBMCs.	*In vitro*: Ishikawa or KLE endometrial cancer cells	Role of $CD45RO^-CD8^+$ T cell-derived sEVs in combating hormone-responsive cancer.	$CD45RO^-CD8^+$ T cell-derived sEVs abolished the stimulatory effects of oestrogen on tumor progression.	$CD45RO^-CD8^+$ T cell-derived sEVs is abundant in tumor-suppressing-miR-765, which limits oestrogen-driven disease development via negatively regulating oncogenic inducing-PLP2.	[103]
T cell	Healthy donors	T cell isolation from PBMCs.	*In vitro*: Primary osteoblasts	Differential effects of T cell-derived sEVs from osteoporotic and non-osteoporotic patients on osteoblasts.	Non-osteoporotic T cell-derived sEVs enhanced ALP activity in osteoblasts for guided bone formation.	Non-osteoporotic T cell-derived sEVs upregulate expression of Runx2, type I collagen, osteopontin, and osteocalcin in osteoblasts for bone construction.	[104]

Table 2 **Studies investigating human immune cell-derived small extracellular vesicles as therapeutic interventions** (continued)

Cell type	Source	Culture conditions	Model organism/cells	Property being assessed	Therapeutic outcome(s)	Important finding(s)	Reference
NK cell	Healthy donors	Expansion and activation of isolated NK cells in medium containing IL-2, IL-12, IL-15, IL-21, galactosyl ceramide, and valproic acid. Followed by co-culture with neuroblastoma cells.	*In vitro*: SK-N-SH NB cell line; *In vivo*: Nude mice	Effect of NB-exposed NK cell-derived sEVs on naïve NK cells.	*In vitro*: NK cells treated with NB-exposed NK cell-derived sEVs showed up to 95% cytotoxicity against NB cells compared to 78% cytotoxicity with standard cytokine activation. *In vivo*: The size of NB tumors was restricted to less than 0.2 g for groups treated with fresh NK cells and NB-exposed NK cell-derived sEVs, in comparison to 1.94 g for groups given with fresh NK cells only.	sEVs from NK cells pre-exposed to NB cell line can educate a new population of NK cells to release more cytokines for better cytotoxic effects against NB tumors.	[105]
NK cell	Healthy donors	Priming of PBMCs with IL-12, IL-15, and IL-18. Followed by PBMC expansion and NK cell isolation.	*In vitro*: MGC803 gastric cancer, A549 non-small-cell lung cancer, and Patu8988t pancreatic cancer cell line; *In vivo*: BALB/c mice	Antitumor effects of memory-like NK cell-derived sEVs.	*In vitro*: Memory-like NK cell-derived sEVs showed enhanced antitumor effect with minimal toxicity on normal cells. *In vivo*: Accumulation of memory-like NK cell-derived sEVs in tumors was higher and tumor growth was more efficiently inhibited compared to mice treated with sEVs derived from conventional NK culture.	sEVs entered cancer cells via macropinocytosis and induced cell apoptosis via a caspase-dependent pathway. Elevated containment of granulysin within sEVs may have contributed to the enhanced therapeutic effect.	[106]
NK cell	Healthy donors	Expansion of CBMCs via co-culture with irradiation-inactivated K562 engineered cells. Followed by NK cell isolation.	*In vitro*: SKOV3 ovarian cancer cell line	Cytotoxicity of sEVs on ovarian cancer cells and effects on immunosuppressed NK cells.	sEVs are cytotoxic against ovarian cancer cells but not normal cells, antitumor activity of NK cells suppressed by the TME was also enhanced.	Molecular load of sEVs contained perforin and granzymes as effector molecules, and CD69 and CD107a for NK cell activation and degranulation. NK cells treated with the sEVs released more perforin and TNF-α than control.	[107]
NK cell	Healthy donors	Expansion of PBMCs in IL-2 containing medium. Followed by NK cell isolation and co-culture with K562 cells.	*In vitro*: K562 erythroleukemic cell line	Antitumor effects of trained NK cell-derived sEVs.	Trained NK cell-derived sEVs showed enhanced inhibitory effect on K562 cell proliferation and induced higher apoptosis compared to the same dose of sEVs derived from non-trained NK cells.	K562 cells treated with trained NK cell-derived sEVs had substantially up-regulated expression of caspase 3 and P53.	[108]

Abbreviations: A549, adenocarcinomic human alveolar basal epithelial cells; ALP, alkaline phosphatase; Arg-1, arginase-1; B16F10, murine melanoma cells; BALB/c, Bagg albino c; BGC823, human gastric adenocarcinoma cells; C57BL/6J, C57 black 6J; CAR T, chimeric antigen receptor T; CAT2, catalase 2; CBMCs, cord blood mononuclear cells; CD3, cluster of differentiation 3; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CD28, cluster of differentiation 28; CD40, cluster of differentiation 40; CD54, cluster of differentiation 54; CD69, cluster of differentiation 69; CD80, cluster of differentiation 80; CD86, cluster of differentiation 86; CD107a, cluster of differentiation 107a (LAMP1); CD45RO, cluster of differentiation 45RO; cGAMP, cyclic guanosine monophosphate–adenosine monophosphate; DC, dendritic cell; EGFR, epidermal growth factor receptor; HCC827, human non-small cell lung cancer cells; HepG2, human liver cancer cells; HPV, human papillomavirus; IFN-γ, interferon gamma; IL-2, interleukin-2; IL-10, interleukin-10; IL-12, interleukin-12; IL-15, interleukin-15; IL-18, interleukin-18; IL-21, interleukin-21; iNOS, inducible nitric oxide synthase; K562, human immortalized myelogenous leukemia cells; KLE, human endometrial cancer cells; LPS, lipopolysaccharide; MDA-MB-231, human breast adenocarcinoma cells; MGC803, human gastric cancer cells; MHC, major histocompatibility complex; NB, neuroblastoma; NK cells, natural killer cells; OX40, tumor necrosis factor receptor superfamily member 4; p53, tumor protein 53; Patu8988t, human pancreatic adenocarcinoma cells; PBMCs, peripheral blood mononuclear cells; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PLP2, proteolipid protein 2; PY8119, murine mammary adenocarcinoma cells; Rab27a, Ras-related protein Rab-27A; Runx2, runt-related transcription factor 2; sEVs, small extracellular vesicles; SK-N-SH, human neuroblastoma cells; SKOV3, human ovarian cancer cells; Smad7, SMAD family member 7; SMMC-7 721, human hepatocellular carcinoma cells; STING, stimulator of interferon genes; TGF-β, transforming growth factor-beta; Th2, T helper 2; Th17, T helper 17; THP-1, human monocytic leukemia cells; TME, tumor microenvironment; TNF-α, tumor necrosis factor-alpha; Treg, regulatory T cells; TSLP, thymic stromal lymphopoietin.

that sEVs might serve as a transfer mechanism of functional MHC-antigen complexes to other DCs for efficient activation of cytotoxic T cell responses[111]. Some of the first reports on phase I clinical trials of DC-sEVs were described in 2005 for advanced non-small cell lung cancer and metastatic melanoma[112,113]. However, although sEVs were well-tolerated in patients, clinical efficacy was unsatisfactory, as demonstrated by a minimal increase in antigen-specific T cell activity[112,113]. More recently, it was found that only sEVs generated from mature DCs, and not immature ones such as those used in the aforementioned clinical trials, could induce circulating monocytes to develop into IL-12p70-secreting mature DCs upon encounter with maturation stimuli[114]. IL-12p70 is a crucial cytokine involved in naïve $CD4^+$ T cell polarization into T helper (Th) 1 cells, which in turn are commonly regarded as the primary Th cell subtype associated with tumor elimination[115,116].

To generate mature DCs from primary monocytes, lipopolysaccharide (LPS)[93,94], cytokine cocktail (IL-1β, IL-6, TNF-α, and PGE2)[114], and thymic stromal lymphopoietin (TSLP)[95,96] stimulation have been used in studies. Studies have shown that sEVs produced by LPS-stimulated DCs outperformed parental cells in T cell recruitment and cytotoxic induction against immune-cold breast cancer and liver cancer models[93,94]. Cytokine cocktail-stimulated DC-sEVs hold promise because the cocktail mimics the physiological conditions for DC maturation. This enhances the homing ability of DCs to lymph nodes[117] and results in superior induction of T cell proliferation and the development of Th1 cells compared to LPS and TNF-α maturation-factor treated groups[118], benefits expected in their sEVs as well. On the other hand, sEVs derived from DCs matured by TSLP have been reported to promote Th2[96] and Th17 differentiation[95]. Although previously postulated to be linked to tumor promotion, one study has demonstrated that Th2 cells directly induced epigenetic reprogramming in breast cancer cells, resulting in enhanced expression of genes involved in normal mammary gland development and suppressed epithelial-mesenchymal transition, ultimately blocking spontaneous carcinogenesis[119]. In another review, it has been highlighted that Th2 cells can promote less tissue-destructive antitumor immunity in low-tolerance organs (*e.g.*, brain, lung)[120]. Th17 cells have been postulated to be beneficial for aggressive tumors due to their more differentiated effector memory phenotype compared to Th1 cells, potentially acting on tumors through direct lysis or synergism with $CD8^+$ T cells[121]. In addition, TSLP-stimulated DC-sEVs may have a protective role in fungal keratitis through regulation of the Th17/regulatory T (Treg) cell balance[95].

Apart from cell maturation, DCs are commonly pulsed with tumor lysates[93], tumor total RNA[97], tumor sEVs[122], or are transduced with tumor antigen-associated genes[94] to prompt tumor-specific immune effector cell responses through secreted sEVs. Alternatively, DC-sEVs can be loaded with certain components including siRNAs[92] and cytokines[123] to prompt other disease-healing mechanisms. For instance, catalase 2 siRNA-loaded DC-sEVs promoted antitumor activity by reversing the immunosuppressive effects of cervical cancer TME and inducing inflammatory macrophage polarization and migration[92]. DC-sEVs loaded with VEGF-A siRNA and doxorubicin reduced tumor angiogenesis moderately without causing severe hypoxia and mesenchymal transition, issues often seen with prolonged use of current anti-angiogenic cancer agents[122]. TGF-β and IL-10-loaded regulatory DC-sEVs also promoted bone-protective Treg responses while inhibiting bone-resorbing Th17 cell induction *in vitro*, making them a potential therapeutic modality for bone loss in periodontitis[123]. Importantly, regulatory DCs differ from typical mature DCs that have been extensively discussed; they have shown resistance to maturation, and their sEVs appear to be rich in immunoregulatory molecules that play a role in promoting transplant tolerance[124].

T cell-derived small extracellular vesicles

As a pivotal part of the adaptive immune system, T cells make up the most common tumor-infiltrating lymphocytes in the TME[125]. Typically, $CD4^+$ T cells are known for their role in promoting tumor regression by interacting with other immune cells, whereas $CD8^+$ T cells have a more direct cytotoxic effect on tumor cells. Thus, $CD4^+$ T cell-derived sEVs (T-sEVs) have been previously suggested to act primarily as activators that enhance the antitumor activity of $CD8^+$ T cells[126]. For instance, with IL-2 stimulation, $CD4^+$ T cells generated higher amounts of sEVs that are more potent in inducing $CD8^+$ T cell cytotoxicity due to increased antitumor miRNA cargo[98]. However, beyond impacting $CD8^+$ T cells, co-stimulation of $CD4^+$ T cells with IL-2 and anti-CD3/anti-CD28 antibodies also leads to the production of sEVs that lower the activation threshold of the stimulator of interferon genes (STING)[99], a protein believed to play a pivotal role in stimulating APCs and reprogramming the TME to counteract cancer immune evasion[127]. Similarly, in addition to

their inherent cytotoxicity, sEVs from genetically engineered CD8+ T cells can enhance the cytotoxicity of other PBMCs against tumor cells by delivering high concentrations of IL-2 in the local area, although the precise mechanisms remain unclear[100]. Furthermore, CD8+ T-sEVs can enhance the immune susceptibility of tumor cells by directly altering their expression of proteins and miRNAs. For instance, upregulation of miR-765 in endometrial cancer caused by unmodified sEVs from a subset of CD8+ T cells impairs tumor progression, possibly acting through negative regulation of the Notch signaling pathway[103]. This finding sheds light on the treatment of challenging cancers, as the Notch signaling pathway has been known for its contribution to the retention of stem-like properties in tumor cells, conferring them with chemoresistance[128].

In addition, engineered mouse T-sEVs overexpressing PD-1 have previously demonstrated their role in neutralizing PD-L1 on melanoma cells, thereby reinvigorating effector T cell activity by preventing PD-1/PD-L1 interaction on T cells[129]. Notably, similar properties were observed in sEVs derived from activated human T cells, with an added ability to induce clathrin-mediated endocytosis of surface PD-L1 on tumor cells[102]. Since these anti-PD-L1 sEVs are also internalized during this process, this suggests reduced systemic exposure, thereby potentially reducing the risk of off-target immune activation and subsequent conditions such as autoimmune insulin-dependent diabetes mellitus, a common complication in cancer patients who have undergone PD-1/PD-L1 antibody immunotherapy[130]. Notably, Schneider *et al* (2021) discovered that activated CD8+ T-sEVs are inherently enriched with CD73, which complements CD39 expression on Tregs to promote immune suppression in inflamed tissues through enzymatic generation of adenosine[131]. They further found that while sEVs isolated from synovial fluid of juvenile idiopathic arthritis patients themselves induce T cell activation and proliferation, the effect can be attenuated *via* addition of factors (*e.g.*, adenosine deaminase inhibitors) that strengthen the purinergic signaling cascade[131]. The presence of this built-in "immunomodulatory switch" in CD8+ T-sEVs, along with their natural enrichment at sites of inflammation, highlights their potential in the treatment of autoimmune diseases.

Given that autoimmunity represents an important contributor to pathogenic bone loss[132], the therapeutic applications of T-sEVs could extend to bone diseases. It was previously shown that T-sEVs from healthy donors upregulate essential genes responsible for promoting bone formation, potentially benefiting patients who suffer from osteoporosis[104]. More recently, sEVs isolated from cord blood Treg cells have demonstrated the ability to heal diabetic ulcers *in vivo* by enhancing fibroblast and endothelial cell migration to the wound in addition to upregulating the M2 macrophage ratio[133], underscoring their ability to crosstalk with non-immune cells. Further understanding of T cell-fibroblast crosstalk, for instance, may also open possibilities for utilizing T-sEVs to mitigate adverse outcomes of medical implants such as biomaterial-mediated fibrosis[134].

Natural killer cell-derived small extracellular vesicles

In comparison to human DC-sEVs and T-sEVs, research on human NK cell-derived sEVs (NK-sEVs) emerged relatively late, with the first report that assessed their functionalities published in 2012. The authors noted that both resting and IL-2-activated NK cells generated sEVs with typical NK cell markers (*i.e.*, CD56) and killer proteins (*i.e.*, Fas ligand [FasL] and perforin), inducing cytotoxicity in all tested hematological cancer cell lines, whereas solid tumor-derived cell lines were relatively resistant[135]. To enhance the cytotoxicity of NK-sEVs, various approaches have been taken, such as induction of memory-like NK cells through cell priming with a cocktail of IL-12, IL-15, and IL-18, thereby successfully generating sEVs with higher granulysin content and enhanced antitumor effects on several solid tumors including gastric, lung, and pancreatic cancers[106]. Another method, previously deemed feasible for large-scale production, was to repeatedly co-culture primary NK cells with irradiated inactivated K562-mbIL21 feeder cells, thereby generating highly activated NK cells that can produce functional sEVs containing several cytotoxic proteins including granulysin, perforin, and granzymes[136]. In addition to having cargo rich in killer effector molecules, another study that cultivated the NK cells in a similar way found that the sEVs derived could reverse the immunosuppressive effects of the TME on native NK cells and reactivate their cytotoxic function by upregulating the expression of multiple chemokine ligands, which in turn have an integral role in several crucial pathways (i.e., TNF, IL-17, and cytokine-cytokine receptor pathways) essential for the body's defense mechanism[107]. Other feeder cells that have comparable NK cell expansion and metabolic activation effects to K562-mbIL21 have also been developed and have demonstrated efficacy in mouse models of human sarcoma and T cell leukemia[137]. On the other hand, non-inactivated K562 cells have also

been utilized for co-culture with NK cells, and researchers found that these cells could "train" NK cells to produce sEVs that are more bioactive in their immunological functions, evidenced by upregulated gene expression of caspase 3 and P53 in cancer cells treated with such sEVs[108]. Similarly, cytokine-activated NK cells co-cultured with non-inactivated NB cell line released sEVs that were able to educate other NK cells to exert greater cytotoxicity of 88% towards NB cells compared to 35% and 78% cytotoxicity of fresh isolated and standard cytokine-activated NK cells, respectively[105].

Given that sEVs not only have a direct impact on tumor cells, but also indirectly induce antitumor effects by influencing native NK cells, perhaps future studies investigating the therapeutic potential of NK-sEVs could also assess their impact on other immune cells in the TME to optimize the production of clinically relevant potent NK-sEVs. While the discussion on NK-sEVs thus far has revolved around peripheral and cord blood-derived NK cells as a source, blood NK cells are rather difficult to obtain and expand due to their low abundance in blood and their need for special culture conditions (*e.g.*, feeder cells and cytokines) [138]. In this regard, NK cell lines are an appealing alternative. In particular, the NK-92 cell line and its variant NK-92MI, originally derived from a patient diagnosed with an aggressive NK-cell lymphoma, have been commonly used in studies as they have consistently demonstrated comparable cytotoxic activity to optimally activated blood NK cells against tumors without compromising safety and efficacy[138,139]. Notably, Zhang *et al* (2022) recently engineered NK-92MI cells that can produce sEVs capable of triggering multiple tumor eradication pathways upon laser irradiation by boosting M1 tumor-associated macrophage polarization, promoting dendritic cell maturation, and restoring the immunological surveillance function of effector T cells in the TME[140]. Beyond cancer, studies have found that NK-92MI-derived sEVs highly express miR-223, which can attenuate TGF-β-induced activation of hepatic stellate cells[141], a prominent driver of liver fibrosis, and have shown preliminary success in alleviating liver fibrosis in mouse models[142].

Less commonly, NK3.3 cell line generated from peripheral blood of healthy donors has also been sourced for sEVs as it is the only NK cell line reported to possess all functional characteristics of healthy primary blood NK cells[143]. Studies have shown that NK3.3-derived sEVs could effectively penetrate and induce tumor cell apoptosis in a three-dimensional breast cancer model, display strong antitumor activity against drug-resistant multiple melanoma and leukaemia, and eliminate cancer stem cell-like tumor subpopulations responsible for recurrence[143,144].

Clinical landscape

Since the first EV clinical trial in 1999, interest in their clinical application has grown rapidly, with trials exploring EV-based interventions for more than 200 diseases to date[145]. Nonetheless, studies investigating EVs as therapeutics accounted for less than one-fifth of all trials, and only 10.62% of studies considered subpopulation specifications, defined using unstandardized parameters such as cell source, particle size, or protein/lipid markers[145]. Van Delen *et al* (2024) were the first to perform a meta-analysis evaluating the safety and efficacy of EV-based interventions reported in clinical studies, and they found that EVs are generally well tolerated, with comparable safety profiles between allogeneic and autologous preparations[146]. However, the authors emphasized that meaningful conclusions regarding efficacy remain premature due to the lack of appropriate controls for comparison, the unclear influence of single versus repeated dosing, and potential confounding factors (*e.g.*, previous treatments and route of administration) that were not consistently accounted for[146]. Transcriptomic evidence has also shown that the therapeutic functions of EVs are largely attributed to only a small subpopulation with a total EV isolate[147], such as sEVs, which have been extensively discussed in this review, underscoring the importance of minimizing EV heterogeneity and focusing on defined EV subpopulations when evaluating therapeutic efficacy. There is, however, an overall lack of explicitly defined methodological reporting in EV clinical trials[145]. Only a limited number of sEV-specific intervention trials that have progressed beyond Phase II can be identified on ClinicalTrials.gov (Phase III: NCT06539273, NCT05354141, NCT05216562, NCT02138331), many of which are still ongoing. ***Table 3*** summarizes representative sEV clinical trials with available results.

Overall, these early-stage clinical trials demonstrate favorable safety profiles for sEV-based therapeutics, with no treatment-related serious adverse events reported across indications. Moreover, preliminary efficacy findings are also encouraging. However, these findings should be interpreted with caution given the early-phase nature of most studies, small cohort sizes, lack of randomized controls, and variability in dosing and administration routes.

Table 3 **Summary of sEV clinical trials that have completed phase I and beyond.**

Registration ID	Phase	sEV source	Disease	Key outcomes	Reference
NCT06539273	III	Foreskin-MSC	Androgenetic alopecia	Foreskin-MSC-sEVs injection increased hair density in patients in the first and third months after application. No treatment-related adverse events were observed.	[148]
NCT04493242	II	BM-MSC	COVID-19-associated acute respiratory distress syndrome	No treatment-related adverse events were observed. 60-day mortality was improved with a 41.9% absolute risk reduction after two doses of BM-MSC-sEVs. A Phase III trial has followed.	[149]
NCT05402748	I	Placental-MSC	Complex perianal fistula in non-Crohn's cases	81% of patients who received three doses of placental-MSC-sEVs experienced complete or partial healing. No serious adverse effects were observed during a 6-month follow-up. Phase II trial has followed.	[150]
NCT04388982	I	AT-MSC	Alzheimer's disease	Alzheimer's disease assessment scale–cognitive section scores decreased continuously by 3.98 points until week 36 after treatment with two doses of AT-MSC-sEVs per week for 12 weeks. No treatment-related adverse events were observed. Phase II trial has followed.	[151]

Abbreviations: AT, Adipose tissue; BM, Bone marrow; COVID-19, Coronavirus disease of 2019; MSC, Mesenchymal stem cell; sEVs, Small extracellular vesicles.

Current challenges and future directions

In earlier sections, the therapeutic potential of sEVs to treat various diseases has been discussed. However, among the key challenges to successfully translating sEVs into clinical applications is the issue of heterogeneity[152]. Batch-to-batch variability of independent MSC-sEV preparations was observed in terms of their immunomodulatory capabilities, despite originating from the same donor MSC stock and the implementation of a standardized production strategy[153]. Such differences in functionality imply inconsistencies in the therapeutic potency of sEVs; hence, the authors suggest potency testing of independent sEV preparations prior to patient administration. However, given the multifaceted nature of sEVs, they might need to be subjected to multiple assays to capture the extent to which the different mechanisms of action are exhibited, presenting yet another challenge. To address this problem, Adamo *et al* (2025) recently developed a simple, versatile, yet sensitive enzymatic assay, termed "DetectEV", that can first predict whether a particular sEV preparation possesses the potential to achieve its intended therapeutic effects by assessing both the luminal esterase-like activity and membrane integrity[154]. Subsequently, further robust function-specific tests that have proven to be indicative of *in vivo* therapeutic outcomes, such as the multi-donor mixed lymphocyte reaction assay[155], may be carried out for functional characterization of sEV preparations. Interest in the use of advanced models, such as disease-specific multi-organ-on-a-chip systems combined with *in silico* modeling, is also growing, as this approach builds on a highly integrated framework that permits extrapolation of *in vitro* therapy responses in a clinically relevant manner while circumventing potential false-positive/negative results from animal models[156].

Another challenge to overcome, particularly for allogeneic sEVs, is their rapid clearance and consequently poor accumulation at target sites after systemic administration[152]. Importantly, phosphatidylserine (PS) is enriched in sEV membranes and is one of the known surface molecules responsible for initiating macrophage phagocytosis of sEVs. Consistent with this, PS-deficient sEV preparations demonstrated approximately a 40-fold slower clearance than bulk sEV preparations and exhibited improved biodistribution[157], underscoring the importance of PS depletion in prolonging circulation time. To achieve this, Kobayashi *et al* (2022) proposed an enzymatic method that involves treatment of sEV preparations with phosphatidylserine decarboxylase; results were promising, as PS-deficient sEV yields increased with minimal changes in their original physicochemical properties[158]. On the other hand, CD47 acts in the direct opposite manner to PS, and sEVs derived from cells engineered to have upregulated CD47 expression exhibit improved retention time *in vivo*[159]. With technological advancement, predicting the impact of these modifications on *in vivo* distribution has also become

easier. For instance, utilizing only standard-of-care medical imaging modalities, a radiomics-based machine learning model developed by Tang *et al* (2025) was reported to aid in accurately predicting NP accumulation in tumors by acting as "virtual biopsies"[160]. Meanwhile, Mi *et al* (2024) trained a publicly accessible and user-friendly deep neural network model that could predict NP delivery efficiency not only to tumors but also to major organs such as the heart, liver, spleen, lung, and kidney[161]. While these models were not developed to study EVs specifically, they serve as a strong starting point to better understand and rationally engineer EVs to enhance their circulatory stability and distribution.

Furthermore, clinical translation and commercialization are also constrained by the poor yield of sEVs and difficulties in scaling up[152]. About a decade ago, sEV mimetics known as cell-derived nanovesicles (CNVs), prepared by membrane extrusion, garnered attention as they possess characteristics similar to sEVs but with substantially higher production efficiency (up to 100-fold)[162]. More recently, another study confirmed high similarity in terms of membrane protein (approximately 71%) and small RNA cargo (approximately 65%) between sEVs and CNVs, supporting previous studies that postulated CNVs can be viable alternatives to natural sEVs given their comparable biodistribution, targeting effects, and therapeutic performance[163]. One study even found that CNVs resemble their parental cells more closely than sEVs based on proteomic and RNA sequencing data comparisons[164]. Nevertheless, their position as a promising alternative to sEVs does not exempt CNVs from challenges currently faced by sEVs for clinical translation, such as the absence of a universally standardized protocol with minimal manual interventions for CNV preparation and purification, as well as batch-to-batch variability[165].

Finally, although current clinical studies consistently report that sEV-based interventions are well tolerated, the potential risks associated with long-term or repeated administration remain insufficiently characterized. Xu *et al* (2025) noted that long-term safety experiments, including neurotoxicity, tumorigenicity, genotoxicity, reproductive toxicity, immunotoxicity, and immunogenicity assessment, may be necessary in different therapeutic settings[166]. Moreover, off-target accumulation of sEVs in organs such as the liver, spleen, lungs, and gastrointestinal tract has been reported in multiple studies[167], raising the possibility that prolonged exposure could lead to unintended biological effects in these tissues. From an ethical and regulatory perspective, the use of human-derived primary cells for sEV production poses specific challenges. Due to the limited expansion capacity and finite lifespan of primary cells (including MSCs), sEV preparations cannot be supplied infinitely, necessitating repeated sourcing of new donor-derived materials[167]. However, every acquisition of human tissues or cells for use in advanced therapy medicinal products requires documented informed consent and compliance with donor screening standards[168]. Furthermore, to ensure ethical sourcing and safeguard donor welfare, EU Member States emphasize that such donations should be voluntary and unpaid[169]. Reliance on recurrent voluntary donations may not be sustainable for large-scale manufacturing. Although immortalized human cells offer a more scalable production platform for sEVs, maintaining long-term cellular homogeneity remains challenging.

Conclusion

sEVs represent well-designed hybrids of cell therapy and synthetic nanomedicine, endowing them with the benefits of both approaches while mitigating some of their respective shortcomings. Popular sources of sEVs include MSCs, DCs, T cells, and NK cells. Current studies on MSC-sEVs are primarily focused on their pro-angiogenic, osteogenic, chondrogenic, and neurogenic differentiation abilities, with different functional capacities according to the tissue from which they were sourced, suggesting that precise MSC-sEV selection and disease matching could maximize therapeutic outcomes. On the other hand, interest in immune cell-sEVs primarily lies in their potential as novel cancer therapeutics, with a few studies exploring their possible applications in treating bone diseases and autoimmune diseases, as well as promoting transplant and implant tolerance. Despite the promising therapeutic potential of sEVs in treating complex diseases through diverse mechanisms, their intrinsic heterogeneity, demand for comprehensive and complex functional potency tests, unsatisfactory biodistribution, unclear risks associated with long-term administration, and difficulty in industrial scale-up continue to slow their progression toward regulatory approval as off-the-shelf commercial therapeutics. Nevertheless, substantial efforts are being made to overcome these limitations, making the future of sEV-based therapies an exciting and rapidly evolving field.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

None.

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