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Review

Milk-Derived Extracellular Vesicles as Multifunctional Therapeutic Platforms in Human Health, Nutrition, and Diagnostics

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Abstract

Extracellular vesicles (EVs) are nanoscale, lipid-bilayer carriers that mediate intercellular communication by transporting proteins, lipids, and nucleic acids, thereby influencing physiological and pathological processes. This review integrates current knowledge on milk-derived and human extracellular vesicles, highlighting their multifunctional roles in therapy, diagnostics, and regenerative medicine. Milk-derived EVs (MEVs), small EVs (sEVs), and human milk EVs (hMEVs) demonstrate unique stability, biocompatibility, and cross-barrier transport, enabling oral and systemic therapeutic applications. These vesicles exhibit immunomodulatory, anti-inflammatory, and drug-delivery potential, making them promising platforms for tissue repair, intestinal integrity, metabolic regulation, and microbiota modulation. In oncology, EVs contribute to tumor progression, metastasis, and immune regulation, while engineered or milk-derived vesicles provide biocompatible platforms for targeted therapy and diagnostics. Integration with biomaterials, nanotechnology, and artificial intelligence enhances EV engineering, cargo optimization, and therapeutic precision. Despite their translational promise, clinical applications remain constrained by heterogeneity, inefficiencies in isolation, lack of standardized protocols, and incomplete mechanistic understanding. Scalable production, reproducible purification workflows, cargo-loading

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strategies, and safety assessments are essential for advancing EV-based therapeutics from preclinical studies to clinical interventions. Continued exploration of cross-species and biofluid-derived EVs, along with improved characterization and functional analyses, will facilitate their integration into regenerative medicine, drug delivery, nutritional interventions, and minimally invasive diagnostics. Overall, this review highlights extracellular vesicles as versatile nanoscale platforms with significant translational potential in both pediatric and adult medicine, offering new avenues for precision therapeutics, functional nutrition, and biomarker discovery.

Keywords
Extracellular vesicles; milk-derived EVs; human milk EVs; drug delivery; immunomodulation; regenerative medicine; biomarkers; therapeutic applications

1. Introduction

Extracellular vesicles (EVs), including exosomes and microvesicles, are nanoscale, membrane-enclosed carriers naturally released by all cell types. They transport proteins, lipids, nucleic acids, and bioactive molecules, enabling precise intercellular communication across physiological and pathological contexts. Their biological functions span immune regulation, microbial interactions, and transfer of antimicrobial resistance. Environmental factors such as temperature and cellular stress influence EV composition and activity. Despite clinical potential, limited mechanistic insight and inconsistent isolation methods hinder translation [1]. Tumor-derived EVs influence angiogenesis, immune modulation, and metastasis. In contrast, milk-derived EVs (MEVs), including human milk-derived EVs (hMEVs) and small EVs (sEVs), offer stable, biocompatible platforms for oral delivery, regenerative medicine, and functional nutrition. The resilience of milk EVs during gastrointestinal transit, coupled with their ability to cross biological barriers and deliver diverse cargos, underscores their therapeutic and diagnostic potential. Their multifunctional roles extend to immune regulation, metabolic signaling, tissue repair, and microbiota modulation, making them highly relevant for translational and clinical applications. MEVs have gained attention due to their scalability, biocompatibility, and therapeutic promise. Exhibiting immunomodulatory and regenerative properties, MEVs support disease treatment and diagnostic innovation. However, milk's complex composition complicates the isolation of pure vesicle fractions. Standardized protocols for extraction and characterization are essential for advancing MEVs toward clinical applications [2]. MEVs isolated via ultracentrifugation and hydrochloric acid-assisted ultracentrifugation were comprehensively characterized using nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM), along with assessments of RNA and protein composition and overall sample purity. These analyses enabled a detailed comparison of particle features, molecular content, and isolation efficiency between the two methods. This methodological refinement provided a robust framework for isolating high-quality MEVs from human, bovine, and caprine milk, facilitating more accurate downstream analyses of MEV composition and biological activity [3].

Milk-derived small extracellular vesicles (MsEVs) are natural, low-immunogenic nanocarriers suitable for oral drug delivery. Their biological effects include microbiota modulation, antioxidant activity, and support for intestinal, bone, and muscle health. Engineering MsEVs enhances targeting and drug bioavailability. Yet, large-scale production remains challenged by heterogeneity, purification limitations, and quality-control concerns requiring robust, standardized workflows [4]. Food-derived extracellular vesicles (FDVs) release bioactive molecules that modulate gut immunity, oxidative stress, and tissue integrity. Isolation typically relies on differential ultracentrifugation, yielding high particle counts across diverse sources. Despite promising preclinical evidence, human studies remain scarce, and scalable production, safety validation, and standardized isolation protocols are needed to enable therapeutic development [5].

Exosomes, capable of transporting diverse molecular cargo, cross physiological barriers such as the blood-brain barrier and hold potential in diagnosing and treating CNS disorders. Numerous isolation strategies-centrifugation, chromatography, filtration, and microfluidics- offer varying yields and purities. The lack of global standardization complicates cross-study comparison and impacts downstream therapeutic and diagnostic applications [6]. Each exosome isolation method imposes trade-offs among purity, yield, scalability, and sample compatibility. Ultracentrifugation offers good purity but is time-intensive, while ultrafiltration and precipitation provide higher throughput with lower purity. Immunoaffinity and size-exclusion chromatography improve selectivity but can pose challenges related to cost, yield, or contamination challenges, reinforcing the need for context-specific method selection [7]. Exosomes are nanoscale in size, have low immunogenicity, and can cross biological barriers. Advances in analytical tools and understanding biodistribution will enhance their clinical translation and therapeutic potential [8]. With their engineering flexibility, enabling targeted delivery and enhanced stability, positioning them as powerful tools for precision medicine and improved therapeutic outcomes across diverse diseases [9].

Exosomes' biocompatibility and targeting capabilities position them as powerful platforms for drug delivery, gene therapy, and vaccine development. Advances in surface engineering, synthetic exosome design, and scalable production support their therapeutic evolution. Remaining challenges include clinical validation, regulatory harmonization, and manufacturing optimization, yet exosome-based therapies are rapidly progressing toward clinical translation [10]. Milk serves not only as a source of postnatal nutrition but also as a vehicle for molecular communication through EVs, which modulate neonatal immunity, gut development, neurodevelopment, and systemic metabolism. EVs from bovine, goat, and human milk demonstrate cross-species bioactivity, enhanced epithelial barrier integrity, and anti-inflammatory effects, supporting their potential applications in gastrointestinal disorders, metabolic dysfunction, and systemic inflammatory conditions. Advances in EV engineering, functionalization, and delivery systems, including hydrogels and nanocarriers, further enhance stability, targeting, and efficacy, positioning milk-derived and exogenous EVs as transformative tools in diagnostics, therapeutics, and precision medicine.

Despite strong evidence supporting therapeutic, diagnostic, and regenerative potential, milk- and food-derived EVs face critical translational gaps. Isolation, purification, and characterization methods remain heterogeneous and unstandardized, limiting reproducibility. Mechanistic insights into cargo-function relationships, oral bioavailability, cross-species efficacy, and pharmacokinetics are insufficient. Large-scale production, quality control, functional assays, and targeted delivery strategies are underdeveloped. Human studies and clinical validation are scarce, while regulatory

frameworks remain immature. Addressing these challenges is essential to unlock the full clinical, nutritional, and biomedical potential of milk- and food-derived EVs as safe, effective, and scalable nanotherapeutics.

This review aims to comprehensively evaluate milk-derived and food-derived EVs, focusing on their biological functions, therapeutic potential, and translational applications. Key objectives include understanding their anti-inflammatory, antioxidant, regenerative, and immunomodulatory activities, oral and systemic delivery capabilities, bioactive cargo composition, and cross-species efficacy. The paper also examines isolation, purification, and characterization methods, scalable production strategies, engineering approaches for targeted delivery, and integration with biomaterials and nanotechnology. By highlighting current limitations, standardization gaps, and mechanistic uncertainties, this review seeks to provide insights that will advance the clinical, nutritional, and biomedical translation of milk- and food-derived EV-based therapeutics. The remainder of this paper is organized as follows. Section 2 describes the methodology adopted for the review. Section 3 provides a comprehensive synthesis of the relevant literature and key findings. Section 4 discusses the major implications, existing challenges, and potential directions for future research. Finally, Section 5 summarizes the main conclusions and implications of the review.

2. Methodology

This review investigated the use of milk-derived extracellular vesicles for nutrition by systematically analyzing peer-reviewed literature from major academic databases. Searches employed keywords including extracellular vesicles, milk-derived EVs, human milk EVs, drug delivery, immunomodulation, regenerative medicine, biomarkers, and therapeutic applications in titles and abstracts. Retrieved studies were screened for relevance to milk-derived EVs in nutritional and gut health contexts. Only English-language, peer-reviewed articles were included. Additionally, ChatGPT was utilized to enhance language clarity, coherence, and grammatical accuracy throughout the manuscript.

3. Literature Review

This literature review explores the field of extracellular vesicle research, emphasizing milk-derived extracellular vesicles and their diverse biological functions. It examines applications in regenerative medicine, cancer therapy, oral drug delivery, metabolic and intestinal health, nutrition, immunity, infant development, and biomarker discovery, while highlighting physicochemical properties supporting therapeutic and diagnostic potential.

3.1 Expanding Biomedical Horizons Through Extracellular Vesicles

Extracellular vesicles enable intercellular communication across physiological and pathological settings. Their heterogeneity, molecular cargo, and ability to mirror parent-cell states make them valuable for non-invasive diagnostics, particularly in cancer, while engineered EVs offer promise as targeted therapeutic carriers [11]. Ocular biofluids-including tears, aqueous humor, and vitreous humor-contain EVs that reflect localized cellular states, supporting their role as biomarkers for glaucoma, macular degeneration, diabetic retinopathy, and ocular tumors. Beyond diagnostics, EVs function as biocompatible, low-immunogenic drug delivery systems suited to the eye's

compartmentalized structure, with bioengineering strategies that improve targeting precision [12]. In oncology, EVs exhibit dual immunomodulatory roles: tumor-derived EVs can suppress antitumor immunity and promote metastasis. In contrast, others stimulate immune responses, highlighting their promise in immunotherapy despite the need for standardized characterization [13]. Similarly, in renal medicine, urinary EVs enable non-invasive diagnostics, and mesenchymal stem cell-derived EVs support regeneration via anti-inflammatory and reparative effects, though regulatory and standardization challenges persist [14].

EVs serve as accessible disease indicators and versatile therapeutic carriers capable of crossing biological barriers and modulating inflammation, angiogenesis, and neuroprotection [15]. MEVs, particularly exosomes, offer low-immunogenic platforms for delivering bioactive molecules, with demonstrated benefits in gastrointestinal health, oxidative stress reduction, wound healing, and anticancer activity [16]. Bovine milk exosomes further influence gene expression, microbiota, and tissue regeneration, supporting applications in functional nutrition and therapeutics [17]. Milk-derived exosomes demonstrate significant regenerative potential for wound healing by delivering bioactive molecules that regulate inflammation, angiogenesis, and matrix remodeling, thereby promoting scar-free repair and restoring vascular function in chronic wounds, and can be engineered to enhance safety and production consistency [18]. Engineered MEVs further exhibit transformative potential in bone disease management through targeted functionalization, restoring bone homeostasis and enabling MRI-guided monitoring while enhancing bone density and tissue-specific drug delivery [19]. Overall, EVs represent a rapidly advancing frontier, and with their natural cargo diversity, targeting capabilities, and engineering adaptability, position them as versatile platforms across oncology, nephrology, ophthalmology, wound repair, and metabolic and skeletal health. Continued innovation in isolation, characterization, and regulatory frameworks will accelerate the translation of these advances into precision medical applications.

3.2 Extracellular Vesicles as Emerging Drivers of Diagnostics and Regenerative Innovation

Extracellular vesicles are lipid bilayer particles released by all cells, enabling precise intercellular communication through cargo shaped by their cellular origin. Their presence in accessible fluids such as blood and urine makes them powerful, minimally invasive biomarkers, while their therapeutic versatility supports emerging roles in drug delivery, immunomodulation, and regenerative applications [20]. Exogenous extracellular vesicles derived from milk, plants, and microbes expand translational potential due to their biocompatibility, stability, and ability to transport functional cargos across biological barriers, with integration of nanotechnology and AI enhancing profiling and targeted applications in precision diagnostics and therapeutics [21]. Advances in biomaterials further demonstrate the utility of yogurt whey-derived EVs in engineering injectable, angiogenic, and immunomodulatory hydrogels, highlighting scalable opportunities for supramolecular platform development [22]. When combined with functionalized hydrogels, EVs are delivered within a biomaterial matrix, enabling sustained release and preserving bioactivity, thereby advancing tissue regeneration and personalized wound healing strategies in regenerative medicine. Hydrogel-based EV delivery systems mitigate rapid degradation and diffusion with advanced composites and AI-assisted design, improving therapeutic precision [23]. Exosomes are gaining attention as effective biomaterials for drug delivery. Unlike traditional therapies and synthetic carriers, exosomes offer a non-cytotoxic, efficient means for targeted delivery of therapeutic agents,

including genes, drugs, and peptides, enhancing precision medicine applications [10]. Additionally, nutraceutical studies incorporating milk fat globule membranes and EVs reveal insights into brain lipid remodeling despite limited cognitive improvement, underscoring their relevance for neurodegenerative research and future therapeutic strategies [24]. These findings underscore EVs as powerful biological mediators with broad potential.

3.3 Multifunctional Roles of Extracellular Vesicles in Emerging Cancer Therapeutics

Extracellular vesicles function as essential messengers within the tumor microenvironment, influencing cancer initiation, progression, and therapeutic resistance. EVs modulate angiogenesis, metastasis, immune evasion, and drug response. Their diagnostic and therapeutic potential continues to expand as EV biology becomes better defined [25]. Tumor-derived EVs (TEVs) and dendritic cell-derived EVs (DC-EVs) exert complementary yet opposing roles in cancer immunity, where TEVs often promote immunosuppression but can be engineered as antigen carriers, while DC-EVs demonstrate superior therapeutic potential compared with conventional cell-based immunotherapies [26]. EVs further regulate tumor progression and metastasis while serving as diagnostic biomarkers and drug delivery systems, although challenges such as isolation standardization and pathway elucidation remain [27]. Their utility as circulating biomarkers for non-invasive cancer detection is promising but requires improved reproducibility and specificity for clinical translation [28]. Milk-derived EVs, particularly from colostrum, exhibit notable anti-cancer activity by inducing reversible growth arrest and enhancing chemotherapeutic efficacy [29]. An overview of the comparative analysis of extracellular vesicle sources: mesenchymal stem, plant, and synthetic vesicles, along with representative references, is summarized in Table 1. Collectively, current evidence highlights EVs as dual cancer facilitators and therapeutic tools, enabling immune regulation, biomarker discovery, and drug delivery, requiring standardization and mechanistic advances for clinical translation.

Table 1 Comparative analysis of extracellular vesicle sources: mesenchymal stem, plant, and synthetic vesicles.

Type of EVs	Scalability	Purification Challenges	Oral Stability	Batch Consistency	References
Mesenchymal stem cell-derived EVs	Moderate-Low due to dependence on cell culture expansion and donor variability	High; complex isolation with co-isolated proteins and heterogeneity requiring ultracentrifugation or advanced purification	Moderate; partially protected but sensitive to degradation in harsh environments	Moderate; variability influenced by donor cells and culture conditions	[30]
Mesenchymal stem cell-derived EVs	Moderate; dependent on MSC expansion efficiency and culture optimization	High; issues include low yield and contamination with soluble proteins	Moderate; requires stabilization for functional maintenance	Moderate; influenced by MSC source and environmental cues	[31]
Mesenchymal stem cell-derived EVs	Moderate; scalable but limited by cell production capacity	High; standardized isolation and characterization remain challenging	Moderate; bioactivity preserved under controlled conditions	Moderate; affected by culture heterogeneity and processing conditions	[32]
Plant-derived EVs	High; abundant plant biomass enables large-scale and sustainable production	Moderate; contamination from plant debris and co-isolated biomolecules	High; strong stability due to lipid composition and resilience to gastrointestinal conditions	Moderate-High; depends on plant species and extraction protocols	[33]
Plant-derived EVs	High; scalable and cost-effective from agricultural sources	Moderate; purification requires removal of cell wall debris and heterogeneous vesicles	High; stable in biological environments and suitable for oral delivery	Moderate-High; influenced by plant origin and processing method	[34]

Plant-derived EVs	High; sustainable production from diverse plant sources	Moderate; variability in isolation and characterization across species	High; biocompatible, and stable under physiological conditions	Moderate-High; affected by plant tissue and extraction technique	[35]
Synthetic EVs	Very high; fully controllable and scalable manufacturing	Low-Moderate; controlled synthesis but requires precise formulation quality control	High; engineered for stability and controlled release	Very high; reproducible batch-to-batch manufacturing	[36]
Microbiota-derived EVs	Moderate; dependent on microbial culture complexity	High; isolation complicated by heterogeneous microbial populations	Moderate; stability influenced by gastrointestinal environment	Moderate; variability due to microbiome diversity	[37]
Milk-derived EVs	High; large-scale availability from milk sources	Moderate-High; casein/lipoprotein contamination requires advanced purification	High; stable through gastrointestinal transit and systemic circulation	Moderate; influenced by species and processing conditions	[38]

3.4 Next-Generation Therapeutics Using Milk-Derived Small Extracellular Vesicles

Milk small extracellular vesicles (sEVs) have gained recognition as biocompatible therapeutic carriers, produced in extraordinary quantities and suitable for industrial applications. Their ability to safeguard bioactive cargo during digestion, cross multiple biological barriers, and remain non-toxic supports their expanding relevance in oral and systemic drug delivery [39]. MEVs exhibit inherent anti-inflammatory, antioxidant, and regenerative activities while demonstrating exceptional stability within gastrointestinal environments. Engineering strategies, including surface modification and hybrid nanoparticle formation, have enhanced their targeting precision and loading capacity, enabling the delivery of sensitive therapeutics and improving oral bioavailability across diverse preclinical disease models [40].

Originating from mammary epithelial cells, MEVs encompass exosomes, microvesicles, and apoptotic bodies that transfer proteins, lipids, and RNAs across species. Their natural resilience supports oral and intravenous administration, while targeted lipid modifications enable delivery to diseased tissues. Clinical translation, however, depends on scalable isolation, optimized loading strategies, and deeper mechanistic understanding [41]. Milk-derived exosomes provide a rich, accessible therapeutic resource, particularly effective in treating intestinal disorders such as inflammatory bowel disease, necrotizing enterocolitis, and colorectal cancer. They restore epithelial integrity, rebalance microbiota, reduce oxidative stress, and attenuate inflammation, supporting their suitability for both clinical and agricultural health interventions [42].

Extracellular vesicles serve as natural delivery systems capable of transferring bioactive molecules with high precision. Their therapeutic promise spans traumatic injuries, inflammation, and cancer. Yet large-scale manufacturing, regulatory standardization, and mechanistic clarification remain critical hurdles that must be addressed before EV-based therapeutics achieve widespread clinical approval [43]. EV biomanufacturing continues to face challenges related to the low productivity of mammalian cell lines. Transitioning from traditional two-dimensional cultures to advanced three-dimensional systems improves EV yields and process consistency. Complementary strategies, including stimulatory treatments and optimized media formulations, further enhance secretion, supporting scalable bioproduction for therapeutic application [44].

Recent progress has established EVs as valuable diagnostic and therapeutic platforms across cancer, neurological disease, and cardiovascular dysfunction. Their ability to deliver diverse molecular cargos, modulate immune responses, and serve as biomarkers supports their expanding translational potential. Engineered EVs further enhance targeting and therapeutic precision [45]. MEVs exhibit microbiome-modulating functions that support applications in inflammatory disorders, tissue repair, cancer therapy, and vaccine development. Their capacity to stabilize and deliver diverse molecular cargoes across physiological barriers positions them as promising nanocarriers requiring continued refinement in loading efficiency, specificity, and safety assessment [46]. This concludes that milk-derived sEVs and related EV platforms offer scalable, biocompatible, and multifunctional solutions for advanced therapeutic delivery. Their unique stability, barrier-crossing ability, and cargo versatility highlight immense translational promise. Continued progress in engineering, production standardization, and regulatory frameworks will be essential to realize their clinical efficacy across diverse biomedical applications. An overview of the therapeutic potential and production challenges of MEVs, along with representative references, is summarized in Table 2.

Table 2 Therapeutic potential and production challenges of milk-derived extracellular vesicles.

Author(s)/Year	Major Outcomes	Challenges
Kim et al., 2023 [47]	Milk exosomes mediate intercellular communication and show promise in musculoskeletal health via antioxidant and anti-inflammatory effects.	Limited research on geriatric applications and standardized isolation/characterization techniques.
Meng et al., 2024 [48]	Goat milk extracellular vesicles (GMVs) possess immunomodulatory properties and potential as drug delivery systems.	Incomplete understanding of biogenesis, cargo selection, and the need for standardized isolation methods.
Panda and Ahmad, 2025 [49]	Bovine milk exosomes (BMEs) act as bioactive nanocarriers with neuroprotective and systemic therapeutic potential.	Lack of standardized protocols for isolation and quality control; limited data on intrinsic cargo.
Santoro, 2023 [50]	Isolation methods refined to reduce contamination; industrial processing reduces milk EV integrity; infant formulas have lower EV bioactivity.	Overcoming processing-induced EV degradation; enhancing EV content in infant formulas.
Hu et al., 2021 [51]	Human milk EVs express coagulation factors; species-specific differences noted; EVs survive gastrointestinal conditions and modulate immunity.	Lack of standardized EV isolation; unclear effects of milk storage and pasteurization on EV function.
Caira et al., 2025 [52]	Donkey milk EVs enriched in proteins for immune defense and tissue repair; distinct cargo in colostrum vs. mature milk.	Limited sample size; need for further validation and functional studies on donkey milk EVs.
Karakülah et al., 2026 [53]	Bovine milk and colostrum exosomes show anticancer activity and enhanced drug delivery via engineered targeting.	Challenges in large-scale production, cargo heterogeneity, and regulatory standardization.
Mousavi et al., 2025 [54]	Milk EVs protect epithelial cells from oxidative stress; lyophilized EVs retain bioactivity; internal cargo is key to function.	Identification of key bioactive molecules and mechanistic studies is needed.
Ni et al., 2025 [55]	Hybrid milk EVs combine high drug loading with preserved membrane integrity, improving oral insulin delivery efficacy in diabetic mice.	Scaling production and maintaining EV consistency for clinical translation.
Vahkal et al., 2025 [56]	Term and preterm human milk EVs differ in their immune-modulating effects; term EVs uniquely suppress pro-inflammatory cytokines and cell death.	There is a need for gestational age-specific therapeutic development and further clinical studies.

3.5 Physicochemical Properties of Milk-Derived Extracellular Vesicles

Milk-derived extracellular vesicles have gained attention as effective natural nanocarriers due to their biogenesis, stability, and bioactivity. MEVs can carry diverse therapeutic payloads such as small molecules, proteins, and nucleic acids, improving drug stability and bioavailability, especially in oral administration. Their scalable, cost-effective production, particularly from bovine milk, supports large-scale pharmaceutical applications [57]. The isolation of high-purity MEVs using different chemicals impacts purity and functionality. Acetic acid (AA) induced partial protein aggregation, leading to higher cellular uptake than sodium citrate (SC). AA-treated MEVs exhibited stronger immunomodulatory effects, particularly in reducing T cell proliferation, while SC maintained MEV integrity, enhanced purity, and preserved the smooth surface [58]. Breast milk, a key nutritional source for infants, contains abundant EVs that help facilitate communication between mother and child. Studies have shown that miRNAs in MEVs are stable and resistant to digestion, thereby influencing immune responses, neuronal development, and the progression of diseases like obesity and diabetes [59].

Milk-derived EVs represent a particularly appealing class of vesicles due to their abundance, accessibility, and stability in harsh physiological environments. Research demonstrates therapeutic promise, especially in drug delivery and targeted treatment. However, progress toward clinical translation requires standardized protocols, batch consistency, and strong regulatory guidance [60]. Advances in EV engineering, including the use of high-density producer cell lines and improved purification technologies, have expanded the feasibility of large-scale EV production. Chromatography and filtration methods yield higher-purity vesicles than centrifugation, supporting reproducible manufacturing and accelerating the development of clinically deployable EV-based therapies [61].

3.6 Milk-Derived Extracellular Vesicles as Emerging Oral Nanocarriers

Oral drug delivery remains the most desirable therapeutic route, yet many drugs face challenges such as low solubility, poor permeability, and gastrointestinal instability. MEVs have emerged as natural nanocarriers capable of enhancing stability, bioavailability, and targeted intestinal delivery. Their biocompatibility and safety highlight substantial promise for future oral therapeutics [62]. Mammalian milk contains abundant EVs that support neonatal development by influencing neural maturation, intestinal integrity, immune regulation, and microbial homeostasis, with their high abundance and scalability highlighting strong translational and commercial potential for nutraceutical and pharmaceutical applications [63]. MEVs demonstrate resistance to digestive degradation and preferential intestinal targeting, enabling modulation of barrier function and immunity for conditions such as inflammatory bowel disease. However, standardized purification and stability assessments remain necessary [64]. Their exceptional stability allows systemic distribution, including potential brain delivery, yet challenges persist in cargo-loading efficiency and pharmacokinetic control [65]. MEVs present a scalable, cost-effective approach for oral drug delivery by crossing the gastrointestinal barrier. Different MEV extraction methods can be tailored to achieve targeted drug delivery, improving treatment outcomes for various diseases [66]. Oral insulin delivery faces challenges like gastrointestinal harshness and hepatic clearance. To address this, adaptive milk-derived nanovesicles were developed to overcome these barriers. Milk-derived

nanovesicles offer scalable, cost-effective solutions for the oral delivery of biomacromolecules, including peptides [67]. The major benefits of MEVs are represented in Figure 1.

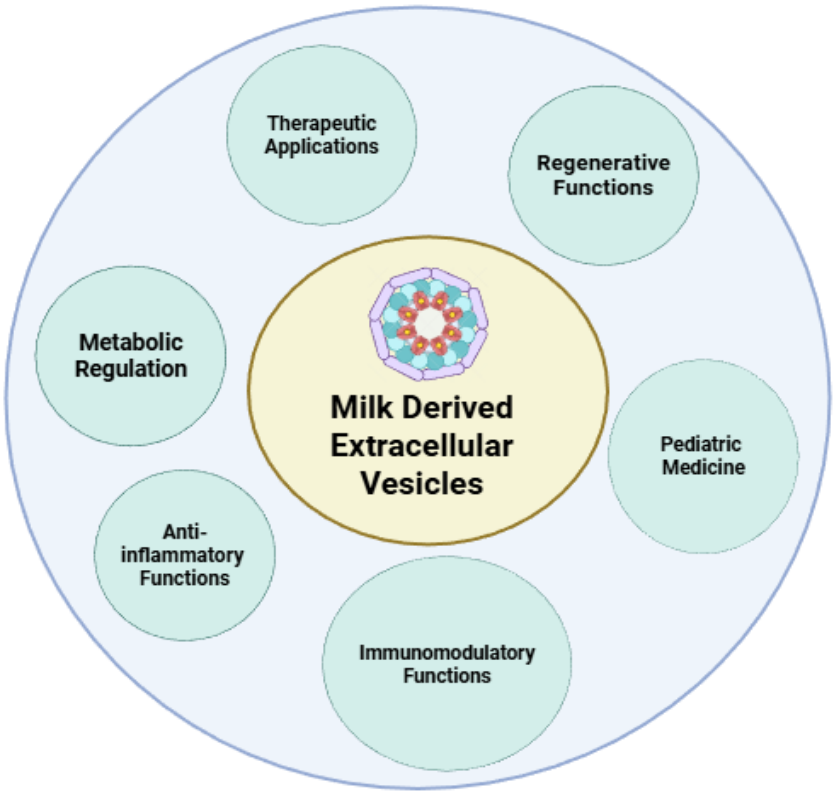

Figure 1 Functional overview of multidisciplinary extracellular vesicles applications.

Milk also acts as a signaling medium mediating cross-species communication, though mechanisms of uptake and variability in EV content require further clarification for clinical translation [38]. Potential uptake mechanisms of EVs are governed by their physicochemical properties and the biological context of recipient cells. EVs can interact with target cells via direct membrane fusion, enabling cargo release into the cytosol. EVs are commonly internalized via endocytic pathways and are influenced by cell type and vesicle size. Surface biomolecules enable receptor-specific binding and targeting. After internalization, vesicles traffic through endosomes for cargo release. Uptake efficiency depends on vesicle properties and environmental conditions, while engineering strategies and experimental models optimize delivery and therapeutic applications [68]. More broadly, EVs serve as versatile drug delivery systems with ongoing improvements in engineering and standardization [69], while MEVs in particular offer low-cost and orally compatible platforms for treating inflammatory, dermatologic, and systemic diseases [70]. Overall, MEVs are a biocompatible, abundant nanoplatform for oral therapeutics and regenerative medicine, with clinical translation dependent on improved scalability, regulation, and mechanistic understanding.

3.7 Extracellular Vesicles as Emerging Regulators of Intestinal Health

Human milk-derived exosomes (HMDEs) are nanosized vesicles central to maternal-infant biological communication, supporting neonatal intestinal protection through cargo delivery and barrier-crossing capability. Their low immunogenicity and ability to enhance epithelial integrity

highlight therapeutic relevance for conditions such as necrotizing enterocolitis, despite persisting challenges in purification and quality control [71]. Milk extracellular vesicles from diverse species exhibit potent anti-inflammatory and microbiota-modulating effects, reinforcing epithelial barriers and mitigating gastrointestinal disorders, including colitis and necrotizing enterocolitis. Their dual anticancer and drug-delivery functionality enhances tumor targeting and reduces toxicity, though translational progress requires improvements in standardization, scalability, storage, and drug-loading efficiency [72].

Milk-derived EVs deliver bioactive molecules across the gastrointestinal environment, supporting intestinal repair, immune modulation, microbiota regulation, and mucosal homeostasis. Their capacity for selective targeting and oral delivery positions them as promising tools for treating inflammatory bowel disease and colorectal cancer, though mechanistic gaps and production challenges remain significant barriers [73]. Milk contains diverse bioactive molecules essential for shaping infant gut immunity and development. EVs and associated microRNAs may influence epithelial and microbial dynamics, although controversy persists regarding their digestive stability and tissue targeting. Deepening molecular characterization and uptake studies may clarify their roles in infant nutrition and adult gastrointestinal health [74]. Sequencing defined the miRNA landscape of sheep milk EVs and enabled comparison with bovine counterparts. Nanoparticle tracking confirmed the presence of abundant nanosized vesicles carrying diverse small RNAs, with miRNAs constituting a major portion. Eighty-four miRNAs were identified, largely conserved across samples. Prominent species such as miR-26a, miR-191, let-7f, let-7b, and miR-10b were highly expressed in both species. Notably, most dominant sheep EV miRNAs, including oar-miR-148a, were immune-associated, while pathway analysis indicated shared roles in lipid metabolism, calcium signaling, and neural processes [75].

Mechanistic studies show that bovine milk EVs enhance tight junction proteins, accelerate barrier maturation, and reduce permeability without inducing inflammation. Specific EV components strengthen junctional pathways, underscoring their capacity to maintain epithelial integrity and support functional food or therapeutic applications [76]. MEVs consistently demonstrate anti-inflammatory activity by modulating host-microbiome interactions and restoring gut integrity. Their cross-species biocompatibility and gastrointestinal stability support oral administration for managing chronic inflammatory diseases. Advancing scalable isolation and validating efficacy in human organoid and clinical systems remain essential for therapeutic translation [77].

Recent studies reveal that human and bovine MEVs reinforce intestinal barriers under inflammatory and metabolic stress, reduce endotoxin translocation, and alleviate liver injury through gut-liver axis modulation. Their durability in gastrointestinal conditions and in vivo colon delivery highlight their potential for non-invasive treatment of colitis and metabolic disorders [78]. Together, evidence shows that HMDEs and MEVs regulate epithelial integrity, immune balance, and microbiota composition while enabling targeted cargo delivery. Their natural stability and cross-barrier transport underscore broad therapeutic promise. Achieving clinical implementation will require refined isolation, mechanistic clarity, optimized drug-loading strategies, and rigorous safety evaluation to harness these vesicles for intestinal and systemic health.

3.8 Extracellular Vesicles as Emerging Modulators of Metabolic Health

Small extracellular vesicles are now recognized as regulators of metabolic homeostasis, which influence gene expression and metabolic signaling. In obesity-related inflammation, sEVs modulate lipid handling, insulin sensitivity, and inflammatory responses. Their miRNA cargos show biomarker potential and therapeutic relevance for metabolic disorders [79]. A 16-week dietary study demonstrated that supplementation with cow milk MFGM/EV-rich concentrate alters systemic lipid metabolism despite unchanged body weight or glucose tolerance. Mice selectively incorporated milk-derived very-long-chain sphingomyelins and ceramides, with enhanced whole-body lipid turnover, suggesting that dietary sphingolipids contribute structurally and functionally to metabolic regulation [80].

Milk fat globule membrane-derived sphingolipids exhibit potent bioactivity, modulating intestinal absorption, hepatic lipid metabolism, and cholesterol transport. Their metabolic impact is shaped by structural attributes and the ceramide ratio, which influences insulin sensitivity, atherogenesis, and muscle physiology [81]. A 25-week preclinical trial revealed that MFGM/EV supplementation promotes the accretion of long-chain sphingolipids in aging rats, lowering ceramide biomarkers associated with cardiometabolic risk. Enhanced lipoprotein turnover and improved hepatic lipid processing underscored metabolic benefits, supporting nutraceutical applications for older adults unable to sustain traditional interventions [82]. Collectively, sEVs and milk-derived lipid nanostructures emerge as influential regulators of metabolic pathways, acting through intercellular signaling and selective integration of sphingolipids. Their roles in lipid turnover, inflammation, and cardiometabolic resilience highlight therapeutic promise. Advancing mechanistic insights, biomarker validation, and translational studies will be essential for developing precision nutrition and metabolic-targeted therapies.

3.9 Milk-Derived Extracellular Vesicles as Mediators of Nutrition and Immunity

Milk provides essential postnatal nutrition while transmitting maternal biochemical signals that shape immune maturation and developmental trajectories. Among their bioactive constituents, EVs have gained prominence for their ability to deliver molecular cargo that modulates inflammation, angiogenesis, and tissue repair. Despite rising interest, inconsistencies in isolation and variability in nomenclature hinder definitive mechanistic interpretation, reinforcing calls for standardized methodologies [83]. Bovine MsEVs can traverse physiological barriers such as the intestine, placenta, and blood-brain barrier. Their pharmacokinetics indicate efficient uptake and high oral bioavailability, though rapid macrophage-mediated clearance and lysosomal degradation remain limitations. Overcoming these hurdles could substantially enhance sEV-based drug delivery platforms [84].

Milk-derived small extracellular vesicles contain conserved structures and bioactivities across mammalian species, contributing to intestinal, immune, skeletal, hepatic, neural, and cardiovascular regulation. Their biocompatibility supports applications in functional foods and natural therapeutic delivery. However, stronger associations between vesicle composition and biological effects, along with optimized purification standards, are required to accelerate translational success [85]. EVs in milk remain stable during digestion and are intentionally enriched with bioactive cargos, yet research has focused predominantly on human and bovine sources. EVs from lesser-studied species may harbor distinct molecular signatures with nutritional and therapeutic implications. Rigorous

isolation and adherence to the MISEV (Minimal Information for Studies of Extracellular Vesicles) guidelines are essential to clarify species-specific EV bioavailability and function [86].

Goat milk-derived EVs demonstrate potent anti-aging activity, showing strong cellular uptake, suppression of oxidative stress, enhanced migration, and regulation of extracellular matrix remodeling in fibroblasts. These EVs support tissue repair, while in vivo results confirm high skin permeability and favorable biosafety profiles [87]. Maternal immune activation during lactation alters milk EV microRNA cargo, reshaping neurodevelopmental pathways in offspring. These miRNAs transfer to the neonate, influencing long-term gene expression and behavior. Environmental enrichment normalizes MEV signatures and rescues neurobehavioral deficits, underscoring MEVs as dynamic conveyors of maternal stress and protective signals [88].

Cross-species metabolomic profiling of MEVs from bovine, goat, and donkey milk reveals metabolites regulating cytokine activity, immune receptor signaling, and intestinal barrier integrity. Goat MEVs exhibit the strongest pathway enrichment, suggesting elevated immunomodulatory potency. These findings highlight MEV metabolites as promising modulators of inflammatory diseases [89]. Milk exosomes possess inherent stability and long circulation time, making them attractive nanoscale carriers for drug and imaging agents. Advances in loading techniques and exosome engineering enhance therapeutic precision and targeting. Their low immunogenicity and scalability across milk species support broad theranostic applications in future clinical technologies [90].

Milk-derived EVs/exosomes play vital roles in guiding immune development and modulating inflammatory pathways, with applications spanning developmental disorders, autoimmunity, gastrointestinal disease, and tissue fibrosis. Their resilience to digestion and capacity to influence macrophage polarization and T-cell activity strengthen their potential as bioactive therapeutic and nutritional platforms, despite regulatory and safety challenges [91]. In summary, MEVs represent a versatile, bioactive system connecting nutrition, immunity, and therapeutic innovation. Their stability, intercellular signaling capacity, and cross-species applicability position them as transformative tools for diagnostics, drug delivery, and functional nutrition. Continued standardization, mechanistic elucidation, and translational research will be essential for unlocking their full clinical and biomedical potential.

3.10 Human Milk Extracellular Vesicles as Mediators of Infant Development

Human milk provides extensive short- and long-term health benefits, now increasingly attributed to human milk extracellular vesicles. Carrying miRNAs, proteins, and lipids, hMEVs regulate infant development through epigenetic modulation, gut maturation, immune conditioning, and antiviral protection. Their composition reflects maternal physiology, offering biomarkers and therapeutic potential across pediatric and adult medicine [92]. Proteomic profiling of MEVs has revealed their distinct molecular identity. Analysis of EVs from seven donors identified 1,963 proteins, including classical vesicle markers and hundreds previously unreported in milk studies. These EV-specific proteins enrich pathways involved in signaling and inflammation regulation, indicating critical contributions to neonatal immunity and gastrointestinal development [93].

Breast milk-derived EVs also demonstrate therapeutic relevance beyond infancy. In endothelial models, human breast milk-EVs suppressed inflammation, reduced oxidative stress, and restored vasorelaxation in obese mice. Their ability to enhance endothelial migration and wound healing

suggests dual anti-inflammatory and pro-angiogenic functions [94]. Milk sEVs also cross the blood-brain barrier, accumulating within key regions such as the hippocampus and cortex. Diets depleted of sEVs and RNAs impaired neuronal gene expression, reduced dendritic complexity, weakened cognitive performance, and heightened seizure susceptibility in mice, underscoring their essential role in neurodevelopment and brain functional maturation [95].

As stable carriers of diverse cargos, MEVs mediate cross-species molecular communication. miRNA-containing vesicles influence immune maturation, synaptic development, and metabolic pathways linked to obesity and diabetes. However, proteins, lipids, and non-coding RNAs remain understudied, emphasizing the need for deeper characterization and optimized isolation techniques [59]. These findings reveal hMEVs as central determinants of infant health, influencing immunity, neurodevelopment, and long-term physiology. Their diagnostic and therapeutic potential extends far beyond nutrition, supporting applications in cardiovascular therapy, biomarker discovery, and engineered biologics. Advancing mechanistic insights and refining methods will be pivotal to integrating milk EVs into clinical and nutritional innovations.

3.11 Milk-Derived Extracellular Vesicles as Emerging Biomarkers

Intensive dairy farming has heightened cattle vulnerability to disease, driving the need for precision diagnostics and innovative health-management strategies. EVs from bovine tissues and fluids provide molecular insights into immunity, metabolism, reproduction, and mammary and intestinal function. Their biomarker potential is promising, though standardization and commercial translation remain essential challenges [96]. Milk-derived exosomes are abundant, biocompatible vesicles enriched with regulatory microRNAs such as miRNA-21. These cargos influence pathways, shaping intestinal and immune functions. Despite therapeutic promise, uncertainties about long-term metabolic effects and isolation variability call for improved methodological rigor and safety evaluations [97].

Milk-derived exosomes resist gastric degradation and retain biological activity upon systemic entry, influencing immune modulation, intestinal integrity, microbiota composition, and musculoskeletal development. Their stability and bioavailability support emerging applications in nutrition, therapeutics, and functional foods. However, deeper mechanistic clarification and clinical validation are required to ensure long-term efficacy and safety [98]. Bovine milk sEVs enable noninvasive, cost-effective sampling for immune regulation and disease monitoring in veterinary medicine. Their cargo reflects physiological states, yet isolation-challenge constraints widespread adoption. Collaborative research that bridges veterinary science and EV specialization is critical to advance sEV-based diagnostics and therapeutics for cattle health [99].

A novel acid-based isolation method improved the purity of bovine milk EVs, producing ≈120-nm vesicles that express canonical markers and demonstrate safe uptake by macrophages. In vivo administration caused no toxicity or anaphylaxis, supporting their suitability as drug-delivery vehicles and highlighting their potential for scalable therapeutic applications [100]. Orally administered bovine sMEVs show substantial systemic absorption, with an apparent bioavailability of ≈45%. Fluorescent tracking revealed peak plasma and intestinal levels at six hours, tissue distribution by twelve hours, and elimination primarily through feces and urine. These findings affirm sMEVs as stable, orally deliverable nanocarriers with translational therapeutic promise [101]. These findings across dairy science, nutrition, and biomedical research position MEVs as

multifunctional mediators of communication, diagnostics, and therapy. Their stability, bioactivity, and scalability support applications ranging from cattle health monitoring to human drug delivery. Continued refinement of isolation, mechanistic understanding, and safety assessment will be crucial for advancing their clinical and agricultural impact.

4. Discussion

4.1 Current Study-Based Therapeutics, Diagnostics, and Nutritional Implications of EVs

Milk-derived and small EVs hold broad implications for human health, offering multifunctional roles in immunomodulation, microbiota regulation, antioxidant activity, and tissue regeneration. Their natural stability, biocompatibility, and ability to transport therapeutic cargos position them as promising platforms for drug delivery, gene therapy, vaccines, and functional nutrition. EVs provide minimally invasive diagnostic tools that reflect physiological and pathological states across systems, while tumor-derived EVs enable biomarker discovery and immunomodulatory interventions. Milk- and human milk-derived EVs support gastrointestinal integrity, neurodevelopment, metabolic regulation, and wound or tissue repair, with applications in inflammatory disorders, cancer, and regenerative medicine. Integration with bioengineering, nanotechnology, and precision medicine strategies enhances targeting, stability, and therapeutic efficacy. Additionally, EV metabolites and microRNAs present novel intervention points, while cross-species bioactivity enables oral delivery and translational applications in both human and veterinary health. Collectively, these properties underscore the potential of EVs as versatile, clinically relevant platforms bridging diagnostics, therapeutics, and personalized medicine.

4.2 Challenges in Translating Milk-Derived Extracellular Vesicle Therapeutics

Milk- and food-derived EVs face multiple critical challenges that impede clinical translation and large-scale therapeutic applications. Heterogeneity within EV populations, co-isolation of non-vesicular components, and low reproducibility of current extraction methods complicate standardization. Isolation techniques such as ultracentrifugation, filtration, and chromatography vary widely in yield, purity, and scalability, while cargo-loading efficiency and batch consistency remain inconsistent. Oral delivery introduces additional obstacles, including digestive stability, absorption variability, and pharmacokinetic control. Regulatory guidance, safety evaluation, and functional validation in human studies are limited, further constraining translational potential. Mechanistic understanding of cargo-function relationships, biodistribution, species-specific effects, and long-term safety is incomplete. Rapid clearance, lysosomal degradation, and biofluid-specific variability undermine therapeutic consistency, while large-scale production is hindered by low vesicle yields and quality-control limitations. Translating MEV therapeutics faces challenges in standardization and reproducibility. The MISEV guidelines provide essential frameworks, emphasizing consistent nomenclature, thorough characterization, miRNA and protein profiling, and quality control. Strict adherence to these methodologies ensures accurate, reproducible results, which are crucial for advancing MEV therapeutics in clinical applications. Isolating and assigning biological effects to EVs is challenging due to milk's complexity, requiring strict adherence to standardized methodologies like MISEV guidelines [86]. EVs are promising mRNA delivery platforms due to low immunogenicity, efficient RNA transport, and natural targeting abilities. Standardized

production, quality control, and adherence to MISEV2023 guidelines, alongside multidisciplinary advances, will support successful clinical translation of EV-based therapies and vaccines [102]. Overcoming these barriers requires robust, reproducible workflows, standardized protocols, rigorous preclinical validation, and integration with bioengineering and nanotechnology strategies to enable safe, scalable, and effective clinical applications of MEVs.

4.3 Prospects of Functionalized Milk Extracellular Vesicles

Milk- and food-derived EVs hold immense promise, driven by advances in engineering, surface functionalization, and scalable production. Engineered milk EVs can enhance oral bioavailability, gut repair, systemic delivery, and CNS-targeted therapies while supporting regenerative and metabolic interventions. Integration with bioengineered, synthetic, or hybrid vesicles, combined with AI-assisted molecular profiling, 3D bioprinting, and supramolecular scaffolds, expands precision targeting and therapeutic versatility. Standardized isolation, regulatory harmonization, and quality control will accelerate clinical translation. Future applications may include dual therapeutic-diagnostic platforms, functional food ingredients, immunomodulation, neuroprotection, cardiovascular repair, and personalized nutrition. Optimization of cargo loading, targeting strategies, and cross-species bioactivity will enable reproducible, large-scale production. Continued research into molecular mechanisms, pharmacokinetics, and functional validation will facilitate industrial and clinical adoption, transforming MEVs into robust nanotherapeutics, nutraceuticals, and biomarker-guided precision medicine tools, bridging the gap between preclinical promise and real-world translational impact. Their multifunctionality positions them as foundational components in next-generation biomedical and nutritional innovations. Artificial intelligence, encompassing machine learning and deep learning, is advancing extracellular vesicle research by enabling precise target identification, optimized delivery strategies, drug system design, cellular network reconstruction, multi-omics integration, and synthetic biology applications. These innovations improve analytical scalability and accuracy. Future progress depends on standardized data pipelines, multicenter validation, privacy-preserving approaches like federated learning, robust regulation, and a ChatGPT-based platform for integrated support [103]. From precision biomarkers to engineered hydrogels and nutritional interventions, EV research is rapidly expanding. Continued mechanistic exploration, optimization of EV sourcing, and technological integration will be essential to transforming EV-based platforms into impactful clinical and biomedical solutions.

5. Conclusion

This review has comprehensively synthesized current knowledge on milk- and food-derived EVs, including their biological functions, therapeutic potential, and translational relevance. Emphasis was placed on understanding functional properties and anti-inflammatory activities, oral and systemic delivery, tumor- and gut-specific applications, microbiota modulation, immune regulation, and engineering strategies to enhance targeting and scalability. Milk-derived EVs, including HMDEs and sEVs, are versatile, biocompatible nanocarriers with significant potential in diagnostics, regenerative medicine, and functional nutrition. Their natural bioactive cargo, stability during digestion, ability to cross physiological barriers, and compatibility with oral delivery position them as promising platforms for intestinal protection, immune modulation, metabolic regulation, and systemic therapy. Beyond infancy, milk EVs demonstrate applicability in cardiovascular support, neuroprotection,

wound healing, and precision drug delivery, bridging nutritional science and biomedical innovation. Their multifunctionality underscores their transformative potential in personalized medicine, functional foods, and translational therapeutics.

Additionally, the review evaluated isolation, characterization, and standardization techniques, translational challenges, and clinical and preclinical applications. The integration of bioengineering, AI-assisted design, and biomaterial platforms was discussed to highlight pathways for personalized and scalable EV-based interventions. Collectively, this work confirms that all objectives have been thoroughly covered, providing a robust foundation for future research and clinical translation. However, fully realizing the clinical and industrial potential of milk-derived EVs requires addressing critical challenges, including standardized isolation, scalable production, mechanistic elucidation, and quality control. Integration of bioengineering, AI-assisted design, and biomaterial-based strategies can enhance cargo targeting, therapeutic precision, and reproducibility. Continued research into pharmacokinetics, cross-species bioactivity, and regulatory frameworks will be pivotal for translating preclinical findings into safe, effective, and scalable interventions. Overall, milk-derived EVs offer a robust foundation for next-generation biomedical, nutritional, and therapeutic applications, bridging fundamental research with clinical innovation.

Abbreviations

DC-EVs	Dendritic cell-derived EVs
EVs	Extracellular vesicles
FDVs	Food-derived extracellular vesicles
hMEVs	Human milk EVs
HMDEs	Human milk-derived exosomes
MEVs	Milk-derived EVs
MsEVs	Milk-derived small extracellular vesicles
sEVs	Small EVs
TEVs	Tumor-derived EVs

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