**Review Article**

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# Overcoming oral drug delivery challenges in cancer treatment: The role of milk exosomes

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**Running title:** Oral Exosome for Cancer Therapy

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**Abstract**

**Background:** Cancer is a leading global cause of mortality, traditionally treated with intravenous (IV) chemotherapy which IV administration has poor patient compliance and causes significant systemic toxicity. While oral chemotherapy is preferred, its effectiveness is limited by poor drug solubility, instability, and low bioavailability. To overcome these challenges, nano-based drug delivery systems, particularly milk-derived exosomes, offer a promising solution by enhancing drug stability and absorption. This review aimed to highlight the application of milk-exosomes in oral cancer therapy.

**Methods:** This narrative review investigated the potential use of milk-derived exosomes in oral cancer treatment by searching the PubMed, Scopus, and Google Scholar databases from their inception through August 2025.

**Results:** According to the findings, milk exosomes are biological nanovesicles with low immunogenicity and a unique capability to pass through biological barriers without degrading in the gastrointestinal tract. Their natural abundance and cost-effectiveness make them preferable carriers for oral drug delivery. In cancer treatment, they enhance tumor targeting and the bioavailability of chemotherapeutic agents, reducing systemic side effects. Furthermore, milk exosomes can be efficiently loaded with a variety of therapeutic agents, including small molecules, nucleic acids, and proteins, while preserving their structural integrity and functionality.

**Conclusion:** In conclusion, milk exosomes represent a highly promising, naturally-derived platform for effective oral anticancer therapy. By overcoming enzymatic degradation and poor mucosal absorption, they could turn potent injectable drugs into effective oral treatments. If successfully developed, milk exosome-based therapies could provide a non-invasive, patient-friendly alternative to intravenous treatments, improving compliance and quality of life.

**Keywords:** Cancer therapy, Oral drug delivery, Exosome, Milk exosome, nanocarriers, biocompatibility

## 1. Introduction

Milk-derived exosomes are nano-sized extracellular vesicles, typically ranging from 30 to 200 nm in diameter, naturally secreted by mammary epithelial cells and present in mammalian milk. They are enclosed by a lipid bilayer and carry diverse biomolecules, including proteins, lipids, deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA), and microRNAs, which enable intercellular communication, immune modulation, and delivery of bioactive molecules. The abundance, biocompatibility, and safety of milk exosomes make them an attractive source compared with exosomes derived from cultured cells or blood, supporting their potential for therapeutic applications.[1] Oral administration of milk exosomes offers unique advantages over intravenous (IV) therapies. Their lipid bilayer protects encapsulated cargo from enzymatic degradation in the gastrointestinal tract (GI), enabling absorption and systemic distribution. Milk exosomes can carry both hydrophilic and hydrophobic drugs, enhancing bioavailability and therapeutic efficacy. Additionally, their natural composition reduces immunogenicity, facilitates efficient cellular uptake, and allows potential surface modification for targeted delivery, making them especially promising for cancer therapies.[2]

Cancer is a major global health challenge, representing one of the leading causes of morbidity and mortality worldwide. IV administration of chemotherapeutic agents presents several inherent limitations. Systemic exposure to potent drugs often leads to severe side effects, including damage to the liver, kidneys, heart, and bone marrow. Additionally, the pharmacokinetics of many anticancer drugs are inadequate, with rapid clearance from the bloodstream reducing effective drug concentrations at tumor sites. Repeated IV administration also causes problems for patients, requiring frequent hospital visits and invasive procedures, which can compromise adherence and quality of life. Moreover, poor solubility and instability of certain

chemotherapeutic compounds in plasma further limit their bioavailability and therapeutic efficacy. These challenges emphasize the need for innovative drug delivery systems that enhance tumor targeting, minimize systemic side effects, and provide more patient-friendly administration routes.[3]

The therapeutic potential of milk exosomes lies in their unique biological composition and functional versatility. Milk exosomes have been demonstrated to contain specific proteins such as CD9, CD63, CD81, CD82, HSP70, HSP90, Alix, and TSG101, as well as nucleic acids capable of regulating gene expression in recipient cells. These molecules can modulate immune responses, promote cellular uptake, and mediate intercellular signaling, making them particularly valuable for delivering chemotherapeutic agents or nucleic acids to target tissues[4]. Notably, milk exosomes can cross biological barriers, including the intestinal epithelium, the blood-brain barrier, and the placental barrier, further expanding their potential applications in treating cancers.[5]

Compared to synthetic nanoparticles, milk exosomes offer distinct advantages that make them highly attractive for therapeutic delivery. Unlike liposomes, polymeric nanoparticles, or inorganic nanocarriers, exosomes are naturally occurring vesicles with intrinsic biological functions, enabling efficient cellular uptake and minimal immunogenicity.[6] Additionally, they are recognized as GRAS (Generally Recognized as Safe), exhibiting low immunogenicity and high biocompatibility. Their endogenous origin allows them to interact with cellular membranes via receptor-mediated pathways, enhancing targeted delivery. Importantly, milk exosomes are abundant, safe, and easily accessible at large scales, providing a cost-effective and biocompatible alternative to conventional nanoparticles, which often require complex chemical synthesis and surface modification. These characteristics position milk-derived exosomes as next-generation nanocarriers with the potential to overcome the limitations of synthetic nanoparticles in cancer therapy and other biomedical applications.[7]

Previous studies have firmly established milk exosomes as natural nanocarriers with remarkable potential for drug delivery. Overall, these intrinsic features emphasize the strong promise of milk-derived exosomes as efficient, safe, and stable vehicles for oral administration.[8,9] This review aims to provide a comprehensive analysis of milk exosomes as oral drug delivery vehicles for cancer therapy. We focus on their biological properties, therapeutic advantages, isolation, and characterization methods. This review guides the translation of milk exosomes into clinical applications by synthesizing recent progress and systematically highlighting the key industrial challenges in scaling up their production including standardization, cost-effective isolation, and quality control. A clear identification of these hurdles provides the crucial foundation for future research and development efforts aimed at overcoming them.

## 2. Methods

A structured narrative literature search was conducted using the databases PubMed, Scopus, and Google Scholar. The search strategy employed keywords and phrases such as "oral drug delivery", "cancer therapy", "exosomes", "milk exosomes", “nanocarriers”, and “biocompatibility. The primary search was limited to studies published between 2015 and 2025 to capture the most recent advancements. However, seminal and highly relevant works published before 2015 were also included to provide an appropriate scientific context, identified through citation tracking of the retrieved articles. Studies that were not directly related to the application of milk exosomes in an oncological context were excluded. The selection process involved an initial screening based on titles and abstracts, followed by a thorough evaluation of the full-text articles for final inclusion. This approach aims to map current trends and highlight translational challenges rather than serve as a full systematic review.

## 3. Routes of cancer drug administration: focusing on intravenous and oral modalities

Common methods for delivering chemotherapy agents include IV, per-oral (PO), intrathecal (IT), and intravesical routes.[10] The various properties of each mode can impact the pharmacokinetic and pharmacodynamic profiles of the drug in the body.[11] Choosing the appropriate route of administration depends on several factors, such as the physicochemical properties of the drug, the site of the tumor, cancer staging, rate, and extent of absorption.[12] With the development of modern treatments, some cancers have transitioned into chronic conditions, requiring long-term medication. Therefore, attending to patient choice in the administration route, when the efficacy is the same, enhances patient adherence to the chemotherapy regimen.[13]

Other less commonly used but clinically relevant administration routes include intramuscular (IM),[14] subcutaneous (SC),[15] intravesical,[16] intrapleural, intraperitoneal (IP),[17] topical,[18] transdermal,[19] intralesional,[20] intratumoral,[21] intra-arterial (IA),[17] ophthalmic,[22] and intranasal.[23] An overview of the administration routes is presented in Figure 1.

Selecting the route of administration is one of the important criteria for patient adherence to treatment and is an essential component of medication use quality.[24] IV administration is preferred for cytotoxic drugs with poor oral absorption, unstable GI profile, and medicines that need immediate therapeutic levels.[25] While IV chemotherapy provides high drug concentrations and controlled dosing, it is associated with several limitations. These include the need for trained healthcare personnel, risk of infection at the injection site, time-consuming, patient discomfort due to frequent hospital visits, and systemic toxicity.[3] In addition, long-term IV therapy may lead to complications such as vein irritation or catheter-related issues.[26] Therefore, IV administration of chemotherapy agents has been associated with a higher incidence of medication errors (MEs) compared to other routes of drug delivery.[27]

In contrast to IV administration, which demands sterile procedures, skilled staff, and medical facilities,[28,29] oral administration enables outpatient treatment, which significantly enhances patient quality of life by avoiding hospitalization and allowing self-management in the home setting; therefore, it can potentially lower overall treatment costs,[30] facilitates ease of administration for healthcare professionals while improving convenience and adherence among patients.[31] PO route can also cause continuous exposure of the tumor to the drugs, which can improve the outcome for some types of cancers.[32] This convenience helps patients follow their daily medication routine.[33]

In the IV route, the risk of thrombophlebitis, infection, and extravasation injuries is probable, which cannot happen with oral.[34] For patients with needle phobia or difficulty with venous access, oral administration is a good route that enhances treatment tolerability.[35] Additionally, home-based treatment reduces stress associated with clinical visits, enhancing psychological well-being.[36]

A favorable treatment will occur by special formulation techniques, oral medication can be designed for controlled release, and also sustained release from a pharmacokinetic perspective.[37] Moreover, oral administration can deliver the drug to a special region of the GI tract, which will be helpful for targeted therapy of tumors in the GI, such as oral carcinoma and colon cancer.[38] Economic studies have demonstrated that oral administration of anti-cancer drugs is more cost-effective in some cases, especially when it is used instead of IV administration.[39] For example, oral vinorelbine or oral capecitabine lowers total annual follow-up costs compared to their IV counterparts.[40]

## 4. Challenges of oral drug delivery for cancer treatment

Oral drug delivery is preferred by patients because it is more convenient and less invasive; however, it encounters several challenges, including GI tract irritation and patient adherence issues, which can affect therapy.[41,42] Some anticancer drugs have low aqueous solubility[43] or can be degraded by the acidic stomach environment and

enzymatic activity.[44] First-pass metabolism can also convert drugs into less effective metabolites. To overcome these barriers, higher doses are often required, which can increase toxicity.[45]

Chronic use of chemotherapy drugs may lead to serious GI tract damage.[46] For example, irinotecan is a chemotherapeutic agent that, upon metabolic activation, produces a toxic metabolite called SN-38. By accumulation of this metabolite in the intestinal lumen, several damages occur, including villous atrophy and crypt ablation in the small intestine, as well as severe colonic mucosal damage, which results in inflammation and chemotherapy-induced diarrhea (CID).[47] Many chemotherapeutic agents have low solubility in aqueous solutions, which causes difficulties for formulating them orally.[48] Also, some drugs have poor absorption from the GI tract because of different barriers, so bioavailability problems are common for these drugs and may result in individual variations.[49] In administering drugs orally, food-drug and drug-drug contraindication have effects that cause more interpersonal differences and also intrapersonal differences.[50]

These challenges are further compounded for agents that are substrates for efflux transporters, such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP); these transporters limit the absorption of these drugs, thus their plasma concentrations decrease.[51] For elderly patients, administering oral anticancer drugs presents additional challenges. Therefore, lowering the frequency of medication taking would be helpful.[52]

Oral administration is widely recognized as the most patient-friendly route for many drugs; however, this advantage has not been fully realized in oncology. The therapeutic efficacy of oral chemotherapeutic agents is often limited by physiological and pharmacological barriers, including GI degradation, poor intestinal permeability, active efflux transport, extensive first-pass metabolism, suboptimal drug physicochemical properties, and dose-limiting toxicity.[53] As a result, only a limited number of anticancer drugs have received Food and Drug Administration (FDA)

approval for oral use, and most of them are listed in Table 1. This gap underscores the urgent need for innovative oral drug delivery systems capable of improving stability, targeting efficiency, bioavailability, and overall therapeutic outcomes. One promising strategy is the use of exosomes as natural nanocarriers to overcome these challenges.[54]

Table 1.FDA-Approved Oral Cancer Drugs

| Year | Brand Name | Generic name | Indication | References |
|---|---|---|---|---|
| 2025 | Ibtrozi | Taletrectinib | ROS1-positive Non-Small Cell Lung Cancer (NSCLC) | 55,56 |
| 2025 | Welireg | Belzutifan | Renal cell carcinoma | 56 |
| 2025 | Nubeqa | Darolutamide | Metastatic castration-sensitive prostate cancer | 55,56 |
| 2025 | Zegfrovy | Sunvozertinib | EGFR exon 20 insertion-mutated metastatic NSCLC | 56 |
| 2024 | Augtyro | Repotrectinib | ROS1-positive NSCLC | 56 |
| 2024 | Lytgobi | Futibatinib | Cholangiocarcinoma with FGFR2 fusions | 56 |
| 2023 | Truqap | Capivasertib | HR-positive, HER2-negative Advanced Breast Cancer | 56 |
| 2023 | Jaypirca | Pirtobrutinib | Relapsed or Refractory Mantle Cell Lymphoma (MCL) | 56 |
| 2022 | Vonjo | Pacritinib | Myelofibrosis with severe thrombocytopenia | 56 |
| 2021 | Scemblix | Asciminib | Philadelphia Chromosome-Positive Chronic Myeloid Leukemia (CML) (Ph+ CML) | 56 |
| 2020 | Retevmo | Selpercatinib | RET-driven Lung and Thyroid Cancers | 56 |
| 2019 | Rozlytrek | Entrectinib | NTRK Fusion-Positive Solid Tumors | 56 |
| 2019 | Tukysa | Tucatinib | HER2-Positive Breast Cancer | 56 |
| 2018 | Vitrakvi | Larotrectinib | NTRK Fusion-Positive Solid Tumors | 56 |
| 2017 | Calquence | Acalabrutinib | Mantle Cell Lymphoma, Chronic Lymphocytic Leukemia | 56 |
| 2016 | Rubraca | Rucaparib | BRCA-Mutant Ovarian Cancer | 56 |
| 2015 | Ibrance | Palbociclib | HR-positive, HER2-negative Breast Cancer | 56 |
| 2015 | Lenvima | Lenvatinib | Thyroid Cancer, Renal Cell Carcinoma | 56 |
| 2014 | Lynparza | Olaparib | BRCA-Mutant Ovarian Cancer | 56 |
| 2014 | Imbruvica | Ibrutinib | Chronic Lymphocytic Leukemia, Mantle Cell Lymphoma | 56 |
| 2013 | Gilotrif | Afatinib | EGFR-Positive NSCLC | 56 |
| 2012 | Xtandi | Enzalutamide | Metastatic Castration-Resistant Prostate Cancer | 56 |
| 2011 | Xalkori | Crizotinib | ALK-Positive NSCLC | 56 |
| 2011 | Zelboraf | Vemurafenib | BRAF V600E Mutation-Positive Melanoma | 56 |

| | | | | |
|---|---|---|---|---|
| 2006 | Sutent | Sunitinib | Gastrointestinal Stromal Tumor (GIST), Renal Cell Carcinoma | 56 |
| 2005 | Nexavar | Sorafenib | Renal Cell Carcinoma, Hepatocellular Carcinoma | 56 |
| 2005 | Revlimid | Lenalidomide | Multiple Myeloma | 56 |
| 2004 | Tarceva | Erlotinib | NSCLC, Pancreatic Cancer | 56 |
| 2001 | Gleevec | Imatinib | CML | 56 |
| 1998 | Xeloda | Capecitabine | Metastatic Breast Cancer, Colorectal Cancer | 56 |

## 5. Exosomes

### 5.1. Biogenesis and properties

Exosomes were first identified in 1983 within the sheep reticulocytes as small extracellular vesicles and they were regarded as a cellular waste product. The term "exosome" was formally introduced by R.M. Johnston in 1987.[9] Exosome biogenesis begins with the formation of intraluminal vesicles (ILVs) within endosomes. This occurs through invagination of the plasmatic membrane, leading to the formation of early endosomes, which then mature into late endosomes. During this maturation process, late endosomes transform into multivesicular bodies (MVBs) that contain ILVs. These MVBs follow different fates within the cell.[57] They may merge with the lysosomes, where ILVs are degraded through the enzymatic reactions, such as hydrolysis. Alternatively, MVBs may move towards the plasma membrane and fuse with it, releasing ILVs into the extracellular space. These released vesicles are known as exosomes.[58]

Exosomes are round-shaped secreted organelles with a diameter ranging from 30 to 200 nm.[59] While they appear spherical in solution, they may take on a biconcave or cup-shaped morphology when artificially dried and arranged for transmission electron microscopy (TEM).[60] The content of exosomes varies depending on their origin, as they contain various cargoes, such as proteins, lipids, and nucleic acids.[61]

Some of the proteins found in exosomes, such as Alix and TSG101, play a role in the Endosomal Sorting Complex Required for Transport (ESCRT) complexes. Additionally, there are over 70 different Rab GTPases present in human exosomes, which are

involved in the transport, secretion, and docking of vesicles. These include Rab 2B, Rab 5A, Rab 7, Rab 11, Rab27a, Rab27b, and Rab35. Other families of GTPases, such as Rho/Rac/cdc42, may also be present in exosomes.[62,63] Tetraspanins, including CD9, CD63, CD81, CD82, CD37, and CD53, are other proteins that play a crucial role in exosome release, penetration, and targeting. Other proteins found in exosomes include lectins, major histocompatibility complex (MHC I and II) proteins, heat shock proteins (HSP), cytoskeleton proteins, glycosylphosphatidylinositol-anchored proteins, and annexins.[59]

The lipid composition of exosomes is also important, as the majority of the bilayer consists of various lipids arranged asymmetrically. Some of the main lipids found in exosomes include cholesterol, sphingomyelins, phosphatidylserine, and glycosphingolipids. Other lipids, such as ceramides, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, may also be present. These lipids play a role in the formation and structure of exosomes, as well as in their secretion, signaling, trafficking, binding, and uptake.[64]

Exosomes naturally encapsulate various types of nucleic acids derived from their parent cells. This cargo includes genomic DNA (such as single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA)), mitochondrial DNA (mtDNA), and numerous forms of ribonucleic acid (RNA), including mRNA, microRNA (miRNA), long non-coding RNA (lncRNA), circular RNA (circRNA), and short hairpin RNA (shRNA).[57,65]

While exosomes were initially thought to be cellular waste products, it has been discovered in recent years that they play a crucial role in transferring signals between cells and facilitating cell-to-cell communication. They can also transport different cargoes to target cells and influence various targets, making them involved in a range of physiological and pathological functions. Additionally, exosomes are involved in intracellular communication and can play a role in gene expression, antigen presentation, immune response, and removal of harmful biomolecules.[66] In viruses, exosomes can also contribute to the spread of viral infections.[67] Exosomes can be

used as therapeutic delivery vehicles in different diseases, such as cancer, neurodegenerative and CNS disorders, cardiovascular diseases, inflammatory and autoimmune diseases, and infectious diseases.[68,69] Also, they can be utilized as biomarkers for non-invasive disease diagnosis,[70] and support tissue and organ regeneration.[71]

### 5.2. Types based on source

Exosomes are derived from various biological sources, including multiple cell types and biological fluids.[72,73] Mesenchymal stem cells (MSCs) are one of the sources that secrete exosomes with regenerative, immunomodulatory, and anti-inflammatory properties of their parent cells.[74] These MSCs can be isolated from various tissues, including bone marrow, adipose tissue, umbilical cord, placenta, liver, and dental tissues such as dental pulp, periodontal ligament, and deciduous teeth, as well as different fluids, each offering a distinct exosomal profile and unique therapeutic potential.[75,76] They contain a broad spectrum of biologically active compounds, including proteins such as tetraspanins (CD9, CD63, CD81, and CD82), HSP70, HSP60,[77] growth factors, cytokines,[78] lipids such as phospholipids, cholesterol, sphingomyelin, and glycosphingolipids, different miRNAs, mRNAs.[79]

Some exosomes are directly isolated from tissues and are known as tissue-derived exosomes (Ti-EVs).[80] Ti-EVs retain tissue-specific signatures, including unique protein, lipid, and nucleic acid cargos, making them valuable tools for studying disease mechanisms, biomarker discovery, and intercellular communication within the in vivo environment.[81] These exosomes can be isolated from cancer tissues,[82] adipose tissues,[83] neural tissues,[84] immune tissues,[85] and liver.[86] They also carry specific pathological or physiological information that can provide insights into their cell of origin.[87] For instance, pancreatic cancer exosomes carry Glypican-1 (GPC1), a biomarker that enables highly specific cancer detection with 100% accuracy,[88] cardiac Ti-EVs transport critical bioactive molecules, including miR-21, miR-1, HSPs, and TGF-β modulators, which contribute to reducing infarct size, suppressing cardiac fibrosis, and enhancing heart function following myocardial infarction.[89-91]

Furthermore, body fluids represent an important source of exosomes, as they are actively secreted into these biofluids by diverse cell types. Exosomes have been identified in a range of biological fluids, including blood, urine, saliva, sweat, bile, semen, amniotic fluid, breast milk, and CSF.[73,92-94] The isolation of body fluid–derived exosomes is more accessible and obtained through less invasive methods compared to other sources.[95] Therefore, they can be used for different purposes. They have been utilized for non-invasive clinical diagnostics.[96] In a 2020 study, researchers used the ExoDx Prostate (IntelliScore) test, also known as the Exosome Prostate IntelliScore (EPI) assay, to assess the risk of high-grade prostate cancer (HGPCa) by analyzing the expression levels of three key biomarker genes: Prostate Cancer Antigen 3 (PCA3), ETS-related gene (ERG), and SAM Pointed Domain Containing ETS Transcription Factor (SPDEF) in urinary exosomes.[97]

Milk-derived exosomes have a unique biological and therapeutic potential. These exosomes are naturally enriched with lipids, proteins, and regulatory RNAs, which support immune development and cellular communication.[7] Their biocompatibility and stability in the GI tract make them particularly attractive as carriers for oral drug delivery and nutritional interventions.[5] Moreover, milk-derived exosomes have shown the ability to cross biological barriers efficiently, enhancing the bioavailability of encapsulated therapeutics.[5] Ongoing research continues to explore their potential in personalized medicine due to their low immunogenicity and capacity for targeted delivery.[7]

Milk exosomes can enhance the growth and colonization of probiotic bacteria such as *Bifidobacterium* and *Lactobacillus* within the infant gut. They also support the maturation of immune tolerance in infants, offering particular benefits for preterm newborns at high risk for immune-related complications. In infants who are unable to consume breast milk, milk exosomes can serve as a bioactive supplement to help prevent gut microbiota dysbiosis (an imbalance in bacterial populations) and reduce the risk of neonatal infections and allergic disorders.[98] Vaccination based on

exosomes derived from body fluids (e.g., blood, milk, exogenous fluids) is an emerging and future-proof platform in medical biotechnology.[99]

## 6. Milk exosomes: properties and advantages

Human and bovine milk are recognized as valuable sources for the isolation of exosomes.[100] Milk exosomes represent a rich and cost-effective source, whereas other sources yield fewer exosomes and present a higher risk of immunogenicity. Milk exosomes exhibit remarkable stability when exposed to the harsh conditions of the GI tract, including acidic environments such as the stomach, pancreas, and bile, as well as various digestive enzymes. Furthermore, they can endure the pasteurization process, highlighting their suitability for oral delivery applications.[101]

As illustrated in Figure 2, milk exosomes contain proteins such as CD9, CD63, CD81, HSP70/90, TSG101, lactadherin, and Rab GTPases, which play a crucial role in regulating immune system functions.[4,102,103] These nano-sized vesicles can influence immune responses by promoting regulatory T cells (Tregs), reducing levels of cytokines such as IL-2 and IFN-γ, and mitigating chronic inflammation.[55,104] These exosomes have emerged as capable nanocarriers for drug delivery due to their ability to pass biological barriers such as the intestinal epithelium, placenta, and even the BBB, enabling their distribution to organs like the brain and liver.[5]

Importantly, milk exosomes exhibit low immunogenicity and high biocompatibility, making them well-tolerated in both human and animal models. Repeated administration has not been associated with adverse immune reactions such as anaphylaxis.[5,103] From an industrial perspective, milk offers a scalable and cost-effective source for exosome isolation. These vesicles demonstrate high stability during storage and remain structurally intact even after pasteurization, which enhances their applicability in pharmaceutical and nutraceutical formulations.[101,103,105] Table 2 provides an overview of selected orally administered exosome-based formulations that have entered clinical trials.

Table 2. Selected clinical trials involving orally administered exosome-based therapeutics

| Trial | Exosome Source | Cargo | Disease | Phase | Reference |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| NCT01294072 | Plant-derived (grape) | Curcumin | Colon cancer | Phase I | [106] |
| NCT04879810 | Plant-derived (grape) | Curcumin | Inflammatory bowel disease | Phase I | [107] |
| NCT05043181 | bone marrow-derived MSCs | LDLR mRNA | Homozygous familial hypercholesterolemia | Phase I | [108] |
| NCT03608631 | Bovine Milk | Paclitaxel | Metastatic Pancreatic Cancer | Phase 1 | [109] |
| NCT01294072 | Plant-derived (grape) | Curcumin | Colon cancer | Phase I | [106] |

## 7. Milk exosomes isolation methods

Several techniques have been developed for the isolation of milk-derived exosomes, such as differential ultracentrifugation, density gradient centrifugation, ultrafiltration, polymer-based precipitation methods, and precipitation kits.[58] These methods are compared in Table 3.

Table 3 Comparing different methods for isolating milk exosomes: Procedures, advantages, and limitations.

| Method | Principle | Key Steps | Advantages | Disadvantages | References |
|---|---|---|---|---|---|
| Differential Ultracentrifugation | Separation based on particle size and density | Low-speed centrifugation (300–2,000 ×g) → Medium-speed (10,000 ×g) → High-speed ultracentrifugation (100,000 ×g) | Gold standard, scalable, reproducible, handles large volumes, no chemical reagents needed | Time-consuming, possible co-isolation of protein aggregates and other vesicles | [110-115] |
| Density Gradient Centrifugation | Separation based on particle density | Pre-centrifugation → Layering on density gradient (sucrose, iodixanol, Percoll) → Ultracentrifugation >100,000 ×g → Collect exosome layer | High purity, separates a broad density range, and is accurate | Time-consuming, low throughput, requires expensive equipment | [116-120] |
| Ultrafiltration | Separation based on particle size using membranes | Pre-treatment (fat removal, casein precipitation) → Pre-filtration (0.22–0.45 µm) → Ultrafiltration (100–500 kDa MWCO) → Washing/purification | Faster, simpler, scalable, preserves exosome integrity, no ultracentrifugation needed | Risk of co-isolation of proteins/vesicles, membrane fouling, may require complementary methods | [113,121-132] |
| Polymer-based Precipitation | Aggregation of exosomes by water-excluding polymers (e.g., PEG) | Add polymer → Incubate 4°C overnight → Low-speed centrifugation → Wash/resuspend pellet | Simple, scalable, minimal specialized equipment, works with complex fluids | Co-precipitation of proteins/other vesicles may require further purification for high purity | [93,121,133,134] |

| | | | | | |
|---|---|---|---|---|---|
| Commercial Precipitation Kits | Polymer-based precipitation (PEG or proprietary polymers) | Add kit reagent → Incubate → Centrifugation → Collect exosomes | Fast, convenient, no specialized equipment, versatile for small/large volumes | Similar to polymer precipitation: possible contaminants, less control over purity | 116,127,135,136 |

### 7.1. Differential ultracentrifugation

Differential ultracentrifugation, the most traditional method, is considered a gold standard for isolating exosomes.[110-112] This technique separates based on differences in particle size and density. By gradually increasing the speed of the centrifuge, cellular components are separated according to their sedimentation rates.[112] The isolation of exosomes from milk is achieved through a multi-step differential centrifugation protocol. The process initiates with low-speed centrifugation (300–2,000 ×g) to eliminate cellular components and large debris. Subsequently, a medium-speed centrifugation step (10,000 ×g) is applied to pellet larger extracellular vesicles, including microvesicles.[113] The final purification is accomplished via high-speed ultracentrifugation (100,000 ×g for 1–2 hours), which sediments the exosomal fraction.[111]

While this method may result in co-isolation of contaminants such as protein aggregates or other extracellular vesicles, differential ultracentrifugation remains the gold standard for exosome isolation due to its well-established protocol, scalability, reproducibility, and capacity to handle large sample volumes without requiring chemical reagents. As such, it is often used as a benchmark for assessing the performance of alternative isolation techniques.[111,115]

### 7.2. Density gradient centrifugation

This method is one of the most common and accurate methods for isolating exosomes from biological fluids or cell culture medium. This operates based on the difference in density of various particles and utilizes centrifugal force.[116] In this method, the sample is first pretreated by sequential centrifugation at lower speeds to remove cells, debris, and larger particles,[116] then it is gently layered onto the density gradient. The density gradient is generated by different concentrations of sucrose, iodixanol, or Percoll solutions.[118] The sample is layered onto the gradient and subjected to ultracentrifugation at high speeds (typically over 100,000 × g) for several hours.[120] Particles in the sample are separated according to their density, forming distinct layers within the gradient.[137] Exosomes typically accumulate at a density range of

1.10–1.19 g/mL.[127] The layer containing the exosomes is carefully collected. This method can separate particles across a broad range of densities with high accuracy and purity; however, it is time-consuming, has low throughput, and requires advanced, expensive equipment.[120]

### 7.3. Ultrafiltration

Ultrafiltration is one of the physical and relatively simple methods for the isolation of exosomes from milk and other biological fluids.[138] This method works based on particle size by membranes with defined pores (typically 100–500 kDa cut-off) to remove larger particles, such as soluble proteins and other undesirable compounds, and to concentrate and purify exosomes.[113,122] For isolating the milk exosome, ultrafiltration is often used as part of a combined process with centrifugation or other methods.[123] Milk pre-treatment involves fat removal and casein protein precipitation, typically achieved via low-speed centrifugation or acidification.[124,125] Following this, pre-filtration is carried out using 0.45 or 0.22 μm filters to eliminate bacteria and large particles.[126,127] Ultrafiltration then concentrates exosomes by passing milk through membranes with specific molecular weight cut-offs (MWCO), effectively removing low-molecular-weight compounds.[128,129] Washing and purification steps, such as buffer exchanges or washes, are optionally performed to further remove residual serum proteins or contaminants.[130] The advantages of ultrafiltration include eliminating the need for ultracentrifugation, reducing time and equipment costs, being faster and simpler than differential centrifugation methods, scalability for industrial production, and better preservation of exosome integrity and functionality compared to harsher techniques.[113,127,131] However, the potential co-isolation of contaminating proteins or other vesicles, the frequent need for complementary techniques like size-exclusion chromatography or immunoaffinity to enhance purity, and the risk of membrane fouling, which may reduce efficiency during repeated cycles.[127,132]

### 7.4. Polymer-based precipitation

Polymer-based precipitation is a common method for isolating exosomes due to its simplicity, scalability, and minimal need for specialized equipment.[139] In this method, water-excluding polymers such as polyethylene glycol (PEG) are added to the milk sample. These polymers reduce the solubility of extracellular vesicles, such as exosomes, by creating a hydrophilic environment that causes them to aggregate and precipitate out of solution.[93]

After adding the polymer solution, the mixture is incubated at 4°C for several hours (typically overnight), followed by low-speed centrifugation (10,000–20,000 ×g) to pellet the exosomes. The pellet can then be washed and resuspended in an appropriate buffer for downstream applications.[133] While this method is efficient for large-scale isolation and works well with complex fluids like milk, it may co-precipitate contaminating proteins or other vesicles. Hence, further purification (e.g., ultracentrifugation or filtration) is sometimes required for downstream applications needing higher purity.[134]

### 7.5. Precipitation kits

Precipitation kits are commercially available products designed to simplify the isolation of exosomes from complex biological fluids such as milk. Exosome isolation kits (for example, ExoQuick™) generally employ polymer-based precipitation agents, such as PEG or proprietary polymers. These agents facilitate the aggregation and precipitation of extracellular vesicles, including exosomes. Polymer-based precipitation kits offer a fast and convenient way to isolate exosomes, often completing the process in just a few hours. These kits are especially appealing because they don't require expensive or specialized equipment; standard laboratory centrifuges are sufficient. In addition, they are highly versatile, allowing researchers to work with both small-scale samples and large volumes, making them suitable for everything from basic research to high-throughput applications.[116,127,135,136]

## 8. Milk exosomes characterization methods

Milk-derived exosomes can be characterized using a variety of techniques. Characterization is essential for understanding their structure, composition, and functional properties. Morphological analysis and size determination are commonly performed using techniques such as TEM, Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA), and Resistive Pulse Sensing (RPS). Biomarker identification is crucial for confirming the exosomal origin and typically involves the detection of surface proteins such as CD9, CD63, and CD81 using flow cytometry or western blotting. Exosome cargo analysis focuses on profiling the diverse lipids, proteins, and regulatory RNAs encapsulated within, often using mass spectrometry (MS) and RNA sequencing techniques. Together, these characterization methods provide a comprehensive understanding of milk-derived exosomes for different applications.[54,140]

### 8.1. Morphology and size

#### 8.1.1. Transmission Electron Microscopy (TEM)

TEM is a gold-standard technique for examining the morphology and structural integrity of exosomes. Studies on milk-derived exosomes describe them as cup-shaped or spherical nanovesicles (30–150 nm). TEM operates by directing a high-energy electron beam through ultra-thin, negatively stained samples, commonly using uranyl acetate, phosphotungstic acid, or lead citrate, which enhance image contrast and prevent vesicle collapse. This technique enables high-resolution visualization, allowing a clear distinction of exosomes from other milk components, such as casein micelles. However, TEM is low-throughput, costly, and sample dehydration may induce structural artifacts. To address this, cryo-TEM was introduced, which preserves native morphology by imaging samples in a rapidly frozen, hydrated state, offering improved structural accuracy.[141-146]

#### 8.1.2. Scanning Electron Microscopy (SEM)

SEM is used to analyze the surface morphology and topology of milk-derived exosomes by imaging them under a high-energy electron beam. After isolation,

exosomes are fixed (e.g., with glutaraldehyde), dehydrated, and coated with a conductive layer such as gold or platinum to enhance imaging and prevent charging. SEM enables high-resolution visualization of surface features and contaminants and can provide semi-quantitative insights into size distribution. Compared to the TEM method, SEM cannot reveal internal structure, and sample preparation steps may introduce artifacts or lead to misinterpretation of native morphology.[147-149]

#### 8.1.3. Dynamic Light Scattering (DLS)

DLS determines the size distribution and polydispersity index (PDI) of milk-derived exosomes by analyzing fluctuations in light scattering caused by their Brownian motion.[58] Following isolation, exosomes are resuspended in filtered PBS and passed through 0.22 µm filters to eliminate casein micelles and aggregates that may interfere with signal accuracy. DLS is rapid, non-destructive, and easy to operate, making it suitable for routine characterization. However, it offers limited resolution, cannot distinguish exosomes from similarly sized particles.[4,102,150]

#### 8.1.4. Nanoparticle Tracking Analysis (NTA)

NTA is based on the Brownian motion of exosomes, similar to DLS, and combines this principle with laser light scattering microscopy. In this technique, after purification and filtration of the milk sample, it is illuminated with a laser beam. Exosomes in the sample scatter the laser light and appear as bright points under a video microscope. Subsequently, the software tracks the movement of individual particles over time. This method can measure the true size, size distribution, and concentration of milk exosomes. NTA is a high-resolution method that can even detect individual exosomes and enable direct particle movement and behavior. Unlike DSC, NTA can calculate the concentration of exosomes and detect fluorescently labeled exosomes. Nevertheless, it has limitations in detecting exosomes smaller than 50 nm and, compared to DLS, offers lower throughput.[6,151,152]

#### 8.1.5. Resistive Pulse Sensing (RPS)

RPS, also known as nanopore sensing, is an analytical technique based on monitoring the transient changes in ionic current when exosomes pass through a micro- or

nanopore filled with electrolyte solution. A constant voltage is applied across the pore, generating an ionic current. As an exosome passes through the pore, it displaces ions and temporarily reduces the current, producing a resistive pulse. The amplitude of the pulse correlates with the particle size, and the number of pulses indicates the concentration of exosomes in the milk sample.[153]

After isolating milk exosomes, they are resuspended in an appropriate electrolyte buffer. The suspension is then introduced into the RPS device, where particle-by-particle measurement is performed. This approach provides quantitative information on size distribution and particle count without fluorescent labeling, making it highly suitable for assessing the heterogeneity of milk exosome populations. RPS technique gives information on size distribution, concentration, and surface charge.[153-155]

### 8.2. Biomarkers

Biomarkers are one of the key features used to characterize milk-derived exosomes. Flow cytometry and western blot are two common methods used for detecting biomarkers. In flow cytometry, milk exosomes suspended in a liquid are passed through a laser. Their optical properties, specifically light scattering and fluorescence emission, are then measured by detectors. It can be used for detecting and quantifying biomarkers. This method is high-throughput and can detect different markers simultaneously using different fluorescent dyes. However, it requires expensive equipment, and there is a possibility of interference with other vesicles and particles.

Western blot is the critical gold-standard method used to confirm the identity, purity, and presence of specific biomarkers of exosomes in a sample after isolation. Proteins extracted from exosomes are first separated based on their size using gel electrophoresis. After separation, they are transferred onto a membrane, and specific antibodies are used to detect the target biomarkers. Western blot is a highly specific and widely used technique for the detection of exosomal proteins. Unlike flow cytometry, it can detect both surface and internal proteins. Although the western blot is a semi-quantitative and time-consuming method that requires a relatively large

amount of protein, it remains a gold standard for characterizing biomarkers in milk-derived exosomes.[102,156-159]

### 8.3. Exosome cargo analysis

Proteomics analysis is used for detecting different protein cargoes encapsulated within milk exosomes. In this technique, after isolating milk exosomes, the membranes of exosomes are lysed, and their protein content is extracted, digested with trypsin into peptides, and prepared for MS. Liquid chromatography-tandem MS (LC-MS/MS) is then employed to separate these peptides, fragment them, and identify them by matching their mass-to-charge ratios against protein databases. This method is useful for the identification and quantification of proteins, revealing functional components involved in immune modulation, signaling pathways, and metabolism. Proteomic analysis of milk exosomes provides high-resolution insight into their molecular composition, enabling researchers to understand their functional roles and biological significance.[160-163]

## 9. Milk exosomes for oral drug delivery in cancer treatment

A meta-analysis by Amitay et al. concluded that breastfeeding for a duration of six months or more is associated with a 19% reduction in the incidence of childhood leukemia.[164] Additionally, research has shown that bovine milk exosomes possess anti-cancer properties by inhibiting the proliferation of cancer cells, including lung, prostate, colon, pancreatic, breast, and ovarian, indicating the potential of these exosomes as anti-cancer drug delivery systems.[165] Oral drug delivery of milk-derived exosomes displays significant advantages for cancer treatment, as they can freely cross the barriers of the GI tract.[166]

### 9.1. Milk exosomes for oral drug delivery in lung cancer treatment

Lung cancer is the leading cause of cancer-related deaths worldwide, with a low five-year survival rate, particularly in cases of metastatic lung cancer.[167] Exosome-based treatment strategies are currently being explored as a novel and biologically driven approach. As an example, celastrol is a terpenoid with anti-cancer effects. However, its efficiency is limited by its low oral bioavailability.[168] Accordingly, in a study,

researchers isolated exosomes from bovine milk and encapsulated celastrol within them (Exo-CEL). The Exo-CEL exosomes were then orally administered to mice with lung cancer, and were found to have superior anti-tumor activity compared to celastrol and a control group. This is likely due to the higher bioavailability of celastrol when encapsulated in exosomes.[169]

In another study by Agrawal et al., the bovine milk exosomes were used to encapsulate paclitaxel (ExoPAC). Both naked exosomes and ExoPAC were found to be stable in simulated GI fluids. The researchers then compared the effectiveness of orally administered ExoPAC and intraperitoneally administered ExoPAC in treating lung cancer in nude mice. The results showed that oral administration was twice as effective in inhibiting tumor growth compared to IP injection. Additionally, encapsulating paclitaxel in exosomes led to reduced toxicity against erythrocytes, the kidney, and the liver. Furthermore, ExoPAC was found to have lower immune toxicity compared to paclitaxel, as it did not cause a reduction in bone marrow cell numbers.[170]

Targeted therapy, a kind of personalized medicine, aims to decrease the side effects of anti-cancer agents on normal cells.[168] Munagala and colleagues employed folic acid as a targeting ligand and withaferin A as a chemotherapeutic agent, co-loading both into exosomes derived from bovine milk. These engineered exosomes were administered to athymic nude mice bearing human lung cancer tumors via either the oral or IV route. The administration pathway profoundly influenced the resulting tissue biodistribution. Intravenously injected exosomes accumulated primarily in the liver. In contrast, orally delivered exosomes demonstrated a wider distribution, with detectable levels found in the liver, spleen, kidneys, ovaries, pancreas, colon, and brain. The oral delivery of milk exosomes induced no observed short-term or long-term toxicity.

Furthermore, in the human lung cancer mouse model, oral administration of withaferin-A-loaded exosomes functionalized with folic acid resulted in a significantly

greater ($p = 0.016$) suppression of tumor growth compared to exosomes loaded only with withaferin A. This enhanced antitumor efficacy is likely attributable to the active targeting mediated by the folic acid ligand.[171]

Colostrum, the first milk released from the breast immediately after giving birth, contains higher amounts of exosomes compared to milk.[172] In a study by Kandimalla and colleagues, paclitaxel was encapsulated within colostrum-derived exosomes conjugated with folic acid to mitigate the dose-dependent toxicity associated with solvent-based paclitaxel formulations. The resulting complex, folic acid-conjugated exosomes loaded with paclitaxel, was administered orally to nude mice bearing lung cancer tumors. Results demonstrated that oral delivery of this complex produced a significant inhibition of tumor growth ($p < 0.001$), reducing it by fifty percent. Furthermore, in non-obese diabetic and severe combined immunodeficient (NOD/SCID) nude mice with lung cancer, orally administered folic acid-conjugated paclitaxel exosomes induced a substantially greater suppression of tumor growth compared to intravenously delivered solvent-based paclitaxel. However, IV administration of the folic acid-conjugated paclitaxel exosomes itself resulted in even higher antitumor efficacy.[173] These studies confirm the stability of milk exosomes in the GI tract, their efficacy in the treatment of lung cancer, and their ability to reduce the adverse effects of anti-cancer drugs.

### 9.2. Milk exosomes for oral drug delivery in breast cancer treatment

Breast cancer is one of the most common types of cancer. In a study by Badawy et al., the anticancer effects of isolated camel milk exosomes were investigated both in vivo and in vitro. The results showed that these exosomes were able to decrease the migration and proliferation of the Michigan Cancer Foundation (MCF-7) cell line. Furthermore, when orally administered to female rats with breast cancer, the exosomes led to a decrease in tumor weight, an increase in DNA damage, and apoptosis in the tumor tissues, compared to both untreated rats and rats treated with camel milk. The induction of apoptosis was mediated by an increase in caspase 3 activity, downregulation of B-cell lymphoma 2 (BCL-2) mRNA levels, and upregulation

of BCL2 Associated X (BAX) mRNA levels. Moreover, the study found that oral administration of milk exosomes reduced oxidative stress, angiogenesis, metastasis, and inflammation in the tumor tissues. However, the number of CD4+, CD8+, and Natural killer (NK1.1) T cells in the spleen of rats that were treated with oral milk exosomes was lower than that in rats that were treated with milk, showing the presence of other immune stimulators in the camel milk. This study confirms the efficiency of orally administered camel milk exosomes in the treatment of breast cancer by promoting immune response and inducing apoptosis in tumor cells, while also reducing oxidative stress, metastasis, and inflammation in tumor tissue.[174]

### 9.3. Milk exosomes for oral drug delivery in ovarian cancer treatment

The prognosis for ovarian cancer remains poor due to the development of drug resistance and significant systemic toxicity associated with chemotherapy.[175] Anthocyanins have been shown to have anti-cancer effects; however, their effectiveness is limited by their low bioavailability.[176] To address this issue, Aqil et al. encapsulated berry Anthocyanins in the milk exosomes. Nude mice with ovarian cancer were treated with oral Anthocyanin milk exosomes or free Anthocyanin at the same dosage. The results showed that Anthocyanin milk exosomes had a significantly higher ($p < 0.01$) tumor growth inhibition effect compared to Anthocyanin, indicating their high stability, uptake, and circulation time. Furthermore, the combination therapy of paclitaxel-loaded exosomes and anthocyanin-loaded exosomes resulted in a significantly greater ($p < 0.001$) anti-tumor effect compared to the monotherapies of either agent alone. This study suggests that oral administration of drug-loaded milk exosomes is a promising approach for treating ovarian cancer. Furthermore, combination therapy with exosomes may also help overcome drug resistance.[177]

### 9.4. Milk exosomes for oral drug delivery in melanoma treatment

Melanoma is a highly aggressive form of skin cancer that arises from the malignant transformation of melanocytes, the pigment-producing cells.[178] Dihydroartemisinin (DHA), a low-soluble derivative of artemisinin known for its anti-malaria effect, has shown a promising therapeutic effect against melanoma by promoting apoptosis and

autophagy while inhibiting metastasis and angiogenesis.[179] Kumar et al. encapsulated DHA in bovine milk exosomes (EXO_DHA) to increase its oral bioavailability. The Sprague-Dawley rats received EXO_DHA or DHA orally. The results showed that maximum plasma concentration ($C_{max}$), ($p < 0.01$), time to peak concentration ($T_{max}$), ($p < 0.05$), and area under the curve (AUC), ($p < 0.001$), of EXO_DHA were significantly higher than those of DHA. This increased oral bioavailability is likely due to the lower degradation of exosomes in the stomach and their improved absorption compared to DHA. In addition, encapsulating DHA within milk exosomes decreased the accumulation of DHA in the liver, thereby decreasing its hepatotoxicity. It should be mentioned that the treatment of melanoma is limited by chemotherapy-induced hepatotoxicity. In the next part of the study, the efficacy of EXO_DHA in the treatment of melanoma in mice was evaluated. The mice bearing melanoma were given DHA, EXO_DHA, dacarbazine, or naked exosomes orally. The results showed that EXO_DHA had a higher percentage of tumor growth inhibition compared to DHA and dacarbazine. EXO_DHA also had the longest tumor volume doubling time and the lowest tumor weight/volume among all the groups.[180] This study further confirms the potential of exosomes to sustain the anti-cancer effects of chemotherapy drugs, increase their ability to inhibit tumor growth, and decrease their adverse effects on normal tissues. These studies are summarized in Table 4.

Table 4.Summary of studies on oral anticancer drug encapsulation in milk exosomes

| Exosome Source | Drug | Encapsulation Method | Encapsulation Efficiency (EE%) | In Vitro Outcomes | In Vivo Outcomes | References |
|---|---|---|---|---|---|---|
| Milk | Celastrol | Simple incubation | 18-20% | Enhanced Anti-Proliferative Effect<br>Induction of Apoptosis<br>Increased expression of cdc25B and p21.<br>Induction of ER Stress and Unfolded Protein Response (UPR) | Superior inhibition of lung tumor growth in xenograft mice compared to free drug.<br>No significant systemic toxicity | 169 |
| Bovine Milk | Paclitaxel (PTX) | Simple incubation | 8% | Enhanced cellular uptake and cytotoxicity in A549 and MD-MB-231 cells. | Significant tumor growth inhibition in lung and breast cancer mouse xenograft models after oral administration. | 170 |
| Bovine Milk | Withaferin A (WA) & Paclitaxel (PTX) | Simple incubation | 10-40% | Dose-dependent cytotoxicity and induction of apoptosis in A549 and MD-MB-231 cells.<br>Enhanced anti-cancer and anti-inflammatory effects by exosomal delivery | Significant inhibition of lung and breast tumor growth in mouse xenograft models following oral delivery. | 171 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Bovine Colostrum | Paclitaxel (PTX) | Simple incubation | 89,8.8% | Enhanced cytotoxicity and cellular uptake in lung (A549) and breast (MD-MB-231) cancer cells. | Oral delivery effectively inhibited tumor growth in lung and breast cancer mouse models; superior to IV paclitaxel. | 173 |
| Camel Milk | Not a drug (whole exosomes) | Not Applicable (Natural exosomes) | Not Applicable | Inhibited proliferation and induced apoptosis in MCF-7 breast cancer cells. | Reduced tumor volume and weight in a rat model of breast cancer. | 174 |
| Bovine Milk | Berry Anthocyanidins (Anthos) | Simple incubation | Not specified | Significant reduction in cell viability and increased apoptosis in A2780 ovarian cancer cells. | Marked inhibition of tumor growth in an A2780 xenograft mouse model following oral administration. | 177 |
| Bovine Milk | Dihydroartemisinin (DHA) | Sonication | ~70% | Enhanced anti-proliferative and pro-apoptotic effects in B16F10 melanoma cells. | Oral delivery significantly suppressed tumor growth and prolonged survival in a B16F10 melanoma mouse model. | 180 |

## 10. Challenges in the industrial production of milk exosomes

The transition of milk-derived exosomes from laboratory research to industrial-scale production presents several difficult scientific and technical challenges. The studies, while demonstrating notable efficacy, also implicitly highlight these critical challenges:

- **Scalable and efficient drug loading：** A major challenge is creating drug loading methods that work on a large scale. The studies show that simple incubation is commonly used for drugs like celastrol and paclitaxel.[169,170,177] However, this passive method often leads to low drug encapsulation efficiency and loading capacity, which may not be strong enough for human treatments. While alternative methods like electroporation exist, they can damage the exosomes and are difficult to control in large batches.
- **Scalable and reproducible isolation:** Developing methods that provide high yield with minimal contamination (caseins, whey proteins, lipoproteins). Also, high-purity techniques are often low-yielding and expensive, necessitating new technologies for industrial production.[181]
- **Standardization of source material and isolation：** There are no uniform global standards for where to get exosomes or how to isolate them. Researchers use different sources such as bovine milk, colostrum, and camel milk.[170,173,174] Although milk is abundant, its exosomes can vary from batch to batch in quantity and quality. Standard isolation methods like ultracentrifugation are not easily scalable and make it hard to produce identical exosomes every time. Also, ultracentrifugation is low-throughput, costly, and may damage exosome structure and function by inducing shear stress.[180,181]
- **Milk variability:** Composition changes with lactation stage, dietary patterns, seasonal changes, and breed, impacting batch-to-batch consistency.
- **Comprehensive biodistribution and safety profiling：** We need a better understanding of what happens to exosomes inside the body. While animal

studies show they are effective and safe in the short term,[169,180] data is often missing on their long-term safety, how they are distributed in the body, and if they cause immune reactions after repeated use. This lack of information makes it difficult to predict their safety and behavior in humans.

- **Cost-Effective and good manufacturing practice (GMP)-compliant manufacturing :** Setting up a large-scale, cost-effective manufacturing process that meets strict GMP standards is a significant hurdle. The current process, involving sourcing, isolation, drug loading, and purification, is complex, time-consuming, and expensive. Relying on ultracentrifugation is not practical for mass production, necessitating the development of scalable technologies. Difficulties in validating exosome identity, purity, dosage, and sterility; GMP requirements increase complexity.[182]
- **Reproducible characterization and quality control :** To meet regulatory standards, exosome products must be consistently and thoroughly characterized. While research studies check for size and some markers,[170,173] industrial production requires a much stricter set of quality controls. This includes testing for potency, purity, stability (shelf-life), and ensuring no harmful residues are left from the manufacturing process.
- **Stability concerns:** Exosomes are sensitive to storage conditions, handling, and formulation.
- **Regulatory pathway:** Exosomes do not fit into existing regulatory categories. It is unclear whether they should be classified as biologics, drug delivery systems, or something else. This lack of a clear regulatory pathway, combined with the need to define their exact mechanism of action, creates significant uncertainty and delay for their approval and commercialization.

## 11. Future prospective

Milk exosomes function as a naturally optimized platform for oral nanomedicine; however, their full therapeutic potential in oncology remains underexploited. To date, most studies have focused on passive delivery or simple surface modification of milk

exosomes using single ligands such as folic acid to enhance tumor uptake and reduce off-target toxicity. While these strategies have demonstrated encouraging outcomes, next-generation targeting approaches should move toward multivalent ligand engineering, incorporating tumor-homing peptides, monoclonal antibodies, or dual-receptor targeting motifs to achieve highly selective tissue accumulation and minimize exposure to healthy tissues.[182,183]

Exosome-nanoparticle hybrid nanoplatforms can be developed by combining exosomes with different types of nanoparticles to increase the encapsulation efficiency and production yield.[58] Conversely, nanoparticles, especially polymeric nanoparticles, retain numerous favorable features for oral drug delivery.[184] Therefore, the development of hybrid nanoplatforms consisting of milk exosomes and polymeric nanoparticles shows promise as a potential approach for cancer treatment through oral administration.

Modular engineering of milk exosomes, including surface display of programmable ligands, gastrointestinal barrier-responsive coatings, and integration with stimuli-sensitive polymeric networks, could establish smart oral delivery systems capable of precise spatiotemporal drug release. Additionally, coupling these platforms with artificial intelligence (AI)-guided ligand screening and high-throughput exosome–drug interaction profiling may further accelerate the rational design of targeted oral cancer nanotherapeutics.[185,186] In addition to therapeutic delivery, AI-engineered exosomes are increasingly being investigated as intelligent diagnostic vesicles, where machine learning–guided surface modification enables selective recognition of tumor biomarkers and real-time disease monitoring.[187]

## 12. Conclusion

Significant physiological and biochemical barriers, including enzymatic degradation, poor intestinal permeability, and active efflux mechanisms, fundamentally obstruct the effectiveness of oral chemotherapeutic regimens. As extensively discussed, the route of administration is a

critical determinant in cancer therapy, influencing not only patient compliance and quality of life but also the pharmacokinetic profile and therapeutic index of anticancer agents. While IV delivery remains a mainstay, it is often associated with systemic toxicity and requires clinical administration.

In this context, milk-derived exosomes emerge as a highly promising and naturally engineered platform for oral drug delivery. Their innate biogenesis gives them stability in the harsh gastrointestinal environment, an exceptional capacity to cross biological membranes, and biocompatibility that mitigates the risk of adverse immune reactions. We have detailed the methodologies for their isolation, from gold-standard ultracentrifugation to scalable precipitation techniques, and characterization, which are paramount for ensuring batch-to-batch reproducibility and clinical translation. Milk exosomes, loaded with chemotherapeutic agents, have been demonstrated preclinically across a spectrum of malignancies, including lung, breast, ovarian cancer, and melanoma.

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## References


1. Yin W, Ma H, Qu Y, Ren J, Sun Y, Guo ZN, et al. Exosomes: The next-generation therapeutic platform for ischemic stroke. *Neural Regen Res* 2025;20(5):1221–35. doi: 10.4103/nrr.Nrr-d-23-02051
2. Barathan M, Ng SL, Lokanathan Y, Ng MH, Law JX. Milk-derived extracellular vesicles: A novel perspective on comparative therapeutics and targeted nanocarrier application. *Vaccines (Basel)* 2024;12(11). doi: 10.3390/vaccines12111282
3. Grobler L, O'Connor D, Rischin D, Putrik P, Karnon J, Rischin K, et al. Delivery of intravenous anti-cancer therapy at home versus in hospital or community settings for adults with cancer. *status and date: New, published in* 2025(4).
4. Yang H, Wuren T, Zhai BT, Liu Y, Er D. Milk-derived exosomes in the regulation of nutritional and immune functions. *Food Sci Nutr* 2024;12(10):7048–59. doi: http://dx.doi.org/10.1002/fsn3.4323
5. Amthaniwala BK, Mohamed ZI, Vllasaliu D. Therapeutic potential of naïve and engineered milk extracellular vesicles. *Health Nanotechnol* 2025;1(1):5. doi: http://dx.doi.org/10.1186/s44301-025-00005-5
6. Marsh SR, Beard CE, Gourdie RG. Milk extracellular vesicles: A burgeoning new presence in nutraceuticals and drug delivery. *Bioeng Transl Med* 2025;10(3):e10756. doi: 10.1002/btm2.10756
7. Kong C, Huang LB, Yang MF, Yue NN, Zhang Y, Tian CM, et al. Milk-derived extracellular vesicles: Nature's nanocarriers for drug delivery and therapeutics. *Front Pharmacol* 2025;16:1595891. doi: 10.3389/fphar.2025.1595891
8. Timofeeva AM, Paramonik AP, Sedykh SS, Nevinsky GA. Milk exosomes: Next-generation agents for delivery of anticancer drugs and therapeutic nucleic acids. *International Journal of Molecular Sciences* 2023;24(12):10194.
9. Zhong J, Xia B, Shan S, Zheng A, Zhang S, Chen J, et al. High-quality milk exosomes as oral drug delivery system. *Biomaterials* 2021;277:121126. doi: https://doi.org/10.1016/j.biomaterials.2021.121126
10. Amjad MT, Chidharla A, Kasi A. Cancer chemotherapy. Statpearls. Treasure Island (FL): StatPearls Publishing





11. Haumschild R, Kennerly-Shah J, Barbarotta L, Zeidan AM. Clinical activity, pharmacokinetics, and pharmacodynamics of oral hypomethylating agents for myelodysplastic syndromes/neoplasms and acute myeloid leukemia: A multidisciplinary review. *J Oncol Pharm Pract* 2024;30(4):721–36. doi: 10.1177/10781552241238979
12. Chen Q, Zhang B, Dong Y, Mo X, Zhang L, Huang W, et al. Comparison between intravenous chemotherapy and intra-arterial chemotherapy for retinoblastoma: A meta-analysis. *BMC Cancer* 2018;18(1):486. doi: http://dx.doi.org/10.1186/s12885-018-4406-6
13. Stoner KL, Harder H, Fallowfield LJ, Jenkins VA. Intravenous versus subcutaneous drug administration. Which do patients prefer? A systematic review. *Patient* 2014. doi: http://dx.doi.org/10.1007/s40271-014-0075-y
14. Zhang H, Ma J, Pan F, Liu Y, Zhang M, Li Y, et al. Prediction of high-performing spleen-targeted lipid nanoparticles using a deep learning model for robust anticancer immunotherapy. *J Mater Chem B* 2025. doi: https://doi.org/10.1039/D5TB01217A
15. Jacquot G, Lopez Navarro P, Grange C, Boudali L, Harlepp S, Pivot X, et al. Landscape of subcutaneous administration strategies for monoclonal antibodies in oncology. *Adv Mater* 2024;36(40):2406604. doi: https://doi.org/10.1002/adma.202406604

16. Abou Chakra M, Luo Y, Duquesne I, O'Donnell MA. Update on the mechanism of action of intravesical bcg therapy to treat non-muscle-invasive bladder cancer. *Front Biosci Landmark* 2024;29(8):295. doi: https://doi.org/10.31083/j.fbl2908295
17. Saito A, Kitayama J, Nagai R, Aizawa K. Anatomical targeting of anticancer drugs to solid tumors using specific administration routes: Review. *Pharmaceutics* 2023;15(6). doi: http://dx.doi.org/10.3390/pharmaceutics15061664
18. Chakraborty P, Roy N, Biswas P, Biswas DS, Datta HK, Dutta A, et al. Exploiting urea-carboxylate synthon for designing supramolecular topical hydrogel via simple organic salt formation: Synthesis, crystal structures, and anticancer behavior against melanoma b16–f10 cells. *Chem Mater* 2024;36(15):7317–31. doi: https://doi.org/10.1021/acs.chemmater.4c01225
19. Rane B, Gawade S. Transdermal drug delivery in oncology charting the road ahead. *Hacet Univ J Fac Pharm* 2025;45(3):286–99. doi: https://doi.org/10.52794/hujpharm.1611956
20. Mittal S, Goorman E, Schreidah C, Nguyen C, Choi J, Zheng L. Lb1145 evaluating intralesional bleomycin for kaposi sarcoma: A retrospective study. *J Investig Dermatol* 2025;145(8):S199. doi: https://doi.org/10.1016/j.jid.2025.06.1434
21. Souri M, Elahi S, Soltani M. Intratumoral implantable drug delivery system for targeted localized chemotherapy in breast cancer. *J Drug Deliv Sci Technol* 2024;94:105519. doi: https://doi.org/10.1016/j.jddst.2024.105519
22. Leelakanok N, Atchaneeyasakul L-o, Songsaeng D, Methaneethorn J, Sanpakit K, Buaboonnam J. Metastatic death following ophthalmic artery chemotherapy for retinoblastoma: A systematic review and meta-analysis. *Siriraj Med J* 2024;76(3):144–51. doi: https://doi.org/10.33192/smj.v76i3.266573
23. Harbi E, Yarar E, Mason CE, Aschner M, Altundag A. Intranasal terpene treatment for glioblastoma: The neuro-oncological potential of perillyl alcohol. *Neurochem Res* 2025;50(4):1–11. doi: https://doi.org/10.1007/s11064-025-04505-9
24. Oedingen C, van Gestel R, Huls SPI, Granic G, de Bekker-Grob EW, Veldwijk J. Association of medication adherence with treatment preferences: Incentivizing truthful self-reporting. *Eur J Health Econ* 2025. doi: http://dx.doi.org/10.1007/s10198-025-01760-z
25. Price G, Patel DA. Drug bioavailability: StatPearls Publishing, Treasure Island (FL); 2025 2025.
26. Nasirizadeh S, Malaekeh-Nikouei B. Solid lipid nanoparticles and nanostructured lipid carriers in oral cancer drug delivery. *J Drug Deliv Sci Technol* 2020;55:101458. doi: https://doi.org/10.1016/j.jddst.2019.101458
27. Al Khawaldeh TA, Wazaify M. Intravenous cancer chemotherapy administration errors: An observational study at referral hospital in jordan. *Eur J Cancer Care (Engl)* 2018;27(4):e12863. doi: http://dx.doi.org/10.1111/ecc.12863
28. Antúnez-Blancat A, Gago-Valiente FJ, García-Iglesias JJ, Merino-Navarro D. The role of nursing in the management of chemotherapy extravasation: A systematic review regarding public health. *Healthcare (Basel)* 2024;12(14). doi: http://dx.doi.org/10.3390/healthcare12141456
29. Piekiełko P, Mucha A, Stawowczyk E, Wójkowska-Mach J. Peripheral intravenous therapy in internal medicine department-antibiotics and other drugs' consumption and characteristics of vascular access devices in 2-year observation study. *Antibiotics (Basel)* 2024;13(7). doi: http://dx.doi.org/10.3390/antibiotics13070664
30. Tejedor Tejada E, Gonzalez Suárez S, Lizondo López T, López-Cabezas C, Soy Muner D. Alternatives for the administration of oral antineoplastics in patients with swallowing

difficulties. *J Chemother* 2025;37(4):293–306. doi: https://doi.org/10.1080/1120009X.2024.2354621
31. Hanna K, Mayden K. The use of real-world evidence for oral chemotherapies in breast cancer. *J Adv Pract Oncol* 2021;12(Suppl 2):13–20. doi: http://dx.doi.org/10.6004/jadpro.2021.12.2.12
32. Segin Chandran AB, Pandiar D, Krishnan RP, Gopinath D. Efficacy of oral metronomic chemotherapy in the management of head and neck squamous cell carcinoma-a systematic review. *Front Oral Health* 2025;6:1632316. doi: https://doi.org/10.3389/froh.2025.1632316
33. Jacobs JM, Ream ME, Pensak N, Nisotel LE, Fishbein JN, MacDonald JJ, et al. Patient experiences with oral chemotherapy: Adherence, symptoms, and quality of life. *J Natl Compr Canc Netw* 2019;17(3):221–8. doi: http://dx.doi.org/10.6004/jnccn.2018.7098
34. Scarano M, D'Arrigo S, De Letteriis S, Grasso S, Pittiruti M, Scoppettuolo G. Risk of thrombophlebitis associated with continuous peripheral infusion of vancomycin: The effect of dilution. *J Vasc Access* 2024;25(1):107–12. doi: http://dx.doi.org/10.1177/11297298221095778
35. Dychter SS, Gold DA, Carson D, Haller M. Intravenous therapy: A review of complications and economic considerations of peripheral access. *J Infus Nurs* 2012;35(2):84–91. doi: http://dx.doi.org/10.1097/NAN.0b013e31824237ce
36. Ahmed S, Maheu C, Gotlieb W, Batist G, Loiselle CG. Feasibility, acceptability, and potential effects of a digital oral anticancer agent intervention: Protocol for a pilot randomized controlled trial. *JMIR Res Protoc* 2025;14:e55475. doi: http://dx.doi.org/10.2196/55475
37. Andrade C. Sustained-release, extended-release, and other time-release formulations in neuropsychiatry. *J Clin Psychiatry* 2015;76(8):e995–9. doi: http://dx.doi.org/10.4088/JCP.15f10219
38. Lou J, Duan H, Qin Q, Teng Z, Gan F, Zhou X, et al. Advances in oral drug delivery systems: Challenges and opportunities. *Pharmaceutics* 2023;15(2). doi: http://dx.doi.org/10.3390/pharmaceutics15020484
39. Sohi GK, Levy J, Delibasic V, Davis LE, Mahar AL, Amirazodi E, et al. The cost of chemotherapy administration: A systematic review and meta-analysis. *Eur J Health Econ* 2021;22(4):605–20. doi: http://dx.doi.org/10.1007/s10198-021-01278-0
40. Noxon V, Wu J. The costs of oral versus intravenous chemotherapy in insured, low income patients with breast or colon cancer. *Value in Health* 2013;16(3):A133. doi: http://dx.doi.org/10.1016/j.jval.2013.03.648
41. Asar TO, Al-Hejaili OD, El-Sawy HS, Abd-Allah FI, Omar AM, Ahmed TA, et al. From oral to sublingual: A redefined avanafil tablet with a breakthrough in bioavailability and first-pass metabolism avoidance. *Drug Des Devel Ther* 2025:2551–76. doi: https://doi.org/10.2147/DDDT.S504291
42. Song T, Yuan L, Wang J, Li W, Sun Y. Advances in the transport of oral nanoparticles in gastrointestinal tract. *Colloids Surf B Biointerfaces* 2025;245:114321. doi: https://doi.org/10.1016/j.colsurfb.2024.114321
43. Almawash S. Oral bioavailability enhancement of anti-cancer drugs through lipid polymer hybrid nanoparticles. *Pharmaceutics* 2025;17(3):381. doi: https://doi.org/10.3390/pharmaceutics17030381
44. Ahmed Saeed Al-Japairai K, Mahmood S, Hamed Almurisi S, Reddy Venugopal J, Rebhi Hilles A, Azmana M, et al. Current trends in polymer microneedle for transdermal drug delivery. *Int J Pharm* 2020;587:119673. doi: http://dx.doi.org/10.1016/j.ijpharm.2020.119673

45. Mandić-Kovacević N, Kasagić-Vujanović I, Gatarić B, Škrbić R, Popović Bijelić A. Study of the acidic, basic, and thermal degradation kinetics of three antihypertensive drugs-individually and in combination. *Pharmaceutics* 2024;16(11). doi: http://dx.doi.org/10.3390/pharmaceutics16111410
46. Eisenmann ED, Talebi Z, Sparreboom A, Baker SD. Boosting the oral bioavailability of anticancer drugs through intentional drug–drug interactions. *Basic Clin Pharmacol Toxicol* 2022;130:23–35. doi: https://doi.org/10.1111/bcpt.13623
47. Arendt N, Lennernäs H, Heindryckx F, Sjöblom M. Irinotecan induces intestinal atrophy and compromises duodenal motility and mucosal permeability: In vivo and in vitro evaluations. *bioRxiv* 2025:2025.03. 14.643345. doi: https://doi.org/10.1101/2025.03.14.643345
48. Rizwan D, Masoodi FA. Brassica-derived isothiocyanates as anticancer therapeutic agents and their nanodelivery. *Phytother Res* 2024;38(1):331–48. doi: http://dx.doi.org/10.1002/ptr.8042
49. Hasan N, Aftab M, Ullah M, Nguyen PT, Agustina R, Djabir YY, et al. Nanoparticle-based drug delivery system for oral cancer: Mechanism, challenges, and therapeutic potential. *Results Chem* 2025;14:102068. doi: https://doi.org/10.1016/j.rechem.2025.102068
50. Stevens M. Pharmacokinetics and toxicity of anticancer drugs. *Am J Pharm Pharmacol* 2024;5(1):5–8.
51. Rani P, Mandal P, Rajak BK, Singh DV. A review on dynamics of permeability-glycoprotein in efflux of chemotherapeutic drugs. *Front Drug Discov* 2024;4:1363364. doi: https://doi.org/10.3389/fddsv.2024.1363364
52. Rousseau A, Géraud A, Geiss R, Farcet A, Spano JP, Hamy AS, et al. Safety of solid oncology drugs in older patients: A narrative review. *ESMO Open* 2024;9(11):103965. doi: http://dx.doi.org/10.1016/j.esmoop.2024.103965
53. Prieložná J, Mikušová V, Mikuš P. Advances in the delivery of anticancer drugs by nanoparticles and chitosan-based nanoparticles. *Int J Pharm:X* 2024;8:100281. doi: https://doi.org/10.1016/j.ijpx.2024.100281
54. Nathani A, Aare M, Sun L, Bagde A, Li Y, Rishi A, et al. Unlocking the potential of camel milk-derived exosomes as novel delivery systems: Enhanced bioavailability of arv-825 protac for cancer therapy. *Pharmaceutics* 2024;16(8). doi: http://dx.doi.org/10.3390/pharmaceutics16081070
55. Administration USFaD. Fda novel drug therapy approvals for 2025. U.S. FDA; 2025 [13 Oct 2025]; Available from: https://www.fda.gov/drugs/novel-drug-approvals-fda/novel-drug-approvals-2025.
56. (NCI) NCI. A to z list of cancer drugs. National Cancer Institute (NCI); 2025 [October 16, 2025]; Available from: https://www.cancer.gov/about-cancer/treatment/drugs/cancer-drugs.
57. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: Biogenesis, biologic function and clinical potential. *Cell & bioscience* 2019;9:19. doi: http://dx.doi.org/10.1186/s13578-019-0282-2
58. Soltanmohammadi F, Gharehbaba AM, Zangi AR, Adibkia K, Javadzadeh Y. Current knowledge of hybrid nanoplatforms composed of exosomes and organic/inorganic nanoparticles for disease treatment and cell/tissue imaging. *Biomed Pharmacother* 2024;178:117248. doi: 10.1016/j.biopha.2024.117248
59. Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: From biogenesis to uptake and intracellular signalling. *Cell communication and signaling : CCS* 2021;19(1):47. doi: http://dx.doi.org/10.1186/s12964-021-00730-1

60. Yellon DM, Davidson SM. Exosomes: Nanoparticles involved in cardioprotection? *Circulation research* 2014;114(2):325–32. doi: http://dx.doi.org/10.1161/CIRCRESAHA.113.300636
61. Cheshmi B, Cheshomi H. Salivary exosomes: Properties, medical applications, and isolation methods. *Molecular biology reports* 2020;47(8):6295–307. doi: http://dx.doi.org/10.1007/s11033-020-05659-1
62. Feng P, Zhang X, Gao J, Jiang L, Li Y. The roles of exosomes in anti-cancer drugs. *Cancer Med* 2025;14(9):e70897. doi: http://dx.doi.org/10.1002/cam4.70897
63. Wang W, Qiao S, Kong X, Zhang G, Cai Z. The role of exosomes in immunopathology and potential therapeutic implications. *Cell Mol Immunol* 2025;22(9):975–95. doi: http://dx.doi.org/10.1038/s41423-025-01323-5
64. Skotland T, Sagini K, Sandvig K, Llorente A. An emerging focus on lipids in extracellular vesicles. *Advanced drug delivery reviews* 2020;159:308–21. doi: http://dx.doi.org/10.1016/j.addr.2020.03.002
65. Andreu Z, Yáñez-Mó M. Tetraspanins in extracellular vesicle formation and function. *Frontiers in immunology* 2014;5:442. doi: http://dx.doi.org/10.3389/fimmu.2014.00442
66. Mashouri L, Yousefi H, Aref AR, Ahadi AM, Molaei F, Alahari SK. Exosomes: Composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Molecular cancer* 2019;18(1):75. doi: http://dx.doi.org/10.1186/s12943-019-0991-5
67. Alenquer M, Amorim MJ. Exosome biogenesis, regulation, and function in viral infection. *Viruses* 2015;7(9):5066–83. doi: http://dx.doi.org/10.3390/v7092862
68. Kim HI, Park J, Zhu Y, Wang X, Han Y, Zhang D. Recent advances in extracellular vesicles for therapeutic cargo delivery. *Exp Mol Med* 2024;56(4):836–49. doi: http://dx.doi.org/10.1038/s12276-024-01201-6
69. Amina SJ, Azam T, Dagher F, Guo B. A review on the use of extracellular vesicles for the delivery of drugs and biological therapeutics. *Expert Opin Drug Deliv* 2024;21(1):45–70. doi: http://dx.doi.org/10.1080/17425247.2024.2305115
70. Li J, Wang A, Guo H, Zheng W, Chen R, Miao C, et al. Exosomes: Innovative biomarkers leading the charge in non-invasive cancer diagnostics. *Theranostics* 2025;15(11):5277–311. doi: http://dx.doi.org/10.7150/thno.113650
71. Rayat Pisheh H, Sani M. Mesenchymal stem cells derived exosomes: A new era in cardiac regeneration. *Stem Cell Res Ther* 2025;16(1):16. doi: http://dx.doi.org/10.1186/s13287-024-04123-2
72. Chen Y-F, Luh F, Ho Y-S, Yen Y. Exosomes: A review of biologic function, diagnostic and targeted therapy applications, and clinical trials. *J Biomed Sci* 2024;31(1):67. doi: http://dx.doi.org/10.1186/s12929-024-01055-0
73. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* 2020;367(6478). doi: http://dx.doi.org/10.1126/science.aau6977
74. Panda B, Sharma Y, Gupta S, Mohanty S. Mesenchymal stem cell-derived exosomes as an emerging paradigm for regenerative therapy and nano-medicine: A comprehensive review. *Life (Basel)* 2021;11(8). doi: http://dx.doi.org/10.3390/life11080784
75. Tang Y, Zhou Y, Li HJ. Advances in mesenchymal stem cell exosomes: A review. *Stem Cell Res Ther* 2021;12(1):71. doi: http://dx.doi.org/10.1186/s13287-021-02138-7
76. Costela-Ruiz VJ, Melguizo-Rodríguez L, Bellotti C, Illescas-Montes R, Stanco D, Arciola CR, et al. Different sources of mesenchymal stem cells for tissue regeneration: A guide to identifying the most favorable one in orthopedics and dentistry applications. *Int J Mol Sci* 2022;23(11). doi: http://dx.doi.org/10.3390/ijms23116356

77. Hassanzadeh A, Rahman HS, Markov A, Endjun JJ, Zekiy AO, Chartrand MS, et al. Mesenchymal stem/stromal cell-derived exosomes in regenerative medicine and cancer; overview of development, challenges, and opportunities. *Stem Cell Res Ther* 2021;12(1):297. doi: http://dx.doi.org/10.1186/s13287-021-02378-7
78. Deng H, Sun C, Sun Y, Li H, Yang L, Wu D, et al. Lipid, protein, and microrna composition within mesenchymal stem cell-derived exosomes. *Cell Reprogram* 2018;20(3):178–86. doi: http://dx.doi.org/10.1089/cell.2017.0047
79. Hade MD, Suire CN, Suo Z. Mesenchymal stem cell-derived exosomes: Applications in regenerative medicine. Cells [Internet]. 2021 2021/08//; 10(8):[1959 p.].
80. Zhang C, Yang X, Jiang T, Yan C, Xu X, Chen Z. Tissue-derived extracellular vesicles: Isolation, purification, and multiple roles in normal and tumor tissues. *Life Sci* 2023;321:121624. doi: http://dx.doi.org/10.1016/j.lfs.2023.121624
81. Ye Z, Chen W, Li G, Huang J, Lei J. Tissue-derived extracellular vesicles in cancer progression: Mechanisms, roles, and potential applications. *Cancer Metastasis Rev* 2024;43(2):575–95. doi: http://dx.doi.org/10.1007/s10555-023-10147-6
82. Wu Y, Fu H, Hao J, Yang Z, Qiao X, Li Y, et al. Tumor-derived exosomal pd-l1: A new perspective in pd-1/pd-l1 therapy for lung cancer. *Frontiers in immunology* 2024;15:1342728. doi: http://dx.doi.org/10.3389/fimmu.2024.1342728
83. Wang Y, Li Q, Zhou S, Tan P. Contents of exosomes derived from adipose tissue and their regulation on inflammation, tumors, and diabetes. *Front Endocrinol (Lausanne)* 2024;15:1374715. doi: http://dx.doi.org/10.3389/fendo.2024.1374715
84. Huang Y, Driedonks TAP, Cheng L, Rajapaksha H, Routenberg DA, Nagaraj R, et al. Brain tissue-derived extracellular vesicles in alzheimer's disease display altered key protein levels including cell type-specific markers. *J Alzheimers Dis* 2022;90(3):1057–72. doi: http://dx.doi.org/10.3233/jad-220322
85. Au - Luo Y, Au - Liu K, Au - Ling D, Au - Gu T, Au - Zhang L, Au - Chen W. *JoVE* 2024(211):e67234. doi: http://dx.doi.org/10.3791/67234
86. Jiao Y, Xu P, Shi H, Chen D, Shi H. Advances on liver cell-derived exosomes in liver diseases. *J Cell Mol Med* 2021;25(1):15–26. doi: http://dx.doi.org/10.1111/jcmm.16123
87. Rafieezadeh D, Rafieezadeh A. Extracellular vesicles and their therapeutic applications: A review article (part1). *Int J Physiol Pathophysiol Pharmacol* 2024;16(1):1–9. doi: http://dx.doi.org/10.62347/qpag5693
88. Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* 2015;523(7559):177–82. doi: http://dx.doi.org/10.1038/nature14581
89. Tang X, Leng M, Tang W, Cai Z, Yang L, Wang L, et al. The roles of exosome-derived micrornas in cardiac fibrosis. *Molecules* 2024;29(6). doi: http://dx.doi.org/10.3390/molecules29061199
90. Bo X, Li Q, Chen S, Zhou T, Yin N, Song W, et al. Evidence and perspectives on mirna, circrna, and lncrna in myocardial ischemia-reperfusion injury: A bibliometric study. *J Cardiothorac Surg* 2025;20(1):66. doi: http://dx.doi.org/10.1186/s13019-024-03238-0
91. Mollajan E, Yazdani S, Ghasemzadeh M, Mozhgani S-H. Mir-21 in cardiovascular disease: New insights and emerging therapeutic potential. *Discov Appl Sci* 2025;7(5):447. doi: http://dx.doi.org/10.1007/s42452-025-06888-4
92. Wu J, Liu G, Jia R, Guo J. Salivary extracellular vesicles: Biomarkers and beyond in human diseases. *Int J Mol Sci* 2023;24(24). doi: http://dx.doi.org/10.3390/ijms242417328

93. Mukerjee N, Bhattacharya A, Maitra S, Kaur M, Ganesan S, Mishra S, et al. Exosome isolation and characterization for advanced diagnostic and therapeutic applications. *Mater Today Bio* 2025;31:101613. doi: http://dx.doi.org/10.1016/j.mtbio.2025.101613
94. Yao Y, Jiao D, Li Z, Zhou X, Li J, Liu Z, et al. Roles of bile-derived exosomes in hepatobiliary disease. *Biomed Res Int* 2021;2021:8743409. doi: http://dx.doi.org/10.1155/2021/8743409
95. Martins TS, Vaz M, Henriques AG. A review on comparative studies addressing exosome isolation methods from body fluids. *Anal Bioanal Chem* 2023;415(7):1239–63. doi: http://dx.doi.org/10.1007/s00216-022-04174-5
96. Keller S, Ridinger J, Rupp A-K, Janssen JWG, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. *J Transl Med* 2011;9(1):86. doi: http://dx.doi.org/10.1186/1479-5876-9-86
97. McKiernan J, Noerholm M, Tadigotla V, Kumar S, Torkler P, Sant G, et al. A urine-based exosomal gene expression test stratifies risk of high-grade prostate cancer in men with prior negative prostate biopsy undergoing repeat biopsy. *BMC Urol* 2020;20(1):138. doi: http://dx.doi.org/10.1186/s12894-020-00712-4
98. Cristóbal-Cañadas D, Parrón-Carrillo R, Parrón-Carreño T. Exosomes in breast milk: Their impact on the intestinal microbiota of the newborn and therapeutic perspectives for high-risk neonates. *Int J Mol Sci* 2025;26(7). doi: http://dx.doi.org/10.3390/ijms26073421
99. Tan D, Li G, Fu W, Lei C. Exosomes: The next frontier in vaccine development and delivery. *Frontiers in immunology* 2024;15:1435426. doi: http://dx.doi.org/10.3389/fimmu.2024.1435426
100. Jabłońska M, Sawicki T, Żulewska J, Staniewska K, Łobacz A, Przybyłowicz KE. The role of bovine milk-derived exosomes in human health and disease. *Molecules* 2024;29(24). doi: http://dx.doi.org/10.3390/molecules29245835
101. Cui Z, Amevor FK, Zhao X, Mou C, Pang J, Peng X, et al. Potential therapeutic effects of milk-derived exosomes on intestinal diseases. *J Nanobiotechnol* 2023;21(1):496. doi: http://dx.doi.org/10.1186/s12951-023-02176-8
102. Timofeeva AM, Paramonik AP, Sedykh SS, Nevinsky GA. Milk exosomes: Next-generation agents for delivery of anticancer drugs and therapeutic nucleic acids. *Int J Mol Sci* 2023;24(12). doi: http://dx.doi.org/10.3390/ijms241210194
103. Rashidi M, Bijari S, Khazaei AH, Shojaei-Ghahrizjani F, Rezakhani L. The role of milk-derived exosomes in the treatment of diseases. *Front Genet* 2022;13:1009338. doi: http://dx.doi.org/10.3389/fgene.2022.1009338
104. Kim KU, Kim J, Jang H, Dan KB, Kim BK, Ji YW, et al. Protective effects of human breast milk-derived exosomes on inflammatory bowel disease through modulation of immune cells. *NPJ Sci Food* 2025;9(1):34. doi: http://dx.doi.org/10.1038/s41538-025-00400-3
105. Lu L, Han C, Wang M, Du H, Chen N, Gao M, et al. Assessment of bovine milk exosome preparation and lyophilized powder stability. *J Extracell Biol* 2024;3(11):e70009. doi: http://dx.doi.org/10.1002/jex2.70009
106. Phase i clinical trial investigating the ability of plant exosomes to deliver curcumin to normal and malignant colon tissue [database on the Internet]. 2011. Available from: https://clinicaltrials.gov/study/NCT01294072.
107. Pilot clinical trial investigating the ability of plant exosomes +/- curcumin to abrogate symptoms of inflammatory bowel disease (ibd) [database on the Internet]. 2018. Available from: https://clinicaltrials.gov/study/NCT04879810.
108. Exosome-based nanoplatform for ldlr mrna delivery in familial hypercholesterolemia [database on the Internet]. 2021. Available from: https://clinicaltrials.gov/study/NCT05043181.

109. Phase i study of mesenchymal stromal cells-derived exosomes with krasg12d sirna for metastatic pancreas cancer patients harboring krasg12d mutation [database on the Internet]. 2018. Available from: https://clinicaltrials.gov/study/NCT03608631.
110. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (misev2018): A position statement of the international society for extracellular vesicles and update of the misev2014 guidelines. *J Extracell Vesicles* 2018;7(1):1535750. doi: http://dx.doi.org/10.1080/20013078.2018.1535750
111. Gao J, Li A, Hu J, Feng L, Liu L, Shen Z. Recent developments in isolating methods for exosomes. *Front bioeng biotechnol* 2023;10:1100892. doi: http://dx.doi.org/10.3389/fbioe.2022.1100892
112. Gorgzadeh A, Nazari A, Ali Ehsan Ismaeel A, Safarzadeh D, Hassan JAK, Mohammadzadehsaliani S, et al. A state-of-the-art review of the recent advances in exosome isolation and detection methods in viral infection. *Virol J* 2024;21(1):34. doi: http://dx.doi.org/10.1186/s12985-024-02301-5
113. Chen J, Li P, Zhang T, Xu Z, Huang X, Wang R, et al. Review on strategies and technologies for exosome isolation and purification. *Front Bioeng Biotechnol* 2021;9:811971. doi: http://dx.doi.org/10.3389/fbioe.2021.811971
114. Langevin SM, Kuhnell D, Orr-Asman MA, Biesiada J, Zhang X, Medvedovic M, et al. Balancing yield, purity and practicality: A modified differential ultracentrifugation protocol for efficient isolation of small extracellular vesicles from human serum. *RNA Biol* 2019;16(1):5–12. doi: http://dx.doi.org/10.1080/15476286.2018.1564465
115. Gao J, Li A, Hu J, Feng L, Liu L, Shen Z. Recent developments in isolating methods for exosomes. *Front Bioeng Biotechnol* 2022;10:1100892. doi: http://dx.doi.org/10.3389/fbioe.2022.1100892
116. Dilsiz N. A comprehensive review on recent advances in exosome isolation and characterization: Toward clinical applications. *Transl Oncol* 2024;50:102121. doi: http://dx.doi.org/10.1016/j.tranon.2024.102121
117. Konoshenko MY, Lekchnov EA, Vlassov AV, Laktionov PP. Isolation of extracellular vesicles: General methodologies and latest trends. *Biomed Res Int* 2018;2018:8545347. doi: http://dx.doi.org/10.1155/2018/8545347
118. Li K, Wong DK, Hong KY, Raffai RL. Cushioned–density gradient ultracentrifugation (c-dguc): A refined and high performance method for the isolation, characterization, and use of exosomes. In: Patel T, editor. Extracellular rna: Methods and protocols. New York, NY: Springer New York; 2018. p. 69–83.
119. Zonneveld MI, Brisson AR, van Herwijnen MJ, Tan S, van de Lest CH, Redegeld FA, et al. Recovery of extracellular vesicles from human breast milk is influenced by sample collection and vesicle isolation procedures. *J Extracell Vesicles* 2014;3. doi: http://dx.doi.org/10.3402/jev.v3.24215
120. Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. *Theranostics* 2020;10(8):3684–707. doi: http://dx.doi.org/10.7150/thno.41580
121. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. *Theranostics* 2017;7(3):789–804. doi: http://dx.doi.org/10.7150/thno.18133
122. Kim JY, Rhim WK, Yoo YI, Kim DS, Ko KW, Heo Y, et al. Defined msc exosome with high yield and purity to improve regenerative activity. *J Tissue Eng* 2021;12:20417314211008626. doi: http://dx.doi.org/10.1177/20417314211008626

123. Li X, Su L, Zhang X, Chen Q, Wang Y, Shen Z, et al. Recent advances on the function and purification of milk exosomes: A review. *Front Nutr* 2022;9:871346. doi: http://dx.doi.org/10.3389/fnut.2022.871346
124. Cetinkaya H, Kongsomros S, Nommsen-Rivers L, Morrow AL, Chutipongtanate S. Which casein micelle removal method is suitable for studies of human milk extracellular vesicles? A systematic comparison of four different treatments for casein depletion before extracellular vesicle isolation from human milk. *Extracell Vesicles Circ Nucl Acids* 2024;5(2):221–32. doi: http://dx.doi.org/10.20517/evcna.2024.02
125. Medel-Martinez A, Redrado-Osta A, Crespo-Barreda A, Sancho-Albero M, Sánchez L, Sebastián V, et al. Isolation and characterization of milk exosomes for use in advanced therapies. *Biomolecules* 2024;14(7). doi: http://dx.doi.org/10.3390/biom14070810
126. D'Acunzo P, Kim Y, Ungania JM, Pérez-González R, Goulbourne CN, Levy E. Isolation of mitochondria-derived mitovesicles and subpopulations of microvesicles and exosomes from brain tissues. *Nat Protoc* 2022;17(11):2517–49. doi: http://dx.doi.org/10.1038/s41596-022-00719-1
127. Yakubovich EI, Polischouk AG, Evtushenko VI. Principles and problems of exosome isolation from biological fluids. *Biochem (Mosc) Suppl Ser A Membr Cell Biol* 2022;16(2):115–26. doi: http://dx.doi.org/10.1134/s1990747822030096
128. Wu X, Shen J, Zhong Y, Zhao X, Zhou W, Gao P, et al. Large-scale isolation of milk exosomes for skincare. *Pharmaceutics* 2024;16(7). doi: http://dx.doi.org/10.3390/pharmaceutics16070930
129. Marsh SR, Pridham KJ, Jourdan J, Gourdie RG. Novel protocols for scalable production of high quality purified small extracellular vesicles from bovine milk. *Nanotheranostics* 2021;5(4):488–98. doi: http://dx.doi.org/10.7150/ntno.62213
130. Liu WZ, Ma ZJ, Kang XW. Current status and outlook of advances in exosome isolation. *Anal Bioanal Chem* 2022;414(24):7123–41. doi: http://dx.doi.org/10.1007/s00216-022-04253-7
131. Wang W, Sun H, Duan H, Sheng G, Tian N, Liu D, et al. Isolation and usage of exosomes in central nervous system diseases. *CNS Neurosci Ther* 2024;30(3):e14677. doi: http://dx.doi.org/10.1111/cns.14677
132. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: Is size exclusion chromatography the best option? *Int J Mol Sci* 2020;21(18). doi: http://dx.doi.org/10.3390/ijms21186466
133. Rider MA, Hurwitz SN, Meckes DG. Extrapeg: A polyethylene glycol-based method for enrichment of extracellular vesicles. *Sci Rep* 2016;6(1):23978. doi: http://dx.doi.org/10.1038/srep23978
134. Aliakbari F, Stocek NB, Cole-André M, Gomes J, Fanchini G, Pasternak SH, et al. A methodological primer of extracellular vesicles isolation and characterization via different techniques. *Biol Methods Protoc* 2024;9(1):bpae009. doi: http://dx.doi.org/10.1093/biomethods/bpae009
135. Reham M. Marzouk1 MAG-A, 2, and Jeongkwon Kim1,. Polyethylene glycol (peg)-based precipitation for exosome enrichment:

A review on recent developments, current challenges,

and future perspectives. 2025. doi: https://doi.org/10.5806/AST.2025.38.2.42
136. Ruan J, Xia Y, Ma Y, Xu X, Luo S, Yi J, et al. Milk-derived exosomes as functional nanocarriers in wound healing: Mechanisms, applications, and future directions. *Mater Today Bio* 2025;32. doi: http://dx.doi.org/10.1016/j.mtbio.2025.101715

137. Lai JJ, Chau ZL, Chen SY, Hill JJ, Korpany KV, Liang NW, et al. Exosome processing and characterization approaches for research and technology development. *Adv Sci (Weinh)* 2022;9(15):e2103222. doi: http://dx.doi.org/10.1002/advs.202103222
138. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. *Cells* 2019;8(4). doi: http://dx.doi.org/10.3390/cells8040307
139. Huang Q, Wang J, Ning H, Liu W, Han X. Exosome isolation based on polyethylene glycol (peg): A review. *Mol Cell Biochem* 2025;480(5):2847–61. doi: http://dx.doi.org/10.1007/s11010-024-05191-x
140. Ming L, Yi L, Ai Y, Ji R. A new insight into the exosome protein and lipid composition in camel colostrum and mature milk using comparative proteome and lipidomics analyses. *Food Chem X* 2025;29:102729. doi: http://dx.doi.org/10.1016/j.fochx.2025.102729
141. Demir Ş, Erdal E, Bagriyanik HA. Imaging of isolated exosomes by correlative microscopy. *J Histochem Cytochem* 2024;72(3):149–56. doi: https://doi.org/10.1369/00221554241233346
142. Wang R, Zhu Z, Peng S, Xu J, Wei S, Liu X. Exosome microrna-125a-5p derived from epithelium promotes m1 macrophage polarization by targeting il1rn in chronic obstructive pulmonary disease. *Int Immunopharmacol* 2024;137:112466. doi: https://doi.org/10.1016/j.intimp.2024.112466
143. Karapanagioti F, Cissé N, Atamas A, Stetsenko A, Punter CM, Zuidersma E, et al. Alternatives for uranyl acetate negative staining with respect to the resolution obtained after single particle analysis of erythrocruorin. *Microsc Res Tech* 2025;88(9):2381–91. doi: http://dx.doi.org/10.1002/jemt.24865
144. Di S, Huang Y, Qiao W, Zhang X, Wang Y, Zhang M, et al. Advances in the isolation and characterization of milk-derived extracellular vesicles and their functions. *Front Nutr* 2024;11:1512939. doi: http://dx.doi.org/10.3389/fnut.2024.1512939
145. Ming L, Yi L, Ai Y, Ji R. A new insight into the exosome protein and lipid composition in camel colostrum and mature milk using comparative proteome and lipidomics analyses. *Food Chemistry: X* 2025;29:102729. doi: https://doi.org/10.1016/j.fochx.2025.102729
146. Kissling VM, Eitner S, Bottone D, Cereghetti G, Wick P. Systematic comparison of commercial uranyl-alternative stains for negative-and positive-staining transmission electron microscopy of organic specimens. *Adv Healthc Mater* 2025:2404870. doi: https://doi.org/10.1002/adhm.202404870
147. Ishii N, Noguchi K, Ikemoto MJ, Yohda M, Odahara T. Optimizing exosome preparation based on size and morphology: Insights from electron microscopy. *Microsc Microanal* 2023;29(6):2068–79. doi: http://dx.doi.org/10.1093/micmic/ozad103
148. Noble JM, Roberts LM, Vidavsky N, Chiou AE, Fischbach C, Paszek MJ, et al. Direct comparison of optical and electron microscopy methods for structural characterization of extracellular vesicles. *J Struct Biol* 2020;210(1):107474. doi: https://doi.org/10.1016/j.jsb.2020.107474
149. Malenica M, Vukomanović M, Kurtjak M, Masciotti V, Dal Zilio S, Greco S, et al. Perspectives of microscopy methods for morphology characterisation of extracellular vesicles from human biofluids. *Biomedicines* 2021;9(6). doi: http://dx.doi.org/10.3390/biomedicines9060603
150. Liu XJ, Ma ZS, Li Y, Fan TB, Ge ZW, Ou ZJ, et al. A simple modification results in a significant improvement in measuring the size of extracellular vesicles. *Curr Med Sci* 2025;45(2):244–52. doi: http://dx.doi.org/10.1007/s11596-025-00045-z

151. Weiskirchen R, Schröder SK, Weiskirchen S, Buhl EM, Melnik B. Isolation of bovine and human milk extracellular vesicles. *Biomedicines* 2023;11(10). doi: http://dx.doi.org/10.3390/biomedicines11102715
152. Aydoğan C, Günyel Z, Alharthi S, Aslan H, Erdoğan İ Y, Rassi ZE. Rapid separation and analysis of exosomes in milk sample by on-line nano-liquid chromatography. *Electrophoresis* 2025. doi: http://dx.doi.org/10.1002/elps.8155
153. Young TW, Kappler MP, Hockaden NM, Carpenter RL, Jacobson SC. Characterization of extracellular vesicles by resistive-pulse sensing on in-plane multipore nanofluidic devices. *Anal Chem* 2023;95(45):16710–6. doi: http://dx.doi.org/10.1021/acs.analchem.3c03546
154. Calado MRC, Lage TC, André DAM, Calaza C, Marques C, Herrero C, et al. Nanofluidic resistive pulse sensing for characterization of extracellular vesicles. *Lab Chip* 2024;24(17):4028–38. doi: http://dx.doi.org/10.1039/d4lc00364k
155. Yang M, Guo J, Fang L, Chen Z, Liu Y, Sun Z, et al. Quality and efficiency assessment of five extracellular vesicle isolation methods using the resistive pulse sensing strategy. *Anal Methods* 2024;16(32):5536–44. doi: http://dx.doi.org/10.1039/d4ay01158a
156. Tan F, Li X, Wang Z, Li J, Shahzad K, Zheng J. Clinical applications of stem cell-derived exosomes. *Signal Transduct Target Ther* 2024;9(1):17. doi: http://dx.doi.org/10.1038/s41392-023-01704-0
157. Alejandre Gonzalez AG, Ortiz-Lazareno PC, Solorzano-Ibarra F, Gutierrez-Franco J, Tellez-Bañuelos MC, Bueno-Topete MR, et al. A modified method for the quantification of immune checkpoint ligands on exosomes from human serum using flow cytometry. *Technol Cancer Res Treat* 2023;22:15330338221150324. doi: http://dx.doi.org/10.1177/15330338221150324
158. Poddar N, Aratikatla A, Gupta A. Therapeutic potential of stem cell-derived exosomes in hair regeneration: A systematic review. *World J Stem Cells* 2025;17(7):108519. doi: https://doi.org/10.4252/wjsc.v17.i7.108519
159. Chen G, Ouyang X, Mu Y, Chen Y. Human breast milk-derived exosomes and their positive role on neonatal intestinal health. *Pediatr Res* 2025:1–8. doi: https://doi.org/10.1038/s41390-025-03813-8
160. Couse AD, Cox-Vazquez SJ, Ghatak S, Trinidad JC, Clemmer DE. Delineating bovine milk derived microvesicles from exosomes using proteomics. *J Proteome Res* 2024;23(6):2288–97. doi: http://dx.doi.org/10.1021/acs.jproteome.4c00352
161. Ahlberg E, Jenmalm MC, Karlsson A, Karlsson R, Tingö L. Proteome characterization of extracellular vesicles from human milk: Uncovering the surfaceome by a lipid-based protein immobilization technology. *J Extracell Biol* 2024;3(11):e70020. doi: http://dx.doi.org/10.1002/jex2.70020
162. Liu Y, Ma Q, Khan MZ, Wang M, Xiang F, Zhang X, et al. Proteomic profiling of donkey milk exosomes highlights bioactive proteins with immune-related functions. *Int J Mol Sci* 2025;26(7). doi: http://dx.doi.org/10.3390/ijms26072892
163. Xia S, Jiang Y, Li W, An Z, Shen Y, Ding Q, et al. Proteomic analysis of differentially expressed plasma exosome proteins in heat-stressed holstein cows. *Animals (Basel)* 2025;15(15). doi: http://dx.doi.org/10.3390/ani15152286
164. Amitay EL, Keinan-Boker L. Breastfeeding and childhood leukemia incidence: A meta-analysis and systematic review. *JAMA pediatr* 2015;169(6):e151025–e. doi: https://doi.org/10.1001/jamapediatrics.2015.1025
165. Kim K-U, Kim W-H, Jeong CH, Yi DY, Min H. More than nutrition: Therapeutic potential of breast milk-derived exosomes in cancer. *Int J Mol Sci* 2020;21(19):7327. doi: http://dx.doi.org/10.3390/ijms21197327

166. Betker JL, Angle BM, Graner MW, Anchordoquy TJ. The potential of exosomes from cow milk for oral delivery. *J Pharm Sci* 2019;108(4):1496–505. doi: https://doi.org/10.1016/j.xphs.2018.11.022
167. Tang FH, Wong HY, Tsang PS, Yau M, Tam SY, Law L, et al. Recent advancements in lung cancer research: A narrative review. *Transl Lung Cancer Res* 2025;14(3):975. doi: https://doi.org/10.21037/tlcr-24-979
168. Wang C, Dai S, Zhao X, Zhang Y, Gong L, Fu K, et al. Celastrol as an emerging anticancer agent: Current status, challenges and therapeutic strategies. *Biomed Pharmacother* 2023;163:114882. doi: https://doi.org/10.1016/j.biopha.2023.114882
169. Aqil F, Kausar H, Agrawal AK, Jeyabalan J, Kyakulaga A-H, Munagala R, et al. Exosomal formulation enhances therapeutic response of celastrol against lung cancer. *Exp Mol Pathol* 2016;101(1):12–21. doi: https://doi.org/10.1016/j.yexmp.2016.05.013
170. Agrawal AK, Aqil F, Jeyabalan J, Spencer WA, Beck J, Gachuki BW, et al. Milk-derived exosomes for oral delivery of paclitaxel. *Nanomedicine: Nanotechnology, Biology and Medicine* 2017;13(5):1627–36. doi: https://doi.org/10.1016/j.nano.2017.03.001
171. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Lett* 2016;371(1):48–61. doi: https://doi.org/10.1016/j.canlet.2015.10.020
172. Bryant J, Thistle J. Anatomy, colostrum: StatPearls Publishing; 2025.
173. Kandimalla R, Aqil F, Alhakeem SS, Jeyabalan J, Tyagi N, Agrawal A, et al. Targeted oral delivery of paclitaxel using colostrum-derived exosomes. *Cancers* 2021;13(15):3700. doi: https://doi.org/10.3390/cancers13153700
174. Badawy AA, El-Magd MA, AlSadrah SA. Therapeutic effect of camel milk and its exosomes on mcf7 cells in vitro and in vivo. *Integrative cancer therapies* 2018;17(4):1235–46. doi: 10.1177/1534735418786000
175. Alemzadeh E, Allahqoli L, Mazidimoradi A, Alemzadeh E, Ghasemi F, Salehiniya H, et al. Deciphering resistance mechanisms and novel strategies to overcome drug resistance in ovarian cancer: A comprehensive review. *Oncology research* 2024;32(5):831–47. doi: http://dx.doi.org/10.32604/or.2024.031006
176. Rosales TKO, Silva FFAd, Rivera AG, Santos SNd, Bustos D, Morales-Quintana LA, et al. A study of the oral bioavailability and biodistribution increase of nanoencapsulation-driven delivering radiolabeled anthocyanins. *Food Res Int* 2024;197:115125. doi: https://doi.org/10.1016/j.foodres.2024.115125
177. Aqil F, Jeyabalan J, Agrawal AK, Kyakulaga AH, Munagala R, Parker L, et al. Exosomal delivery of berry anthocyanidins for the management of ovarian cancer. *Food Funct* 2017;8(11):4100–7. doi: 10.1039/c7fo00882a
178. Joshi UM, Kashani-Sabet M, Kirkwood JM. Cutaneous melanoma: A review. *Jama* 2025. doi: 10.1001/jama.2025.13074
179. Dai X, Zhang X, Chen W, Chen Y, Zhang Q, Mo S, et al. Dihydroartemisinin: A potential natural anticancer drug. *International journal of biological sciences* 2021;17(2):603–22. doi: http://dx.doi.org/10.7150/ijbs.50364
180. Kumar DN, Chaudhuri A, Dehari D, Gamper AM, Kumar D, Agrawal AK. Oral delivery of dihydroartemisinin for the treatment of melanoma via bovine milk exosomes. *Drug Deliv Transl Res* 2025:1–16. doi: https://doi.org/10.1007/s13346-024-01785-6
181. Salehi M, Negahdari B, Mehryab F, Shekari F. Milk-derived extracellular vesicles: Biomedical applications, current challenges, and future perspectives. *J Agric Food Chem* 2024;72(15):8304–31. doi: https://doi.org/10.1021/acs.jafc.3c07899

182. Li D, Gong L, Lin H, Yao S, Yin Y, Zhou Z, et al. Hyaluronic acid-coated bovine milk exosomes for achieving tumor-specific intracellular delivery of mirna-204. *Cells* 2022;11(19). doi: 10.3390/cells11193065
183. Kumar DN, Chaudhuri A, Dehari D, Shekher A, Gupta SC, Majumdar S, et al. Combination therapy comprising paclitaxel and 5-fluorouracil by using folic acid functionalized bovine milk exosomes improves the therapeutic efficacy against breast cancer. *Life* 2022;12(8):1143. doi: https://doi.org/10.3390/life12081143
184. Mohammadzadeh R, Javadzadeh Y. An overview on oral drug delivery via nano-based formulations. *Pharm Biomed Res* 2018. doi: https://doi.org/10.18502/pbr.v4i1.139
185. Hui L, Jin Z, Tianzhuo S, Wenbing J, Han L, Jiacan S. Harnessing artificial intelligence for engineering extracellular vesicles. *J Unexplored Med Data* 2025;6(3):522–46. doi: 10.20517/evcna.2025.35
186. Premchandani T, Tatode A, Taksande J, Umekar M, Qutub M, Hussain UM, et al. Engineered exosomes as smart drug carriers: Overcoming biological barriers in cns and cancer therapy. *Drugs Drug Candidates* 2025;4(2):19. doi: 10.3390/ddc4020019
187. Choi JY, Park S, Shim JS, Park HJ, Kuh SU, Jeong Y, et al. Explainable artificial intelligence-driven prostate cancer screening using exosomal multi-marker based dual-gate fet biosensor. *Biosens Bioelectron* 2025;267:116773. doi: 10.1016/j.bios.2024.116773

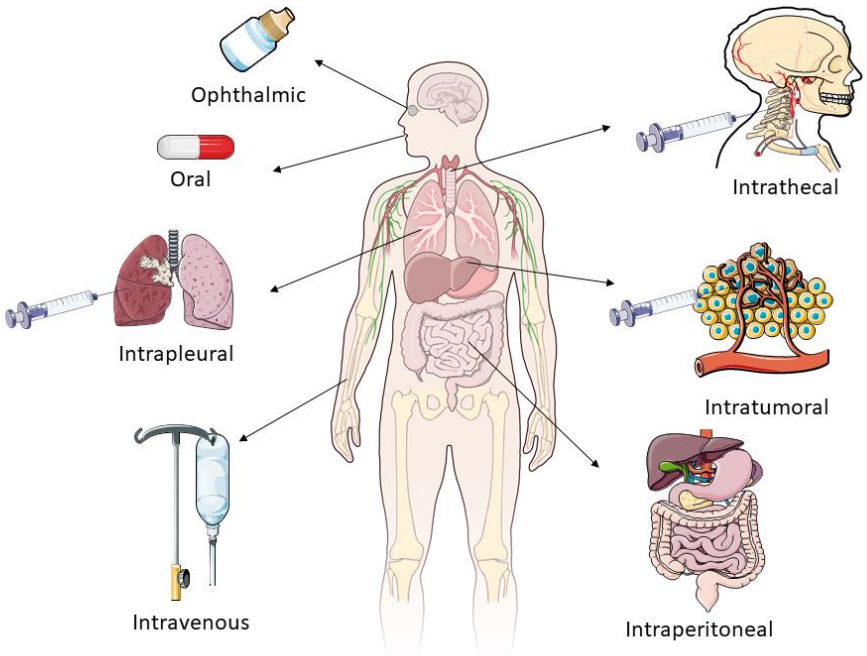


**Figure 1.** Schematic overview of selected drug administration routes reported for cancer therapy.

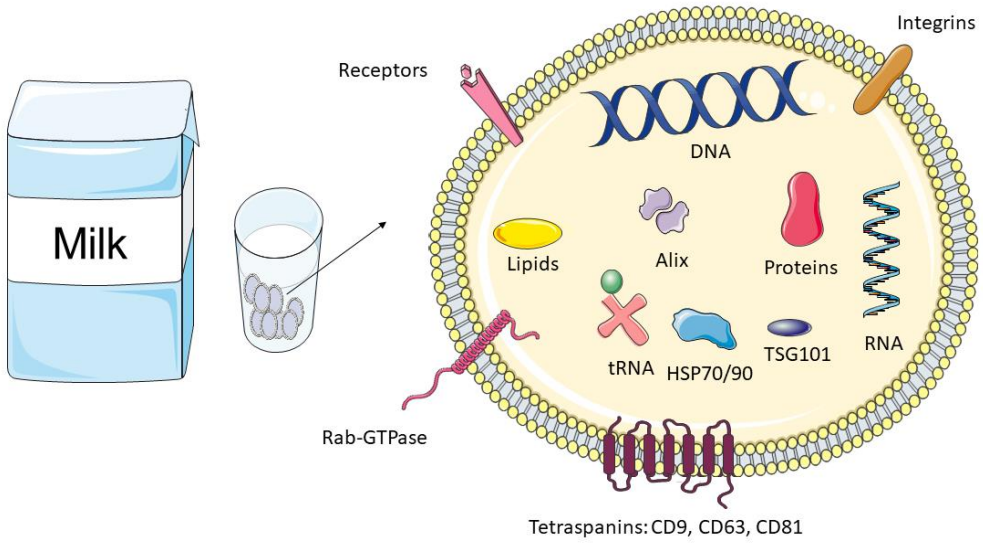


**Figure 2.** Schematic representation of the key structural and molecular components of a milk exosome.