


Review

# Insight into the Functional Dynamics and Challenges of Exosomes in Pharmaceutical Innovation and Precision Medicine

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**Abstract:** Of all the numerous nanosized extracellular vesicles released by a cell, the endosomal-originated exosomes are increasingly recognized as potential therapeutics, owing to their inherent stability, low immunogenicity, and targeted delivery capabilities. This review critically evaluates the transformative potential of exosome-based modalities across pharmaceutical and precision medicine landscapes. Because of their precise targeted biomolecular cargo delivery, exosomes are posited as ideal candidates in drug delivery, enhancing regenerative medicine strategies, and advancing diagnostic technologies. Despite the significant market growth projections of exosome therapy, its utilization is encumbered by substantial scientific and regulatory challenges. These include the lack of universally accepted protocols for exosome isolation and the complexities associated with navigating the regulatory environment, particularly the guidelines set forth by the U.S. Food and Drug Administration (FDA). This review presents a comprehensive overview of current research trajectories aimed at addressing these impediments and discusses prospective advancements that could substantiate the clinical translation of exosomal therapies. By providing a comprehensive analysis of both the capabilities and hurdles inherent to exosome therapeutic applications, this article aims to inform and direct future research paradigms, thereby fostering the integration of exosomal systems into mainstream clinical practice.

**Keywords:** exosome therapy; regenerative medicine; EV-based therapeutics regulatory affairs; cell–cell crosstalk; FDA; precision medicine



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## 1. Introduction

The expanding domain of exosome research represents a significant frontier in the realm of pharmaceutical innovation and precision medicine, offering a convergence of novel opportunities and complex challenges [1,2]. Exosomes, defined as nanosized extracellular vesicles (EVs) of endosomal origin, secreted by almost all cell types, have gained prominence as potential vectors for therapeutic delivery [3,4]. Their intrinsic characteristics, such as biological stability, minimal immunogenicity, and the capability for cell-specific targeting, position them as pivotal candidates in the advancement of drug delivery systems, nucleic acid, and vaccine therapeutics [5,6]. In this review, we have used the term “exosomes” with the intent of discussing the small EVs of endosomal origin having diameters ranging from 30 to 150 nm. It is critically important to acknowledge the fact that the terms “exosome” and “small EV” are not synonymous and should be used with precision. This review seeks to dissect the multifaceted landscape of exosome therapy and show how it is more advantageous than liposomal and microvesicle-based therapies (Table 1) within the pharmaceutical innovation sphere and precision medicine, by focusing on the crucial aspects of regulatory scrutiny, particularly by the United States Food and Drug Administration (FDA). It also aims to analyze the hurdles intrinsic to translating exosome-based therapies from benchtop research to bedside clinical practices [7,8]. The contemporary strategies devised to address the challenges endemic to the therapeutic application of exosomes encompass advancements in research methodologies, data standardization, and the precision

characterization of these nanovesicles, alongside innovations aimed at enhancing targeting specificity and cargo delivery efficiency [9]. It is time to critically explore the emerging techniques for single-exosome analysis and the refinement of surface modification approaches [10,11]. Such advancements underline the transition of exosomes from conceptual entities to practical therapeutic vectors, highlighting the importance of biochemical engineering in optimizing their clinical applicability [12]. By navigating through this discourse, we aim to trace the developmental trajectory of exosome-based therapeutic applications, emphasizing the dynamic synergy between scientific research and regulatory governance, as well as the commitment to ensuring patient safety and therapeutic efficacy [13]. This study not only seeks to contribute to the scholarly discourse on exosome therapy but also aspires to catalyze future advancements in therapeutic modalities, ushering in a new epoch of patient-focused care and therapeutic delivery.

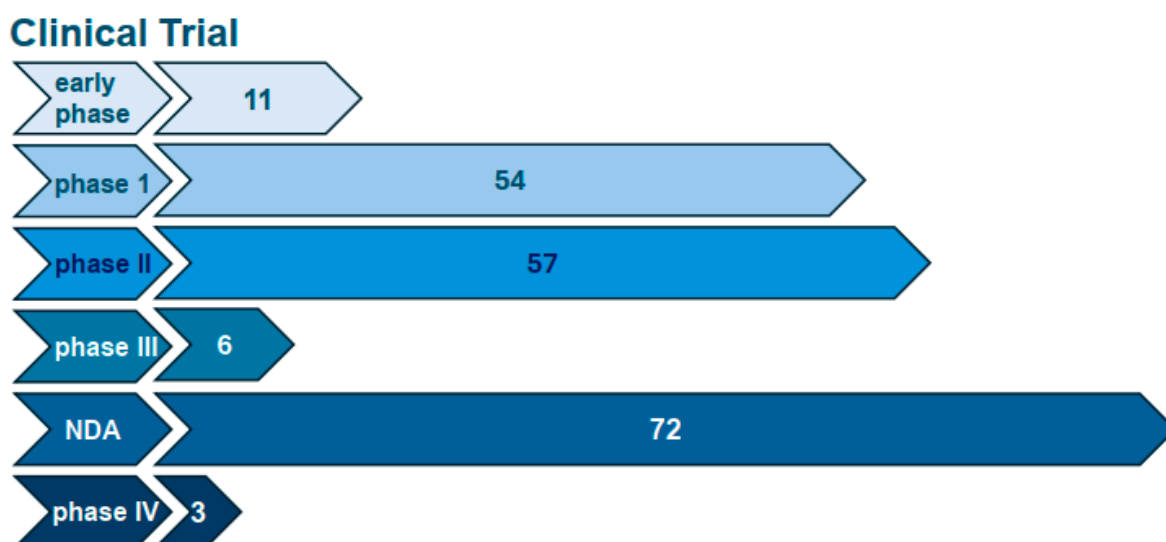
**Table 1.** Advantages and disadvantages of exosome, microvesicle, and liposome-based therapies.

| Vesicle Type  | Advantages  | Disadvantages   |
|---------------|---|---|
| Exosomes      | <ul style="list-style-type: none"> <li>- Small and stable structures; have a high capacity for carrying and protecting therapeutic cargoes such as miRNAs, mRNAs, proteins, and drugs</li> <li>- Ability to cross biological barriers, including the blood–brain barrier</li> <li>- Ability to deliver therapeutics to difficult-to-reach tissues due to small size</li> <li>- Active packaging of cargo</li> <li>- Fast uptake</li> <li>- More targeting capacity</li> <li>- Lower toxicity</li> </ul> | <ul style="list-style-type: none"> <li>- Limited clinical trials</li> <li>- Scalability for clinical use</li> <li>- Need to standardize the isolation techniques</li> <li>- Difficult to perform modifications because of complex structure</li> </ul>                |
| Microvesicles | <ul style="list-style-type: none"> <li>- Nonimmunogenic</li> <li>- Lower toxicity</li> <li>- Easily targetable</li> <li>- Can carry a more diverse array of cargo, including larger molecules and organelles</li> <li>- Passive packaging of cargo</li> </ul>   | <ul style="list-style-type: none"> <li>- Need to standardize the isolation techniques</li> <li>- Potential safety concerns related to the heterogeneous nature of microvesicles</li> <li>- Limited clinical trials</li> <li>- Scalability for clinical use</li> </ul> |
| Liposomes     | <ul style="list-style-type: none"> <li>- Diversity of lipid compositions</li> <li>- Scalable synthesis</li> <li>- Ability to load with desired cargo</li> <li>- Surface and cargo can be easily modified</li> <li>- Active clinical research</li> </ul>   | <ul style="list-style-type: none"> <li>- Not easily targetable</li> <li>- Low cellular uptake and potential complement activation</li> <li>- Premature drug release</li> <li>- Higher toxicity</li> </ul>   |

**2. Where Does the FDA Stand?**

Within the broader domain of regenerative medicine products, the U.S. FDA categorizes therapies derived from stem cells as somatic cellular therapies. It is important to note that the FDA employs a tailored regulatory framework for stem cell-based products based on their characteristics and intended uses. Recent work by Han et al. highlighted the significant influence of paracrine factors such as exosomes on the therapeutic potential of stem cells [14]. The progression of therapeutic applications utilizing exosomes delineates an intricate balance between innovation in biomedical research and stringent oversight by regulatory entities, notably the US FDA [12]. The FDA’s comprehensive regulatory framework for extracellular vesicle-based therapies, particularly those derived from exosomes, is predicated on ensuring the transition of these modalities from theoretical constructs to practical clinical applications, adhering to the highest safety and efficacy standards [15,16]. Such a regulatory approach is predicated on the principles of scientific accuracy, robust quality control mechanisms, and adherence to regulatory compliance stan-

dards [17,18]. Exosomes, a subclass of EVs, are recognized for their promising therapeutic potential across various medical fields [19]. These vesicles provide several key advantages over conventional cell-based therapies by their inherent capability to circumvent the proliferative and differentiation-associated risks post-administration, unlike that of stem cells [20]. Furthermore, they offer the potential for enhanced consistency in quality control standards across different production batches, which is a critical consideration in the context of biological drug development [5]. However, the path to clinical application of these vesicles is fraught with complexities, necessitating a comprehensive elucidation of their biological mechanisms, the establishment of scalable production methodologies that ensure reproducibility, and a tangible enhancement of their intrinsic therapeutic potential [21]. A primary challenge in this developmental trajectory is the establishment of uniform manufacturing protocols that guarantee consistency across product batches. Given the classification of exosomes as biological drugs by the FDA [22,23], they are subjected to exhaustive evaluations to validate their safety and efficacy through extensive clinical trials [24]. This stringent evaluative process highlights the critical need for refined manufacturing processes, comprehensive human trials, and conclusive evidence demonstrating clinical benefits [25,26]. Figure 1 shows an analysis of exosome-based clinical trials, as listed on [clinicaltrials.gov](https://clinicaltrials.gov) (20 April 2024). A search on [clinicaltrials.gov](https://clinicaltrials.gov) using ‘exosome’, ‘exosome therapy’, and ‘exosome treatment’, as keywords generated 204 records and listed in Table 2.



**Figure 1.** Exosome-based therapy in clinical trials (as listed on [clinicaltrials.gov](https://clinicaltrials.gov) as accessed on 20 April 2024).

To address the potential risks associated with unregulated exosome therapies, the FDA has proactively issued guidance and warnings concerning the marketing of unapproved exosome products [27]. These regulatory actions reflect the FDA’s dedication to safeguarding public health and maintaining the integrity of emerging therapeutic modalities. Through the enforcement of regulatory standards and the meticulous review of clinical trial submissions, the FDA endeavors to mitigate the risks posed by unauthorized exosome therapies. While the regulatory pathway for exosome-based therapies is devoid of specific guidance on delivery systems, applying the existing regulatory requirements for biological products remains imperative [27]. These include the submission of Investigational New Drug (IND) applications, adherence to Good Manufacturing Practice (GMP) protocols, implementation of quality control measures, and the selection of appropriate cell sources for therapeutic production [28].

**Table 2.** List of exosome-based clinical trials (as of 10 May 2024, listed on [clinicaltrials.gov](https://clinicaltrials.gov)).

| NCT Number  | Study Title   | Conditions  | Phases            |
|-------------|---|---|-------------------|
| NCT05218759 | Exosomes Detection for the Prediction of the Efficacy and Adverse Reactions of Anlotinib in Patients with Advanced NSCLC            | Non-Small-Cell Lung Cancer  | NA                |
| NCT02822131 | Phosphate in Blood Pressure Regulation  | Hypertension  | NA                |
| NCT02579460 | Reflux-Induced Oxidative Stress in Barrett’s Esophagus: Response, Repair, and Epithelial-Mesenchymal-Transition                     | Barrett’s Esophagus   Gastroesophageal Reflux Disease   | NA                |
| NCT06072794 | A Proof-of-Concept Study to Evaluate Exosomes from Human Mesenchymal Stem Cells in Women with Premature Ovarian Insufficiency (POI) | Premature Ovarian Insufficiency   Diminished Ovarian Reserve  | Phase 1           |
| NCT05658094 | Exosome Effect on Prevention of Hair loss   | Hair Loss   Alopecia  | NA                |
| NCT02649465 | SGLT2 Inhibitor Versus Sulfonylurea on Type 2 Diabetes With NAFLD   | Non-alcoholic Fatty Liver Disease   | Phase 4           |
| NCT03109873 | Metformin Hydrochloride in Affecting Cytokines and Exosomes in Patients with Head and Neck Cancer                                   | Larynx   Lip   Oral Cavity   Pharynx  | Early Phase 1     |
| NCT04184076 | Randomized Controlled Trial of Time-Restricted Feeding (TRF) in Acute Ischemic Stroke Patients                                      | Fasting   | NA                |
| NCT05490173 | The Pilot Experimental Study of the Neuroprotective Effects of Exosomes in Extremely Low Birth Weight Infants                       | Premature Birth   Extreme Prematurity   Preterm Intraventricular Hemorrhage   Hypoxia-Ischemia, Cerebral   Neurodevelopmental Disorders | NA                |
| NCT02138331 | Effect of Microvesicles and Exosomes Therapy on $\beta$ -cell Mass in Type I Diabetes Mellitus (T1DM)                               | Diabetes Mellitus Type 1  | Phase 2   Phase 3 |
| NCT04459182 | Circulating Exosomes and Endothelial Dysfunction in Patients with Obstructive Sleep Apneas Hypopneas Syndrome                       | Endothelial Dysfunction   | NA                |
| NCT03608631 | iExosomes in Treating Participants with Metastatic Pancreas Cancer with KrasG12D Mutation   | KRAS NP_004976.2: p. G12D   Metastatic Pancreatic Adenocarcinoma Pancreatic Ductal Adenocarcinoma Stage IV Pancreatic Cancer AJCC v8    | Phase 1           |
| NCT04388982 | the Safety and the Efficacy Evaluation of Allogenic Adipose MSC-Exos in Patients with Alzheimer’s Disease                           | Alzheimer’s Disease   | Phase 1   Phase 2 |
| NCT01104220 | Role of Immune System in Obesity-related Inflammation and Cardiometabolic Risk  | Non-alcoholic Fatty Liver Disease   Metabolic Syndrome   Metabolically Abnormal Obesity   Metabolically Normal Obesity   Obesity        | NA                |
| NCT05073627 | The Effect of Dicloxacillin on Oral Absorption of Drugs   | Healthy Volunteers   Drug–Drug Interaction  | Phase 1           |
| NCT04153539 | Acute Health Effects of Traffic-Related Air Pollution Exposure  | Cardiovascular System   Respiratory System  | NA                |
| NCT05871463 | Effect of Mesenchymal Stem Cells-derived Exosomes in Decompensated Liver Cirrhosis  | Decompensated Liver Cirrhosis   | Phase 2           |

Table 2. Cont.

| NCT Number  | Study Title   | Conditions  | Phases            |
|-------------|---|---|-------------------|
| NCT05692635 | Reducing the Incidence of Symptomatic Brain Metastases with MRI Surveillance  | Brain Metastases   Non-Small-Cell Lung Cancer Stage III   | Phase 2           |
| NCT05163626 | Combined Aerobic Exercise and Cognitive Training in Seniors at Increased Risk for Alzheimer's Disease                         | Alzheimer's Disease   | NA                |
| NCT04131166 | Precision Nutrition and Metabolic Function  | Obesity   Insulin Resistance  | NA                |
| NCT03217266 | Navtemadlin and Radiation Therapy in Treating Patients with Soft Tissue Sarcoma   | Resectable Soft Tissue Sarcoma   Soft Tissue Sarcoma  | Phase 1           |
| NCT03027726 | Prevention of Diabetes in Overweight/Obese Preadolescent Children   | Overweight Children with Type 2 Diabetes Risk   | NA                |
| NCT06259435 | Modulating Energy Density in Time-Restricted Eating   | Insulin Resistance   Body Weight  | NA                |
| NCT04134676 | Therapeutic Potential of Stem Cell Conditioned Medium on Chronic Ulcer Wounds   | Chronic Ulcer   | Phase 1           |
| NCT05777876 | Early Identification of TRD and Construction and Clinical Validation of NTBS Precision Technology                             | Treatment-Resistant Depression  | NA                |
| NCT05563766 | A Phase II Trial to Evaluate the Effect of Itraconazole on Pathologic Complete Response Rates in Resectable Esophageal Cancer | Esophageal Adenocarcinoma   Esophageal Squamous Cell Carcinoma   Gastroesophageal Junction Carcinoma                                | Phase 2           |
| NCT04460963 | Role of Adrenomedullin in Leukemic Endosteal/Vascular Niches  | Acute Myeloid Leukemia  | NA                |
| NCT04258735 | Genetic Characteristics of Metastatic Breast Cancer Patients  | Metastatic Breast Cancer  | NA                |
| NCT03410030 | Trial of Ascorbic Acid (AA) + Nanoparticle Paclitaxel Protein Bound + Cisplatin + Gemcitabine (AA NABPLAGEM)                  | Pancreatic Cancer   Pancreas Cancer   Pancreatic Adenocarcinoma Resectable   Pancreatic Ductal Adenocarcinoma   Pancreas Metastases | Phase 1   Phase 2 |
| NCT04747574 | Evaluation of the Safety of CD24-Exosomes in Patients With COVID-19 Infection   | SARS-CoV-2  | Phase 1           |
| NCT05969717 | Induced Pluripotent Stem Cell Derived Exosomes for the Treatment of Atopic Dermatitis   | Atopic Dermatitis   | Early Phase 1     |
| NCT02653339 | Effectiveness of Qufeng Shengshi Fang on Treatment of Allergic Rhinitis.  | Rhinitis, Allergic, Perennial   | NA                |
| NCT04266639 | Rheo-Erythrocrine Dysfunction as a Biomarker for RIC Treatment in Acute Ischemic Stroke                                       | Stroke, Acute   Ischemic Stroke   Cerebrovascular Disorders   Central Nervous System Diseases                                       | NA                |
| NCT05354141 | Extracellular Vesicle Treatment for Acute Respiratory Distress Syndrome (ARDS) (EXTINGUISH ARDS)                              | Acute Respiratory Distress Syndrome   ARDS  | Phase 3           |
| NCT05616234 | Exercise-induced Changes in Exosomes  | Exosomes   Connective Tissue   Exercise   | NA                |
| NCT06279039 | Clinical Observation of Exosomes in Patients After Q-switched Laser Surgery   | Exosome   Skin Regeneration   Laser   | NA                |

Table 2. Cont.

| NCT Number  | Study Title  | Conditions   | Phases            |
|-------------|--|--|-------------------|
| NCT03230019 | Two-Lumen Catheterization for Lung Wedge Resection   | Thoracic Surgery, Video-Assisted   | NA                |
| NCT03392441 | Insulin Deprivation on Brain Structure and Function in Humans with Type 1 Diabetes   | Diabetes Mellitus, Type 1   Diabetes   Diabetes Complications  | NA                |
| NCT04483219 | Tyrosine Kinase Inhibitor (TKI) + Anti-PD-1 Antibody in TKI-responded Microsatellite Stability/Proficient Mismatch Repair (MSS/pMMR) Metastatic Colorectal Adenocarcinoma. | MSS   pMMR   Metastatic Colorectal Adenocarcinoma  | Phase 2           |
| NCT05475418 | Pilot Study of Human Adipose Tissue Derived Exosomes Promoting Wound Healing   | Wounds and Injuries  | NA                |
| NCT06110130 | Effect of Empagliflozin on Podocyte Specific Proteins in African American Veterans With NDKD   | Chronic Kidney Diseases  | Phase 4           |
| NCT05413148 | The Effect of Stem Cells and Stem Cell Exosomes on Visual Functions in Patients with Retinitis Pigmentosa  | Retinitis Pigmentosa   | Phase 2   Phase 3 |
| NCT04213248 | Effect of UMSCs Derived Exosomes on Dry Eye in Patients With cGVHD   | Dry Eye Disease  | Phase 1   Phase 2 |
| NCT05402748 | Safety and Efficacy of Injection of Human Placenta Mesenchymal Stem Cells Derived Exosomes for Treatment of Complex Anal Fistula   | Fistula Perianal   | Phase 1   Phase 2 |
| NCT03985696 | Exosomes and Immunotherapy in Non-Hodgkin B-cell Lymphomas   | Lymphoma, B-cell, Aggressive Non-Hodgkin (B-NHL)   | NA                |
| NCT05166616 | Minnelide and Osimertinib for the Treatment of Advanced EGFR Mutated Non-Small Cell Lung Cancer  | Advanced Lung Non-Small-Cell Carcinoma   Locally Advanced Lung Non-Small-Cell Carcinoma   Stage III Lung Cancer AJCC v8   Stage IIIA Lung Cancer AJCC v8   Stage IIIB Lung Cancer AJCC v8   Stage IIIC Lung Cancer AJCC v8   Stage IV Lung Cancer AJCC v8   Stage IVA Lung Cancer AJCC v8   Stage IVB Lung Cancer AJCC v8   Unresectable Lung Non-Small-Cell Carcinoma | Phase 1           |
| NCT04556916 | Early Detection of Prostate Cancer   | Prostate Cancer  | NA                |
| NCT05815524 | Physical Activity in Patients with Parkinson's Disease: A "Disease Modifying" Intervention?  | Parkinson's Disease  | NA                |
| NCT04998058 | Autogenous Mesenchymal Stem Cell Culture-Derived Signaling Molecules as Enhancers of Bone Formation in Bone Grafting   | Bone Loss, Osteoclastic   Bone Loss, Alveolar   Alveolar Bone Loss   Alveolar Bone Atrophy   Grafting Bone   | Phase 1   Phase 2 |
| NCT02892734 | Ipilimumab and Nivolumab in Treating Patients with Recurrent Stage IV HER2 Negative Inflammatory Breast Cancer   | HER2/Neu Negative   Recurrent Inflammatory Breast Carcinoma   Stage IV Breast Cancer   Stage IV Inflammatory Breast Carcinoma  | Phase 2           |
| NCT04493242 | Extracellular Vesicle Infusion Treatment for COVID-19 Associated ARDS  | COVID-19   ARDS  | Phase 2           |

Table 2. Cont.

| NCT Number  | Study Title  | Conditions   | Phases            |
|-------------|--|--|-------------------|
| NCT05698524 | A Study of Temodar with Abexinostat (PCI-24781) for Patients with Recurrent Glioma   | Recurrent High-Grade Glioma   Anaplastic Astrocytoma   Anaplastic Oligodendroglioma   Glioblastoma   Gliosarcoma   | Phase 1           |
| NCT04798716 | The Use of Exosomes for the Treatment of Acute Respiratory Distress Syndrome or Novel Coronavirus Pneumonia Caused by COVID-19                             | COVID-19   Novel Coronavirus Pneumonia   Acute Respiratory Distress Syndrome   | Phase 1   Phase 2 |
| NCT04939324 | Molecular Profiling of Exosomes in Tumor-draining Vein of Early-staged Lung Cancer   | Lung Cancer   Exosomes   Non-Small-Cell Lung Cancer  | NA                |
| NCT05326724 | The Role of Acupuncture-induced Exosome in Treating Post-stroke Dementia   | Exosome   Post-stroke Dementia   Acupuncture   | NA                |
| NCT05621109 | PRE-Pregnancy Weight Loss and the Reducing Effect on Childhood Overweight—Copenhagen   | Overweight and Obesity   Weight Loss   Pregnancy Related   | NA                |
| NCT04641585 | Brugada Syndrome and Artificial Intelligence Applications to Diagnosis   | Brugada Syndrome 1   | NA                |
| NCT05610332 | Clinical Efficacy in Neoadjuvant Treatment of Locally Advanced Gastric Cancer with Different Immunotypes   | Locally Advanced Gastric Cancer  | Phase 3           |
| NCT02869685 | Consistency Analysis of PD-L1s in Advanced NSCLC Tissues and in Plasma Exosomes Before and After Radiotherapy  | NSCLC  | NA                |
| NCT06361485 | Safety and Feasibility of Umbilical Cord Wharton's Jelly Allograft Injections for Lumbar Pain  | Low Back Pain  | Phase 1           |
| NCT02928432 | SWITCH: Study of the Prednisone to Dexamethasone Change in mCRPC Patients Treated with Abiraterone   | Prostate Cancer  | Phase 2           |
| NCT04765137 | Evaluate the Effect of Atorvastatin on Cerebrovascular Reactivity in MCI   | Mild Cognitive Impairment  | Phase 2           |
| NCT03854032 | Nivolumab and BMS986205 in Treating Patients with Stage II-IV Squamous Cell Cancer of the Head and Neck  | Lip   Oral Cavity Squamous Cell Carcinoma   Pharynx   Larynx   Squamous Cell Carcinoma   | Phase 2           |
| NCT04585932 | Androgen Deprivation Therapy and Apalutamide With or Without Radiation Therapy for the Treatment of Biochemically Recurrent Prostate Cancer, RESTART Study | Biochemically Recurrent Prostate Carcinoma   Metastatic Prostate Carcinoma   Oligometastatic Prostate Carcinoma   Stage IV Prostate Cancer AJCC v8   Stage IVA Prostate Cancer AJCC v8   Stage IVB Prostate Cancer AJCC v8 | Phase 2           |
| NCT05669144 | Co-transplantation of Mesenchymal Stem Cell Derived Exosomes and Autologous Mitochondria for Patients Candidate for CABG Surgery                           | Myocardial Infarction   Myocardial Ischemia   Myocardial Stunning  | Phase 1   Phase 2 |
| NCT04029740 | Exosomal microRNAs as a Biomarker in Panic Disorder and in Response to CBT   | Panic Disorder   | NA                |
| NCT06339840 | The Impact of Lifestyle Intervention on Weight and Fertility in Obese Males  | Obesity   Weight Loss   Male Fertility   Artificial Insemination   IVF-ET  | NA                |

Table 2. Cont.

| NCT Number  | Study Title   | Conditions  | Phases            |
|-------------|---|---|-------------------|
| NCT05192694 | Evaluation of Fapi-pet in Prostate Cancer.  | Prostate Cancer   | NA                |
| NCT04781062 | Development of a Horizontal Data Integration Classifier for Noninvasive Early Diagnosis of Breast Cancer  | Breast Cancer   | NA                |
| NCT03762629 | Exercise and Diet Restriction on Cardiovascular Function in Obese Children and Adolescents  | Childhood Obesity   Adolescent Obesity  | NA                |
| NCT04849429 | Intra-discal Injection of Platelet-rich Plasma (PRP) Enriched with Exosomes in Chronic Low Back Pain  | Chronic Low Back Pain   Degenerative Disc Disease   | Phase 1           |
| NCT05927129 | Research on the Biological Mechanism of the Efficacy of Psychotherapy for Depression Based on the fNIRS   | Depressive Disorder   | NA                |
| NCT05738629 | Safety and Efficacy of Pluripotent Stem Cell-derived Mesenchymal Stem Cell Exosome (PSC-MSC-Exo) Eye Drops Treatment for Dry Eye Diseases Post Refractive Surgery and Associated with Blepharospasm | Dry Eye Disease   | Phase 1   Phase 2 |
| NCT05798494 | PRE-Pregnancy Weight Loss and the Reducing Effect on Childhood Overweight - Aarhus  | Overweight and Obesity   Weight Loss   Pregnancy Related   Child Nutrition Sciences   Body Composition  | NA                |
| NCT05261360 | Clinical Efficacy of Exosome in Degenerative Meniscal Injury  | Knee; Injury, Meniscus (Lateral) (Medial)   Meniscus Tear   Meniscus Lesion   Meniscus; Degeneration   Meniscus; Laceration   Meniscus Injury, Tibial   Knee Injuries   Knee Pain Swelling   Arthralgia           | Phase 2           |
| NCT03406780 | A Study of CAP-1002 in Ambulatory and Non-Ambulatory Patients with Duchenne Muscular Dystrophy  | Muscular Dystrophies   Muscular Dystrophy, Duchenne   Muscular Disorders, Atrophic   Muscular diseases   Neuromuscular Diseases   Nervous System Diseases   Genetic Diseases, X-Linked   Genetic Diseases, Inborn | Phase 2           |
| NCT04334603 | Exercise Intervention in Heart Failure  | Heart Failure   | NA                |
| NCT04595903 | Treatment of SARS-CoV-2 Virus Disease (COVID-19) in Humans with Hemopurifier <sup>®</sup> Device  | COVID-19  | NA                |
| NCT03711890 | Ultra-High Resolution Optical Coherence Tomography in Detecting Micrometer Sized Early-Stage Pancreatic Cancer in Participants With Pancreatic Cancer   | Pancreatic Carcinoma   Pancreatic Intraductal Papillary Mucinous Neoplasm, Pancreatobiliary-Type  | NA                |
| NCT05955521 | Exosome as the Prognostic and Predictive Biomarker in EBC Patients  | Triple Negative Breast Cancer   HER2-positive Breast Cancer   | NA                |
| NCT03493984 | Plant Exosomes and Patients Diagnosed with Polycystic Ovary Syndrome (PCOS) 17  | Polycystic Ovary Syndrome   | NA                |
| NCT03260179 | Study to Evaluate the Safety, Preliminary Efficacy and Pharmacokinetics of 3810   | Advanced Solid Tumor   Advanced/Metastatic Colorectal Cancer  | Phase 1           |



Table 2. Cont.

| NCT Number  | Study Title  | Conditions   | Phases            |
|-------------|--|--|-------------------|
| NCT05286684 | Feasibility of Exosome Analysis in Cerebrospinal Fluid During the Diagnostic Workup of Metastatic Meningitis (Exo-LCR)   | Breast Cancer  | NA                |
| NCT04173650 | MSC EVs in Dystrophic Epidermolysis Bullosa  | Dystrophic Epidermolysis Bullosa   | Phase 1   Phase 2 |
| NCT05243368 | Evaluation of Personalized Nutritional Intervention on Wound Healing of Cutaneous Ulcers in Diabetics  | Foot, Diabetics  | NA                |
| NCT04421872 | The Disorder of Circadian Clock Gene and Early Cognitive Dysfunction After General Anesthesia  | Postoperative Delirium   General Anesthesia   Exosomes   Clock Gene Circadian Rhythm Disorders                       | NA                |
| NCT04969172 | A Phase II Randomized, Double-blind, Placebo-controlled Study to Evaluate the Safety and Efficacy of Exosomes Overexpressing CD24 to Prevent Clinical Deterioration in Patients with Moderate or Severe COVID-19 Infection | COVID-19 Disease   | Phase 2           |
| NCT01294072 | Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue  | Colon Cancer   | NA                |
| NCT04879810 | Plant Exosomes +/- Curcumin to Abrogate Symptoms of Inflammatory Bowel Disease   | Irritable Bowel Disease  | NA                |
| NCT01550523 | Pilot Immunotherapy Trial for Recurrent Malignant Gliomas  | Malignant Glioma of Brain  | Phase 1           |
| NCT02439008 | Early Biomarkers of Tumor Response in High Dose Hypo Fractionated Radiotherapy Word Package 3: Immune Response   | Carcinoma, Hepatocellular   Colorectal Neoplasms   Melanoma   Kidney Neoplasms                                       | NA                |
| NCT03974204 | Analyses of Exosomes in the Cerebrospinal Fluid for Breast Cancer Patients with Suspicion of Leptomeningeal Metastasis.  | Breast Cancer   Leptomeningeal Metastasis  | NA                |
| NCT05913960 | Accelerated Intermittent Theta-Burst Stimulation Ameliorate Major Depressive Disorder by Regulating CAMKII Pathway   | Major Depressive Disorder   Major Depressive Disorder, Recurrent   | NA                |
| NCT05375604 | A Study of exoASO-STAT6 (CDK-004) in Patients with Advanced Hepatocellular Carcinoma (HCC) and Patients with Liver Metastases from Either Primary Gastric Cancer or Colorectal Cancer (CRC)                                | Advanced Hepatocellular Carcinoma (HCC)   Gastric Cancer Metastatic to Liver   Colorectal Cancer Metastatic to Liver | Phase 1           |
| NCT02706262 | Complex Effects of Dietary Manipulation on Metabolic Function, Inflammation and Health   | Obesity   Insulin Resistance   | NA                |
| NCT05216562 | Efficacy and Safety of EXOSOME-MSC Therapy to Reduce Hyper-inflammation In Moderate COVID-19 Patients  | SARS-CoV2 Infection  | Phase 2   Phase 3 |

Table 2. Cont.

| NCT Number  | Study Title  | Conditions   | Phases            |
|-------------|--|--|-------------------|
| NCT05775146 | SBRT of Metastases Following Neo-Adjuvant Treatment for Colorectal Cancer with Synchronous Liver Metastases  | Colorectal Cancer   Liver Metastasis<br>Colon Cancer   | Phase 2           |
| NCT02737267 | Development of a Nutrigenetic Test for Personalized Prescription of Body Weight Loss Diets (Obekit)  | Body Weight Changes  | NA                |
| NCT05524974 | Clinical Study of Camrelizumab, Apatinib Mesylate and Nab-paclitaxel Combined with Oxplatin and S-1 in the Neoadjuvant Treatment of Locally Advanced Gastric Cancer With Different Genotypes | Locally Advanced Gastric Cancer  | Phase 2           |
| NCT06116903 | Clinical Relevance of Detecting Molecular Abnormalities in Glial Tumor Exosomes  | Glioma   | NA                |
| NCT04664738 | PEP on a Skin Graft Donor Site Wound   | Skin Graft   | Phase 1           |
| NCT04530890 | Interest of Circulating Tumor DNA in Digestive and Gynecologic/Breast Cancer   | Breast Cancer   Digestive Cancer   Gynecologic Cancer   Circulating Tumor DNA   Exosomes   | NA                |
| NCT02565264 | Effect of Plasma Derived Exosomes on Cutaneous Wound Healing   | Ulcer  | Early Phase 1     |
| NCT04542902 | Non-coding RNAs Analysis of Eosinophil Subtypes in Asthma  | Allergic Asthma   Severe Eosinophilic Asthma   | NA                |
| NCT02662621 | Pilot Study with the Aim to Quantify a Stress Protein in the Blood and in the Urine for the Monitoring and Early Diagnosis of Malignant Solid Tumors   | Cancer   | NA                |
| NCT05813379 | Mesenchymal Stem Cells Derived Exosomes in Skin Rejuvenation   | Anti-Aging   | Phase 1   Phase 2 |
| NCT03255408 | Cerebral Blood Flow and Ventilatory Responses During Sleep in Normoxia and Intermittent Hypoxia  | Obstructive Sleep Apnea of Adult   Hypoxia, Brain   Sleep Apnea   Sleep Disorder   Stroke   Blood Pressure   Endothelial Dysfunction   Oxidative Stress  | Phase 1   Phase 2 |
| NCT05043181 | Exosome-based Nanoplatfrom for Ldlr mRNA Delivery in FH  | Familial Hypercholesterolemia  | Phase 1           |
| NCT05110781 | Atezolizumab Before Surgery for the Treatment of Regionally Metastatic Head and Neck Squamous Cell Cancer with an Unknown or Historic Primary Site   | Locally Advanced Cutaneous Squamous Cell Carcinoma of the Head and Neck   Resectable Cutaneous Squamous-Cell Carcinoma of the Head and Neck   Stage III Cutaneous Squamous Cell Carcinoma of the Head and Neck AJCC v8 | Phase 2           |
| NCT05243381 | Inflammation, NK Cells, Antisense Protein and Exosomes, and Correlation with Immune Response During HIV Infection  | HIV Infections   | NA                |
| NCT05373381 | The KetoGlioma (Ketogenic Glioma) Study  | Glioma   | NA                |

Table 2. Cont.

| NCT Number  | Study Title   | Conditions   | Phases            |
|-------------|---|--|-------------------|
| NCT03202212 | Effect of Mixed On-line Hemodiafiltration on Circulating Markers of Inflammation and Vascular Dysfunction       | Chronic Kidney Failure   Dialysis-Related Complication   | Phase 1   Phase 2 |
| NCT01811381 | Curcumin and Yoga Therapy for Those at Risk for Alzheimer's Disease   | Mild Cognitive Impairment  | Phase 2           |
| NCT03459703 | Effect of Time-Restricted Feeding on Fat Loss and Cardiometabolic Risk Factors in Overweight Adults             | Obesity  | NA                |
| NCT05624203 | Clinical Efficacy of Extracorporeal Cardiac Shock Wave Therapy in Patients with Ischemia-reperfusion Injury     | Myocardial Reperfusion Injury   Treatment Outcome   Prognosis   ST Elevation Myocardial Infarction | NA                |
| NCT04235023 | PTP1B Implication in the Vascular Dysfunction Associated with Obstructive Sleep Apnea                           | Sleep Apnea   Inflammation   Atherosclerosis   | NA                |
| NCT05480150 | Chinese Longitudinal and Systematic Study of Bipolar Disorder   | Major Depressive Disorder   Bipolar Disorder, Mixed   Affective; Disorder, Organic                 | NA                |
| NCT05832255 | An Investigation of Psilocybin on Behavioural and Cognitive Symptoms of Adults with Fragile X Syndrome          | Fragile X Syndrome   Behavior   Cognitive Dysfunction  | Phase 2           |
| NCT06245746 | UCMSC-Exo for Chemotherapy-induced Myelosuppression in Acute Myeloid Leukemia                                   | Acute Myeloid Leukemia   Neutropenia   Anemia   Thrombocytopenia   Infections   Bleeding           | Phase 1           |
| NCT04453046 | Hemopurifier Plus Pembrolizumab in Head and Neck Cancer   | Squamous Cell Carcinoma of the Head and Neck   | NA                |
| NCT04142138 | When the Kidney Reacts to Nutritional Changes   | Prehypertension  | NA                |
| NCT05833568 | Five-day 20-min 10-Hz tACS in Patients with a Disorder of Consciousness   | Brain Injury Traumatic Severe   Disorder of Consciousness   Brain Injuries                         | NA                |
| NCT02977468 | Effects of MK-3475 (Pembrolizumab) on the Breast Tumor Microenvironment in Triple Negative Breast Cancer        | Triple Negative Breast Cancer  | Phase 1           |
| NCT06236568 | Impact of Graft Reconditioning with Hypothermic Machine Perfusion on HCC Recurrence After Liver Transplantation | Hepatocellular Carcinoma   Liver Transplant; Complications   | NA                |
| NCT02748369 | In vivo Assessment of Cellular Metabolism in Humans   | Normal Cellular Metabolism   | Phase 1           |
| NCT04500769 | Training Induced Muscle Exosome Release   | Metabolism   | NA                |
| NCT02051101 | Pathogenic Mechanisms of Port Wine Stain and Repository of Port Wine Stain Biopsy Samples                       | Port-Wine Stain  | NA                |
| NCT04602104 | A Clinical Study of Mesenchymal Stem Cell Exosomes Nebulizer for the Treatment of ARDS                          | Acute Respiratory Distress Syndrome  | Phase 1   Phase 2 |
| NCT04491240 | Evaluation of Safety and Efficiency of Method of Exosome Inhalation in SARS-CoV-2 Associated Pneumonia.         | COVID-19   SARS-CoV-2 PNEUMONIA   COVID-19   | Phase 1   Phase 2 |

Table 2. Cont.

| NCT Number  | Study Title   | Conditions   | Phases            |
|-------------|---|--|-------------------|
| NCT04653740 | Omic Technologies to Track Resistance to Palbociclib in Metastatic Breast Cancer  | Advanced Breast Cancer   | NA                |
| NCT03096340 | Safety and Pharmacokinetic Study of IT-141 in Monotherapy in Patients with Advanced Cancer  | Cancer   Neoplasms   Tumors   Refractory Solid Tumors   Recurrent Solid Tumors                         | Phase 1           |
| NCT03478410 | Role of Exosomes Derived from Epicardial Fat in Atrial Fibrillation   | Atrial Fibrillation  | NA                |
| NCT06138210 | The Effect of GD-iExo-003 in Acute Ischemic Stroke  | Acute Ischemic Stroke  | Phase 1           |
| NCT05787288 | A Clinical Study on Safety and Effectiveness of Mesenchymal Stem Cell Exosomes for the Treatment of COVID-19.                               | COVID-19 Pneumonia   | Early Phase 1     |
| NCT04965961 | The Effect of Micro-doses Erythropoietin on Exercise Capacity in Male and Females   | Sports Drug Abuse  | NA                |
| NCT03384433 | Allogenic Mesenchymal Stem Cell Derived Exosome in Patients with Acute Ischemic Stroke  | Cerebrovascular Disorders  | Phase 1   Phase 2 |
| NCT06319287 | Phase 2a Multi-Center Prospective, Randomized Trial to Evaluate the Safety & Efficacy of Topical PEP-TISSEEL for Diabetic Foot Ulcers (DFU) | Diabetic Foot Ulcer  | Phase 2           |
| NCT04276987 | A Pilot Clinical Study on Inhalation of Mesenchymal Stem Cells Exosomes Treating Severe Novel Coronavirus Pneumonia                         | Coronavirus  | Phase 1           |
| NCT06051461 | Deciphering the Role of Dietary Fatty Acids on Extracellular Vesicles-mediated Intercellular Communication                                  | Obesity   Metabolic Syndrome   Metabolic Disorder   Inflammation   Immune System and Related Disorders | NA                |
| NCT06239207 | Efficacy and Safety of Exosomes Versus Platelet Rich Plasma in Patients of Androgenetic Alopecia  | Androgenetic Alopecia  | Phase 2           |
| NCT05933707 | Small Extracellular Vesicles and Insulin Action   | Obesity   Insulin Resistance   Metabolically Healthy Obesity   Obesity, Metabolically Benign           | NA                |
| NCT01159288 | Trial of a Vaccination with Tumor Antigen-loaded Dendritic Cell-derived Exosomes  | Non-Small-Cell Lung Cancer   | Phase 2           |
| NCT04536688 | A Study of RGLS4326 in Patients with Autosomal Dominant Polycystic Kidney Disease   | Polycystic Kidney Disease, Autosomal Dominant  | Phase 1           |
| NCT05060107 | Intra-articular Injection of MSC-derived Exosomes in Knee Osteoarthritis (ExoOA-1)  | Osteoarthritis, Knee   | Phase 1           |
| NCT04250493 | Insulin Resistance in Multiple System Atrophy   | Multiple System Atrophy  | NA                |
| NCT05523011 | Safety and Tolerability Study of MSC Exosome Ointment   | Psoriasis  | Phase 1           |

Table 2. Cont.

| NCT Number  | Study Title  | Conditions  | Phases            |
|-------------|--|---|-------------------|
| NCT05669261 | Treatment of Long COVID Symptoms Utilizing Autologous Stem Cells Following COVID-19 Infection  | Long COVID  | Phase 1           |
| NCT05499156 | Safety of Injection of Placental Mesenchymal Stem Cell Derived Exosomes for Treatment of Resistant Perianal Fistula in Crohn's Patients                                | Perianal Fistula in Patients with Crohn's Disease   | Phase 1   Phase 2 |
| NCT02507583 | Antisense102: Pilot Immunotherapy for Newly Diagnosed Malignant Glioma   | Malignant Glioma   Neoplasms  | Phase 1           |
| NCT03660683 | Effect of Saxagliptin and Dapagliflozin on Endothelial Progenitor Cell in Patients with Type 2 Diabetes Mellitus   | Diabetes Mellitus, Type 2   Cardiovascular diseases   | Phase 4           |
| NCT05318898 | Effect of Dietary Protein on the Regulation of Exosome microRNA Expression in Patients with Insulin Resistance.  | Insulin Resistance  | NA                |
| NCT06244849 | Toward a Better Understanding of the Autophagy Machinery for the Identification of Potential Novel Biomarkers and Therapeutic Targets in Crohn's Disease - TOPIC Study | Crohn's Disease   | NA                |
| NCT03824275 | 18F-DCFPyL Positron Emission Tomography (PET)/Computed Tomography (CT) in Men with Prostate Cancer   | Prostatic Neoplasms   | Phase 2   Phase 3 |
| NCT05864534 | Phase 2a Immune Modulation with Ultrasound for Newly Diagnosed Glioblastoma  | Newly Diagnosed Glioblastoma   Glioblastoma, Isocitric Dehydrogenase (IDH)-Wildtype   Gliosarcoma   Glioblastoma Multiforme | Phase 2           |
| NCT04202770 | Focused Ultrasound and Exosomes to Treat Depression, Anxiety, and Dementias  | Refractory Depression   Anxiety disorders   neurodegenerative diseases  | NA                |
| NCT01668849 | Edible Plant Exosome Ability to Prevent Oral Mucositis Associated with Chemoradiation Treatment of Head and Neck Cancer  | Head and Neck Cancer   Oral Mucositis   | Phase 1           |
| NCT05843799 | A Study to Evaluate the Safety and Tolerability of ILB-202   | Healthy   | Phase 1           |
| NCT05228899 | Zofin to Treat COVID-19 Long Haulers   | COVID-19  | Phase 1   Phase 2 |
| NCT04270006 | Evaluation of Adipose Derived Stem Cells Exo.in Treatment of Periodontitis   | Periodontitis   | Early Phase 1     |
| NCT05259449 | Efficacy of Educational Nutrition and Exercise on the Regulation of Appetite Through Exosomes in Type 2 Diabetics  | Diabetes Mellitus, Type 2   | NA                |
| NCT02890849 | Clinical Research for the Consistency Analysis of PD-L1 in Cancer Tissue and Plasma Exosome  | NSCLC   | NA                |
| NCT02823613 | The Influence of High and Low Salt on Exosomes in the Urine  | Healthy   | NA                |

Table 2. Cont.

| NCT Number  | Study Title   | Conditions   | Phases        |
|-------------|---|--|---------------|
| NCT05871359 | Transcranial Direct Current Stimulation and Dual Tasks  | Parkinson's Disease  | NA            |
| NCT03437759 | MSC-Exos Promote Healing of MHs   | Macular Holes  | Early Phase 1 |
| NCT03419000 | Circulating microRNAs as Biomarkers of Respiratory Dysfunction in Patients with Refractory Epilepsy   | Drug-Resistant Epilepsy  | NA            |
| NCT04427475 | Prediction of Immunotherapeutic Effect of Advanced Non-small Cell Lung Cancer   | NSCLC Patients   | NA            |
| NCT04602442 | Safety and Efficiency of Method of Exosome Inhalation in COVID-19 Associated Pneumonia  | COVID-19   SARS-CoV-2 PNEUMONIA   COVID-19                                   | Phase 2       |
| NCT03837470 | Evaluation of Renal Sodium Excretion After Salt Loading in Heart Failure with Preserved Ejection Fraction   | Heart Failure with Preserved Ejection Fraction                               | Early Phase 1 |
| NCT03083678 | Afatinib in Locally Advanced and Metastatic Chordoma  | Chordoma   | Phase 2       |
| NCT05977088 | Prediction of Effectiveness of rTMS Application in Alzheimer's Patients   | Alzheimer's Disease  | NA            |
| NCT02706288 | Effects of a Calorie Restricted, Very Low-Fat Plant-based Diet and Multi-component Exercise Program on Metabolic Health   | Obesity   Insulin Resistance   | NA            |
| NCT06391307 | The Role of Mesenchymal Stem Cell and Exosome in Treating Pilonidal Sinus Disease in Children   | Pilonidal Sinus   Pilonidal Disease  | NA            |
| NCT04636788 | Circulating Extracellular Exosomal Small RNA as Potential Biomarker for Human Pancreatic Cancer   | Pancreas Adenocarcinoma  | NA            |
| NCT03700515 | Exosome Proteomics to Detect EPO  | Healthy  | NA            |
| NCT05387278 | Safety and Effectiveness of Placental Derived Exosomes and Umbilical Cord Mesenchymal Stem Cells in Moderate to Severe Acute Respiratory Distress Syndrome (ARDS) Associated with the Novel Corona Virus Infection (COVID-19) | COVID-19 Acute Respiratory Distress Syndrome   Respiratory Distress Syndrome | Phase 1       |
| NCT05834413 | Clinical Study on the Prevention of Driver Gene Negative II-IIIa Lung Cancer Recurrence and Metastasis by Staged Chinese Herbal Medicine Combined With Chemotherapy and Immune Checkpoint Inhibitors                          | Lung Cancer  | NA            |
| NCT04389385 | COVID-19 Specific T Cell Derived Exosomes (CSTC-Exo)  | Corona Virus Infection   Pneumonia   | Phase 1       |
| NCT05808400 | Safety and Efficacy of Umbilical Cord Mesenchymal Stem Cell Exosomes in Treating Chronic Cough After COVID-19   | Long COVID-19 Syndrome   | Early Phase 1 |

Table 2. Cont.

| NCT Number  | Study Title  | Conditions  | Phases            |
|-------------|--|---|-------------------|
| NCT04356300 | Exosome of Mesenchymal Stem Cells for Multiple Organ Dysfunction Syndrome After Surgical Repair of Acute Type A Aortic Dissection            | Multiple Organ Failure  | NA                |
| NCT03537599 | Daratumumab and Donor Lymphocyte Infusion in Treating Participants with Relapsed Acute Myeloid Leukemia After Stem Cell Transplant           | Minimal Residual Disease   Recurrent Acute Myeloid Leukemia with Myelodysplasia-Related Changes   Recurrent Adult Acute Myeloid Leukemia   Recurrent Childhood Acute Myeloid Leukemia   | Phase 1   Phase 2 |
| NCT04544215 | A Clinical Study of Mesenchymal Progenitor Cell Exosomes Nebulizer for the Treatment of Pulmonary Infection                                  | Drug-resistant  | Phase 1   Phase 2 |
| NCT03627611 | Identification of Non-responders to Levothyroxine Therapy  | Hypothyroidism   Biomarkers   Endocrine System Diseases   | Phase 2           |
| NCT04202783 | The Use of Exosomes in Craniofacial Neuralgia  | Neuralgia   | NA                |
| NCT05886205 | Induced Pluripotent Stem Cell Derived Exosomes Nasal Drops for the Treatment of Refractory Focal Epilepsy                                    | Refractory Focal Epilepsy   | Early Phase 1     |
| NCT04902183 | Safety and Efficacy of Exosomes Overexpressing CD24 in Two Doses for Patients with Moderate or Severe COVID-19                               | COVID-19  | Phase 2           |
| NCT02535247 | Study of MK-3475 Alone or in Combination with Copanlisib in Relapsed or Refractory NK and T-cell Non-Hodgkin Lymphoma                        | Lymphoma, T-Cell, Peripheral  | Phase 1   Phase 2 |
| NCT03228277 | Olmutinib Trial in T790M (+) NSCLC Patients Detected by Liquid Biopsy Using BALF Extracellular Vesicular DNA                                 | Non-Small-Cell Lung Cancer  | Phase 2           |
| NCT04313647 | A Tolerance Clinical Study on Aerosol Inhalation of Mesenchymal Stem Cells Exosomes in Healthy Volunteers                                    | Healthy   | Phase 1           |
| NCT04623671 | Intravenous Infusion of CAP-1002 in Patients With COVID-19   | COVID-19  | Phase 2           |
| NCT00285805 | The Influence of Rosiglitazone on the Diuretic Effect of Furosemide and Amiloride  | Insulin Resistance  | NA                |
| NCT04177498 | Rigosertib in Patients with Recessive Dystrophic Epidermolysis Bullosa Associated SCC  | Recessive Dystrophic Epidermolysis Bullosa  | Early Phase 1     |
| NCT01629498 | Image-Guided, Intensity-Modulated Photon or Proton Beam Radiation Therapy in Treating Patients with Stage II-IIIB Non-small Cell Lung Cancer | Recurrent Lung Non-Small-Cell Carcinoma   Stage II Non-Small-Cell Lung Cancer AJCC v7   Stage IIA Non-Small-Cell Lung Carcinoma AJCC v7   Stage IIB Non-Small-Cell Lung Carcinoma AJCC v7   Stage IIIA Non-Small-Cell Lung Cancer AJCC v7   Stage IIIB Lung Non-Small-Cell Cancer AJCC v7 | Phase 1   Phase 2 |

Table 2. Cont.

| NCT Number  | Study Title   | Conditions   | Phases            |
|-------------|---|--|-------------------|
| NCT03927898 | Phase II Study of Toripalimab Plus Stereotactic Body Radiotherapy in Colorectal Cancer Patients with Oligometastasis              | Metastatic Colorectal Cancer   | Phase 2           |
| NCT05947747 | Safety and Efficacy of EXO-CD24 in Preventing Clinical Deterioration in Patients with Mild-Moderate ARDS                          | ARDS   | Phase 2           |
| NCT02594345 | Effect of Exosomes Derived from Red Blood Cell Units on Platelet Function and Blood Coagulation                                   | Blood Coagulation   Platelet Function  | NA                |
| NCT04384445 | Zofin (Organicell Flow) for Patients With COVID-19  | Corona Virus Infection   COVID-19   SARS   Acute Respiratory Distress Syndrome | Phase 1   Phase 2 |
| NCT04298398 | Impact of Group Psychological Interventions on Extracellular Vesicles in People Who Had Cancer                                    | Cancer   | NA                |
| NCT03109847 | Metformin Hydrochloride in Mitigating Side Effects of Radioactive Iodine Treatment in Patients with Differentiated Thyroid Cancer | Thyroid  | Phase 2           |
| NCT04350177 | A Study to Assess Single and Multiple Doses of Ikt-148009 in Healthy Elderly Participants and Parkinson's Patients                | Healthy Elderly   Parkinson's Disease  | Phase 1           |
| NCT05559177 | An Open, Dose-escalation Clinical Study of Chimeric Exosomal Tumor Vaccines for Recurrent or Metastatic Bladder Cancer            | Recurrent or Metastatic Bladder Cancer   | Early Phase 1     |
| NCT05607654 | The Mechanisms of Underlying Accelerated Repetitive Transcranial Magnetic Stimulation for Treatment-resistant Depression          | Treatment-Resistant Depression   | NA                |
| NCT06221787 | Stem Cell Derived Exosomes in the Treatment of Melasma and Its Percutaneous Penetration   | Melasma  | NA                |
| NCT02921854 | Detection of Circulating Biomarkers of Immunogenic Cell Death   | Non-Small-Cell Lung Cancer   | NA                |
| NCT02310451 | Study of Molecular Mechanisms Implicated in the Pathogenesis of Melanoma. Role of Exosomes  | Metastatic Melanoma  | NA                |

### 3. Challenges and Prospects Associated with the Translation of Exosome Therapy

The clinical adoption of exosome-based therapeutic strategies is impeded by challenges associated with low exosomal yield and efficiency [7]. In a controlled laboratory setting, extraction from one milliliter of cell culture medium frequently results in less than one microgram of exosomal protein [29]. Strategies to augment exosome production have been explored, encompassing mechanical (e.g., three-dimensional culture systems and the application of shear stress), biochemical (e.g., treatment with lipopolysaccharide (LPS), interferon-gamma (IFN- $\gamma$ ), bone morphogenetic protein 2 (BMP-2), tumor necrosis factor-alpha (TNF- $\alpha$ ), and hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ )), and physical (e.g., thermal stress, hypoxic conditions, and nutrient deprivation) methodologies [30–32].

Exosome isolation is complicated by their intrinsic heterogeneity, manifested in variations in size, composition, surface markers, and biological origin [33]. Predominant



isolation and purification methodologies are based on immunoaffinity, leveraging surface charge characteristics, or size and density differences [34]. Each methodology presents specific advantages and drawbacks, and no singular approach is universally applicable [35]. Ultracentrifugation, while often deemed the gold standard, is constrained in its scalability by factors such as cost, efficiency, vesicle aggregation, and the co-isolation of lipoproteins, despite minimal chemical and expertise requirements [36]. Conversely, immunoaffinity chromatography, exploiting specific antigen–antibody interactions, is noted for its high specificity, yield, and purity, albeit dependent on the presence of appropriate exosomal surface antigens [37]. Techniques predicated on size differentiation, including size-exclusion chromatography and ultrafiltration, are advantageous for scalability but are limited by issues such as pore clogging, loss of exosomal material, and reduced purity [38–40]. The amalgamation of disparate isolation techniques, incorporating precipitation- and microfluidics-based approaches, may offer a comprehensive solution to effectively address the multifaceted requirements for exosome isolation and purification [41]. For instance, in our research endeavors, a synergistic application of differential ultracentrifugation and immunomagnetic separation facilitated the isolation of cell-specific exosomes from murine tissue, and a parallel strategy was employed for the extraction of exosomes from human wound effluents [42,43].

Anticoagulants, notably EDTA, have been documented to markedly influence both the composition of exosomes and their isolation processes from blood specimens [44]. Subsequent investigations have elucidated that during the procedural phases of blood collection and processing, EDTA plays a stabilizing role in maintaining the integrity of platelet-derived EVs [45]. However, it has also been substantiated that the utilization of EDTA-containing tubes does not forestall alterations in the exosomal profile. The effects of EDTA substitutes on the characterization and profiles of exosomes remain underexplored, presenting a significant gap in current research [46]. This issue bears considerable importance for the reproducibility of quantifications of blood exosome concentrations and the isolation of platelet-derived exosomes, which pose considerable challenges in biomarker discovery for conditions not associated with platelets [47]. This aspect becomes critically pertinent when processing single-spun plasma samples, potentially leading to contaminations in the exosome isolates due to the presence of frozen platelets [48].

The selection of an anticoagulant that preserves the analytes of interest without compromising the integrity of the exosomes is of paramount consideration [47]. Reports indicate that blood collection tubes incorporating non-aldehyde-based stabilizers for cell-free nucleic acids are conducive for exosome handling, as they also contribute to exosome stabilization. [49]. Another essential aspect for consistent determination of exosome composition in blood involves understanding how various pre-analytical factors, such as collection, processing, and storage conditions, impact the protein corona and the co-isolation of exosomes with other blood components, such as platelets, lipoproteins, soluble protein aggregates, viruses, cell-free DNA/histones, or circulating mitochondria [50].

Furthermore, the quantification and characterization of blood-derived exosomes are also contingent upon the centrifugation parameters and storage conditions [51]. The integrity of microRNA analyses in exosome samples can be significantly compromised by hemolysis during blood sample collection [52]. Addressing these variables is crucial in the standardization of methodologies for exosome research.

#### 4. Factors Taken into Consideration for Exosomal Function Dictation

The function of exosomes is determined by isolation techniques, molecular characterization, functional tests, and imaging approaches. Despite progress, challenges remain due to the need for standardization, as well as the limitations of current technology [53]. Understanding the methods of exosome uptake, such as endocytosis, membrane fusion, or receptor-mediated interactions, is essential for the successful delivery of functional biomolecular cargo such as proteins, RNA, or miRNA [54]. Advanced *in vitro* assays such as fluorescent labeling and reporter gene assays, *in vivo* models, molecular analysis,

such as proteomics, transcriptomics, Western blotting, qPCR, functional impact studies, such as cell proliferation assays, apoptosis assays, migration and invasion assays, and immunomodulation assays, help elucidate the functional impacts of exosomes on recipient cells [55]. The issues of cell specificity, dose, safety, and scalability must be addressed to maximize therapeutic efficacy, while minimizing unwanted effects. Exosomes have intrinsic targeting capability influencing both physiological and pathological states through lipids, RNA and proteins. Not surprisingly, various biotechnology companies like Stem Cell Medicine Ltd., Evox Therapeutics, Pharmaceutics Inc. are working to create different therapies using exosomes [55]. Much research has been conducted to elucidate the functioning of exosomes following uptake into recipient cells, overriding their inherent targeting ability. Exosomes can enter a recipient cell by macropinocytosis, phagocytosis, lipid-raft-mediated uptake, and membrane fusion. The primary route of exosome uptake is clathrin/caveolin-mediated endocytosis [54,56,57]. Functional delivery requires exosome-encapsulated material to “escape” from the endosome and enter the cytoplasm [56]. To support the exosome-encapsulated content’s communicative qualities, robust functional transport to recipient cells must be shown. Endosomal escape and effective cargo delivery are desired outcomes for exosome therapeutic usage, but there is also a risk of cargo loss due to breakdown, recycling in the cell, or the re-release of intact vesicles into the extracellular environment [56].

#### 4.1. Protein Corona

The concept of the protein corona refers to the phenomenon where proteins adhere to the surface of exosomes upon their introduction into biological fluids such as blood plasma, forming a coating (Figure 2) [58]. This process alters the surface properties, such as shape, size, and structural modifications of exosomes, significantly affecting their interactions with the cell membrane of recipient cells by modifying recognition, uptake, targeting, and the biological responses elicited, with profound implications for health and diseases like cancer, Alzheimer’s, wound healing, etc. [59]. The protein corona comprises not merely a random assortment of plasma proteins, but includes specific proteins, such as anti-thrombin III, factor V, complement C3, IgG, fibronectin, and complement factor H, which influence the biological behavior and interactions of exosomes within the bloodstream [60]. This aspect facilitates the targeted delivery of exosomes to specific cell types or tissues by promoting interactions with the receptors. Nonetheless, the presence of a protein corona can compromise therapeutic efficacy through mechanisms such as the accelerated clearance of exosomes, induction of adverse immunological responses or reduction in the efficiency of cargo delivery to target cells [61].

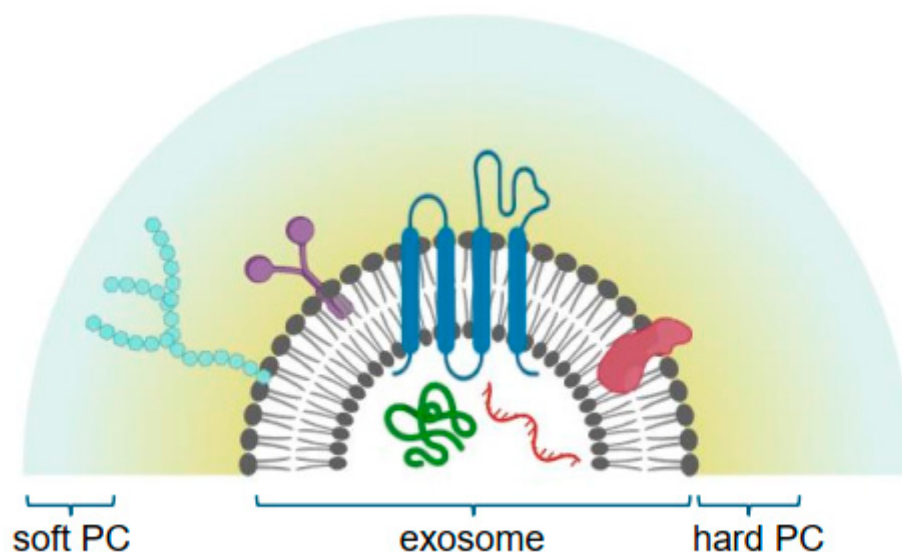


Figure 2. Illustration of the protein corona formation around exosomes.

Strategies involving engineering the exosome surface with specific ligands or coatings can alter the protein corona composition, enhancing targeting specificity and reducing unwanted immune reactions [62]. Pre-exposing exosomes to plasma or specific proteins, such as anti-thrombin III, factor V, complement C3, IgG, fibronectin, and complement factor H, before therapeutic applications, such as cancer, nervous system diseases, and immune diseases, can induce a favorable corona formation, potentially augmenting their therapeutic efficacy *in vivo*. Wolfram et al. highlighted the role of nanoparticles high surface free energy in the adsorption of molecules, predominantly proteins, leading to the formation of the protein corona [61]. The binding forces facilitating such interactions encompass Van der Waals forces, hydrophobic interactions, hydrogen bonds, electrostatic attractions, and  $\pi$ - $\pi$  stacking [61].

Conceptually, the protein corona is divided into a ‘hard’ and a ‘soft’ layer, distinguished by the proximity of biomolecules such as proteins, lipids, and sugars to the nanovesicles [63]. The soft layer is characterized by a more dynamic exchange of biomolecules. However, there is debate regarding the heterogeneity of the hard corona formed in human blood plasma, with some sources indicating fewer than 100 distinct proteins, while others suggest greater than 100 [64]. Table 3 lists protein coronas adsorbed around exosomes [58].

The composition of the protein corona is influenced by the size, shape, and surface charge of the nanovesicles, with prevalent proteins including albumin, complement proteins, apolipoprotein 1, and immunoglobulins [58]. Protein attachment to nanovesicles induces both reversible and irreversible structural changes in the proteins, occurring in stages, with the least stable conformations exhibiting the quickest misfolding kinetics [65]. Protein adsorption is enhanced, with an increase in size due to the lower curvature, facilitating more extensive surface interaction and consequent protein conformational changes. Additionally, a positive surface charge correlates with increased protein adsorption [61].

**Table 3.** List of protein coronas adsorbed around exosomes.

| S. No. | Hard and Soft PC  | Types of Interactions                                   | Effect  | References                   |
|--------|---|---|---|------------------------------|
| 1.     | Albumin   | Connects to RNA/DNA on exosomes                         | Phagocytosis of exosomes  | Malonga et al. 2006 [66]     |
| 2.     | ApoE and ApoB100  | Protein–protein interactions                            | Increases transfer rate in peripheral tissue                                  | Bertrand et al. 2017 [67]    |
| 3.     | Apolipoprotein A1   | Interacts with CD63 on Exosome surface                  | Phagocytosis of exosomes  | Toth et al. 2021 [60]        |
| 4.     | Apolipoprotein B  | Interacts with CD63 on Exosome surface                  | Increases phagocytic activities and triggers secondary inflammation reactions | Toth et al. 2021 [60]        |
| 5.     | Complement factors 3  | Interacts with CD63 on Exosome surface                  | Increases phagocytic activities and triggers secondary inflammation reactions | Toth et al. 2021 [60]        |
| 6.     | Complement proteins C3b and C3ib                            | Protein–protein interactions                            | Prolonged chronic inflammatory conditions                                     | Conde et al. 2012 [68]       |
| 7.     | Immunoglobulin heavy chains of ( $\gamma$ 2 and $\gamma$ 4) | Protein–protein interactions                            | Reduces transit time and affects bio-distribution                             | Toth et al. 2021 [60]        |
| 8.     | Mismatched MHC-I and II                                     | On the surface of Exosomes                              | T-cell immune responses   | Hiltbrunner et al. 2016 [69] |
| 9.     | S100-A8, LDL-receptor, CD14, HLA class I                    | Phosphatidylserine and tissue factor on Exosome surface | Dynamic activity  | Buzas et al. 2018 [70]       |

The protein corona on exosomes can also influence the cellular uptake of nanovesicles by modulating their adhesion to the cell membrane, a process which can either inhibit or enhance cellular internalization [71]. Despite the consensus on the dynamic nature of the protein corona, specific binding events occur at the nano–plasma interface, and their sequential order remains underexplored. Recent studies indicate that the quantity of protein in the corona varies over time, although the types of proteins bound tend to remain stable [58,61]. Nevertheless, the variability in protein corona composition among extracellular vesicle populations presents challenges in controlling the protein corona via surface engineering strategies [61].

#### 4.2. Interactions of Lipoproteins with Exosomes

The potential interactions between exosomes and lipoproteins have received minimal attention in research conducted over the last few decades [62]. Previous investigations have rigorously examined lipoproteins as exogenous impurities in EV samples. Notably, lipoproteins are identified at a minimum quintuple excess in comparison to exosomes in biological specimens processed for exosome isolation, posing a significant challenge due to their overlap in size with exosomes [72,73]. Both exosomes and lipoproteins are ubiquitously found in the bloodstream, sharing similar dimensions and densities. Plasma specimens comprise exosomes (40–120 nm), microvesicles (100 nm–1  $\mu$ m), and apoptotic bodies (50 nm–2  $\mu$ m), while lipoprotein subclasses, such as HDL (5–12 nm), LDL (18–25 nm), IDL (25–35 nm), VLDL (30–80 nm), chylomicron remnants (30–80 nm), and chylomicrons (75–1200 nm), exhibit size ranges overlapping with those of exosomes [62]. This congruence in size renders their separate isolation unfeasible [73–75]. Prior research has documented the presence of complexes formed between exosomes and lipoprotein-like structures in plasma from healthy human subjects [76]. Subsequent research corroborated these findings, observing exosome complexation with lipoproteins under physiological conditions [73,75,77]. Subsequent investigations, including the work of Lozano-Andres et al., utilizing cryogenic transmission electron microscopy, corroborated the association of EVs with lipoproteins and elucidated the consequential effects on EV detection and characterization [78]. Historically, the focus has been on treating lipoproteins as inert contaminants, neglecting the potential interactions between these two biological nanoparticles [39]. Proteomic analyses and immunogold transmission electron microscopy have indicated that lipoprotein components may engage in dynamic interactions with exosomes, leading to complex formation in both artificial and physiological milieus [79]. These complexes could influence exosome detection, characterization, cellular uptake, and subsequent biological effects. Techniques such as atomic force microscopy (AFM) or transmission electron microscopy (TEM) have been proposed to investigate the complexation phenomena between these. Collectively, these insights suggest that exosomes and lipoproteins can form complexes in various environments [80]. It is also paramount to acknowledge that exosome isolation techniques might foster non-physiological interactions between exosomes and lipoproteins [81]. Given these recent discoveries, it becomes imperative to explore the nature of these interactions and their potential mechanistic, therapeutic, and diagnostic ramifications at the exosome–lipoprotein interface [62].

#### 4.3. Effects of Size, Morphology, and Surface Modification on Cellular Uptake of Exosomes

The physicochemical characteristics of nanovesicles significantly influence cellular internalization dynamics. It is pertinent to examine whether the methodologies employed for isolating enriched exosomal fractions impact their cellular uptake [82]. A comparative analysis of exosomes isolated via ultracentrifugation and polymer-based precipitation techniques demonstrated that the latter results in a narrower particle size distribution, accelerated absorption by target cells, and enhanced cellular motility [83]. Furthermore, differential efficiencies in exosome uptake were observed between vesicles of different cellular origins, highlighting the specificity of interaction between exosomes and target cells [2,84]. Endocytic pathways, including clathrin/caveolae-mediated endocytosis, phago-

cytosis, macropinocytosis, and pinocytosis, play critical roles in the size-dependent uptake of exosomes [84]. Smaller exosomes, typically ranging from 30 to 100 nm, are more likely internalized through clathrin-mediated endocytosis, while larger vesicles may prefer alternative pathways, such as caveolin-mediated endocytosis or macropinocytosis [85,86]. The preference for specific endocytic pathways based on size impacts the efficiency of uptake and subsequent intracellular routing, thereby affecting cargo delivery to targeted cellular compartments [87]. Additionally, the morphological characteristics of exosomes, such as their cup-shaped or spherical appearance under TEM, influence their binding affinity to cellular receptors and internalization rates [88]. Variations in preferred morphology are observed across different cell types [89]. The composition of the exosome membrane also affects its fusogenic capacity, facilitating direct cargo delivery into the cytoplasm by fusion with the cell membrane [90].

Exosomes present a diverse array of surface molecules, including proteins lipids, and glycans which can be exploited for targeted cellular interactions [91]. However, their targeting potential is sometimes limited. Innovative approaches such as surface functionalization can enhance targeting specificity [92]. For instance, Tian et al. enhanced the bioavailability of curcumin-loaded exosomes through peptide functionalization, enabling them to cross the blood-brain barrier post-intravenous injection [93]. Similarly, Liang et al. developed miR-140-loaded exosomes that, when administered intra-articularly, effectively penetrated dense extracellular matrices to alleviate osteoarthritis symptoms, suggesting a viable cell-free treatment strategy [94]. Moreover, Li et al. demonstrated the therapeutic potential of targeted engineered exosomes in diabetic wound healing [95]. Enhancements in the bio-functional engineering of exosomes could improve their targeting accuracy and circulation time, optimizing the delivery of therapeutic cargos to specific cells or tissues [95,96].

#### 4.4. Cellular Environment

Paracrine intercellular communication via exosomes utilizes the cellular milieu as a conduit and modulates exosome–cell interactions to varying extents [97]. This milieu includes not only the extracellular matrix (ECM) but also external biophysical variables such as pH, temperature, and oxidative or hypoxic conditions [98]. For instance, ECM rigidity may influence exosome uptake, while its mechanical properties can dictate exosomal transit by interacting with water permeability [99]. Additionally, the extracellular environment's pH and extracellular ion concentrations can significantly affect the stability, absorption, and release of exosomes [100]. Variations in ion levels might alter the composition and structural integrity of exosomal membranes, thereby modifying their interactions with recipient cells [101]. A low pH environment, commonly encountered in tumor settings, may enhance the malignancy of cancer cells by modulating exosome release and uptake [102].

Temperature also profoundly affects the cellular uptake of exosomes, with reduced temperatures markedly impeding this process, indicating its energy-dependent nature [103]. Pathological conditions such as cancer, inflammation, or infection led cells to alter their exosome secretion and composition [104]. For example, cancer cells typically secrete more exosomes containing oncogenic factors that facilitate tumor growth, angiogenesis, and metastatic site preparation [105].

Under conditions of oxidative stress or hypoxia, cells might emit exosomes laden with stress-response proteins or RNAs, influencing the survival and functionality of recipient cells [106]. The local tissue environment, which encompasses components of the ECM, cell–cell interactions, growth factors, and cytokines, also plays a crucial role in modulating exosome activity. Specific receptors within the tissue microenvironment can enhance the selectivity of exosome uptake by recipient cells [107]. Furthermore, oxidative stress can alter the protein content of exosomes from amnion-epithelial cells, promoting the release of inflammatory mediators that trigger inflammation [106]. Additionally, external factors such as ionizing radiation have been shown to enhance exosomal secretion pathways in breast cancer cells, as demonstrated by Jabbari et al., suggesting a potential mechanism for developing treatment resistance [108].



Exosomes play an important function in immunological regulation. They are released by antigen-presenting cells and can provide therapeutic advantages by suppressing or increasing the immune response [109,110]. Exosomes from antigen-presenting cells include major histocompatibility peptide complexes and costimulatory molecules that influence antigen-specific CD8<sup>+</sup> and CD4<sup>+</sup> responses. Antigen-specific T cells directly contact MHC-peptide complexes on exosomes, activating T cells. Exosomes produced by dendritic cells (DCs) can stimulate T and B cells and have been studied for their immunostimulatory characteristics in cancer treatment. Exosomes generated by dendritic cells (DCs) can activate T and B cells and have been investigated for immunostimulatory properties in cancer therapy [111,112]. They can increase immunological responses in vivo by transferring MHC-peptide complexes from DCs that have been exposed to an antigen to another DC that has not encountered the antigen [113].

#### 4.5. Entry and Departure of Exosomes from the Circulation

Exosomes function as pivotal carriers of bioactive molecules, facilitating intercellular communication and playing a significant role in both paracrine and endocrine-signaling processes within the tissue interstitium and circulatory system [114,115]. Nevertheless, the precise biological mechanisms facilitating their ingress and egress from the circulatory system have yet to be fully elucidated. Prior research has delineated the traversal of exosomes through both paracellular and transcellular pathways, with paracellular transport assuming significance in pathological contexts, such as inflammation [116]. The transcellular pathway entails the endocytosis of exosomes by endothelial cells, followed by their transport across the cellular body for subsequent release on the opposite side [117]. Moreover, the lymphatic system is implicated in the transit of exosomes from various organs into the bloodstream, suggesting its critical role in the systemic dissemination of exosomes [114].

Endothelial cells are known to internalize exosomes through diverse mechanisms, including clathrin-dependent endocytosis, caveolin-mediated uptake, macropinocytosis, and lipid raft-mediated internalization [54]. These uptake pathways are influenced by the exosomes biophysical attributes, which may, in turn, modulate the endothelial internalization profile [117]. This inherent variability in exosome properties could be consequential in determining their cellular uptake dynamics, a phenomenon similarly observed with synthetic nanoparticles [54,117]. For instance, larger particles are predominantly internalized through phagocytosis or macropinocytosis, whereas smaller counterparts are primarily subject to alternative endocytic pathways [54,118]. This intricate interplay between exosomes and endothelial cells highlights the complexity of cellular internalization mechanisms and the potential for differential pathway engagement based on exosomal characteristics [114].

#### 4.6. Functional Delivery of Exosomal Cargo

Cells encapsulate specific molecular cargoes within exosomes, which are then released into the extracellular milieu [88]. The lipid bilayer membrane of exosomes serves a critical protective role, shielding the encapsulated cargo from enzymatic degradation in the extracellular environment [1,119]. This process of paracrine signaling involves the direct transfer of this cargo into recipient cells, thereby exerting control over multiple levels, including genetic, signaling pathways, and overall cellular activities [97]. A key aspect of the therapeutic application of exosomes is ensuring the efficient release of the encapsulated content from the endosome before its internal environment becomes acidic and degrades the cargo [120]. However, challenges such as cargo degradation, intracellular recycling, or re-release of intact vesicles into the extracellular space can impede effective therapeutic delivery [12].

Once internalized by target cells, the exosomal cargoes are released, impacting various cellular processes based on the nature of the cargo and the physiological state of the donor cell [121]. For instance, mRNA and miRNA within exosomes can modify gene expression patterns in recipient cells, potentially leading to the synthesis of new proteins. Similarly, proteins and lipids carried by exosomes can also activate or inhibit specific signaling

pathways [32], while antigens delivered via exosomes may modulate immune responses, enhancing or suppressing them as required. For example, cargos carried by exosomes activate the Wnt/ $\beta$ -catenin pathway for collagen deposition; the PI3K/AKT/mTOR pathway can activate endothelial cells or fibroblast functions, while the VEGF pathway promotes angiogenesis in wound healing processes [122].

The specificity with which exosomes target recipient cells offers a significant advantage in designing targeted therapeutic strategies, thereby increasing efficacy, and minimizing side effects [119]. This targeting capability also allows for the *in vitro* manipulation of exosomes to load them with specific therapeutic agents, including drugs such as curcumin, doxorubicin, paclitaxel [123], RNA interference molecules, or other bioactive compounds. Due to their natural origin and biocompatibility, exosomes generally evade immediate immune detection, which is particularly advantageous in drug delivery applications. For example, in oncological therapies, exosomes derived from immune cells can be engineered to carry tumor-suppressive agents such as miRNA, miR-199a-3p [124], cisplatin, doxorubicin [124,125] directly to cancer cells, thus inhibiting tumor growth and progression [126]. This nuanced understanding of exosomal functions and their potential applications underscores the transformative possibilities of exosomes in medical science, particularly in targeted and precision therapies [127].

## 5. Current Attempts to Overcome Challenges Associated with the Therapeutic Application of Exosomes

To address these concerns, a task force from the International Society for Extracellular Vesicles (ISEV) Rigor and Standardization Committee has outlined the pre-analytical factors essential for the research on blood-derived exosomes, aiming to enhance the reproducibility of exosome isolation from blood samples.

### 5.1. Standardization of Research

Efforts to standardize clinical research on exosomes have predominantly concentrated on the optimization of isolation and characterization protocols [53]. However, the preanalytical phases, encompassing specimen handling, storage, and collection protocols, substantially influence the reproducibility and integrity of research outcomes [128,129]. Among the critical preanalytical variables, the transportation duration of biological specimens, such as blood, wound exudates, urine, and other bodily fluids, exhibits significant variability in terms of temperature and transit time, even within identical specimen types [130]. This variability can markedly affect experimental results and should be meticulously considered in data interpretation, especially when samples originate from multiple collection sites. Explicit documentation of these preanalytical variables in the methods section is imperative to evaluate their impact on the research findings and to facilitate cross-study comparisons.

The present market offers a variety of commercial kits, such as exosome isolation kits by System Biosciences, Thermo Fischer Scientific, Qiagen, Miletyi, Norgen Biotek, designed to isolate exosomes from specific biological sources [130]. These kits employ various methodologies, including polymer precipitation, membrane affinity, antibody capture, and filtration to separate or concentrate exosomes. These kits, while beneficial under certain conditions, must be used with caution, as they lack comprehensive documentation on the principles of exosome isolation and enrichment [130–132]. The absence of detailed methodological information can lead to the introduction of unknown impurities, such as polyethylene glycol, which can compromise the purity of the exosome preparations and potentially skew the experimental results. Such contaminants could mislead the interpretation of the data, affecting the validity of the research findings [37].

Therefore, it is recommended to select commercial exosome isolation kits that provide detailed procedural information for reproducibility. Standardizing these procedures not only enhances the reliability of the research but also supports the broader scientific community in achieving consistent and verifiable results in exosome-based studies.

### 5.2. Reporting Standards

The integrity of the data within Vesiclepedia (<http://www.microvesicles.org/>), derived from both curated and author-submitted publications, is directly linked to the quality of the corresponding EV research [133,134]. The field of exosomes is characterized by a lack of stringent nomenclature and variability in the exosome isolation protocols across different studies and laboratories, significantly affecting the purity and type of the isolated exosomes, as well as their associated cargoes [1]. Furthermore, it is imperative to note that the recurrent identification of certain proteins in exosome studies does not necessarily qualify them as definitive exosome markers or proteins enriched in exosomes. Consequently, users are advised to exercise caution when employing Vesiclepedia data for further analysis, paying particular attention to the isolation methods and meta-annotations to select high-quality datasets pertinent to their research [135]. As of the latest update, Vesiclepedia comprises data from 3533 EV-related studies, marking an over twofold increase in the database's catalog since the 2019 update. This expansion includes contributions of 56,691 proteins, 50,550 RNA, 3839 lipids, 192 metabolites, and 167 DNA entries [133]. Quantitative data, now available for 62,822 entries derived from 47 studies, represent a significant augmentation of Vesiclepedia's utility. The database presently catalogs 252 sample sources from 56 distinct organisms. The latest enhancement to Vesiclepedia introduces EVQUANT, a novel feature facilitating the relative quantification of extracellular vesicle (EV) proteins, RNA, and lipid cargoes within individual studies [133]. Given the diversity in experimental methodologies and sample processing across studies, it is currently not feasible to perform quantitative cross-comparisons. Nevertheless, the advent of high-throughput data generation and the potential for establishing uniform analytical pipelines herald the possibility of cross-study comparisons, contingent upon the standardization of experimental procedures.

### 5.3. Accurately Defining the Vesicles

As per the MISEV 2023 guidelines [136], exosomes are defined as endosomal-derived intraluminal vesicles that form a specific subtype of small EVs with diameters less than 200 nm. This classification distinguishes them from other small EVs, such as ectosomes, which also form part of the broader EV population, but originate through different biogenetic processes. The terms "exosome" and "small EV" are not synonymous and should be used with precision. The lack of universal molecular markers for exosomes, ectosomes, and other EV subtypes presents a significant challenge in definitively characterizing these entities based on their origin. Consequently, much of the current research focuses broadly on mixed EV populations rather than exclusively on exosomes, pending clear evidence of their specific cellular origins [136].

### 5.4. Single-Exosome Studies

In the context of therapeutic applications, the characterization and validation of exosomes necessitate a comprehensive assessment, focusing on their size, shape, and molecular content [137]. Current methodologies for exosome characterization are broadly categorized into three primary domains: morphological analysis, size determination, and cargo profiling [138].

Morphological analysis employs techniques such as Scanning Electron Microscopy (SEM) and TEM, which facilitate the direct visualization of exosomal internal structures and surface topography, respectively [138]. Despite the detailed resolution offered by TEM, its intricate operation requirements [139] and labor-intensive sample preparation procedures limit its suitability for high-throughput analysis [140]. High-throughput analysis enables the simultaneous profiling of thousands of exosomes, facilitating the categorization of these vesicles based on their molecular signatures, such as lipids, proteins, and mRNA. This technology assists in identifying the origins and potential functions of exosomes [141]. Additionally, it can be employed to engineer exosomes and to screen them from various biological fluids, including blood, urine, and cerebrospinal fluid, enhancing their utility in disease diagnostics and prognostics [142]. This approach is instrumental



in advancing our understanding of exosome-mediated processes and their implications in health and disease [143]. For size determination, methodologies such as nanoparticle tracking analysis (NTA), Dynamic Light Scattering (DLS), and tunable resistive pulse sensing (TRPS) are employed [144]. Among these, NTA stands out for its ability to provide high-resolution measurements, enabling the rapid identification and real-time observation of exosomes [145]. However, NTA's capability to distinguish between exosomal particles and protein contaminants remains a challenge [146]. To overcome the limitations inherent in each characterization technique, it is a common practice to employ a multifaceted approach, integrating methods from each of the three domains, such as a combination of TEM, NTA, and protein-marker studies. This integrated strategy ensures a comprehensive characterization of exosomes, balancing the advantages and disadvantages of each method [137].

The diversity in biophysical and biochemical characteristics of exosomes is significantly influenced by the originating cell line and the employed isolation technique, leading to variability in the outcomes of biological analyses such as Western blots, PCR, and Dynamic Light Scattering (DLS) [147]. While traditional ensemble analysis methods have provided insights into the general biological and physical properties of exosomes, they offer limited resolution regarding the heterogeneity and individual characteristics of these vesicles. Consequently, these ensemble approaches, encompassing Western blot, enzyme-linked immunosorbent assay (ELISA), and Polymerase Chain Reaction (PCR), have been recognized for their limitations in accurately reflecting the complexity and diversity of exosomal populations [148].

In response to these limitations, advancements in analytical technologies have facilitated the shift towards single-particle analyses, enabling detailed investigation into the unique roles and properties of individual exosomes [149]. The guidelines proposed by the Minimal Information for Studies of Extracellular Vesicles (MISEV) advocate for the implementation of at least two single-particle analysis techniques to evaluate both the shape and biological attributes of single exosomes, emphasizing the importance of characterizing these vesicles at the individual level [150]. The development and refinement of single-exosome analysis techniques aim to address critical challenges associated with exosome research, including their intrinsic heterogeneity, measurement precision, complex biochemical composition, and nanoscale dimensions [11]. The increasing recognition of the need for precise sorting and phenotyping of specific exosome subpopulations has spurred the development of over twenty innovative single-vesicle methodologies [151]. Despite these advancements, the direct visualization of exosomes remains challenging due to their size, which often approaches the diffraction limit of standard optical microscopy. This limitation hampers the detailed examination of extracellular vesicle interactions and behaviors within cellular environments [152]. However, the advent of super-resolution imaging techniques, such as direct stochastic optical reconstruction microscopy (dSTORM), has opened new avenues for understanding the formation, function, and intracellular dynamics of extracellular vesicles [153]. dSTORM offers unprecedented sensitivity from free dyes or dye aggregations and resolution, enabling the precise determination of individual exosome arrangement, localization, and clustering through the tracking of vesicles in fluorescence mode with ultra-high single-molecule sensitivity [154–156]. Total internal reflection fluorescence (TIRF) microscopy, in synergy with single-molecule localization techniques, significantly enhances the signal-to-noise ratio and reduces the duration of imaging sessions [157]. However, super-resolution fluorescence microscopy demands sophisticated instrumentation and powerful data processing software, often resulting in low throughput [158]. While fluorescence microscopy techniques are pivotal for elucidating the mechanisms of exosome secretion, characterization, and uptake, the potential of fluorescent markers to interfere with their localization, activity, and functionality cannot be overlooked [150]. Additionally, lipid markers may exhibit non-specificity in their labeling properties or might aggregate [159].

In this context, flow cytometry (FCM) and nanoparticle tracking analysis (NTA) emerge as alternative approaches, leveraging the analysis of scattered light patterns from single particles and the tracking of light-scattering signals from particle diffusion, respectively, to derive physical insights at sub-wavelength scales [160]. Furthermore, fluorescence-based methodologies harness the interactions between light and molecules, employing fluorophore-conjugated target markers for labeling and detection [161]. The innovation of nano-flow cytometry (nFCM) by Prof. Yan Xiaomei's laboratory represents a significant leap forward. By amalgamating Rayleigh light scattering with sheath flow single-molecule fluorescence detection technology, nFCM markedly enhances the sensitivity of both scattering and fluorescence detection relative to conventional FCM [162]. Capable of analyzing up to 10,000 particles per minute, this technology permits the multi-parametric quantitative measurement of single vesicles, down to a minimum particle size of 40 nm [150]. However, the advanced equipment and expertise required for nFCM impose limitations on its widespread clinical application. Furthermore, electron microscopy (EM) and atomic force microscopy (AFM) offer high-resolution imaging capabilities that allow for the direct visualization of exosomes, thus facilitating the characterization of their morphological and particle size attributes [163]. The application of these diverse nanotechnologies enables the comprehensive examination and cross-validation of the distinct traits and biological functions of individual extracellular vesicles (EVs) [164]. Consequently, researchers must judiciously select a combination of these techniques to effectively conduct their analyses. Therefore, the strategic integration of single-exosome analysis methodologies is a critical prerequisite for advancing our understanding of the physiological and pathological roles of exosomes [165]. This integrative approach not only elucidates the mechanisms underlying exosomal-mediated cell-to-cell communication but also paves the way for establishing a novel paradigm in exosome research [10,166].

### 5.5. Exosome Storage

Cryopreservation, lyophilization, and spray-drying are the predominant methodologies for the extended preservation of therapeutic exosomes, primarily relying on controlled temperature regulation and the inclusion of cryoprotective agents [167]. Although it is imperative to analyze exosomes in their native state post-isolation, for therapeutic purposes, extended shelf life is typically necessary. The stability of exosomes can vary, with some remaining intact without the need for freezing, depending on their biochemical composition and source of origin. Previous research has demonstrated that for long-term storage, a standard temperature of  $-80\text{ }^{\circ}\text{C}$  is often used [168]. Specific studies have demonstrated that urinary exosomes can be preserved for up to four years at  $-20\text{ }^{\circ}\text{C}$ , while saliva-derived exosomes may retain their protein composition and membrane integrity for up to 20 months when stored at  $4\text{ }^{\circ}\text{C}$ , although storage at this temperature can potentially diminish their protein content and biological activity [169].

The process of lyophilization offers an alternative storage solution, allowing exosomes to be easily preserved and reconstituted with the addition of an appropriate buffered solvent [170]. Recent studies suggest that lyophilization, particularly with the inclusion of cryoprotectants, can maintain the functionality of exosomal proteins and RNA for approximately four weeks, even at ambient temperatures [169]. The application of cryoprotectants is crucial in mitigating the adverse effects of freeze-thaw cycles, preventing cryodamage and aggregation of exosomes [171]. Formulations such as phosphate-buffered saline enriched with human albumin and the non-permeable disaccharide trehalose (PBS-HAT) have been found to enhance both short- and long-term stability and enhance both the short- and long-term exosome stability of therapeutic exosomes at  $-80\text{ }^{\circ}\text{C}$  and across multiple freeze-thaw cycles [172,173]. The specifics of the freezing protocol (e.g., snap-freezing in liquid nitrogen vs. gradual freezing), the composition of the suspension buffer, storage duration, thawing techniques, and the number of freeze-thaw cycles are critical variables that need documentation, since optimal storage conditions can vary based on the exosomes' origin and therapeutic composition [174]. To minimize detrimental freeze-thaw cycles,

careful aliquoting is recommended, and it is crucial to recognize that samples subjected to varying numbers of freeze–thaw cycles may not be directly comparable. Proper labeling and documentation of storage containers are also essential to prevent the loss of exosomes due to adhesion to the container surfaces [174,175].

### 5.6. Modification of Exosomes for Specific Targeting

Exosomes, with their natural stability, low immunogenicity, and innate ability to target specific recipient cells represent an ideal vehicle for drug delivery [8]. These nanovesicles present a versatile platform for the bioengineering of therapeutic agents with minimal biochemical modification to enhance, broaden, or alter their therapeutic potentials [176]. The techniques for cargo loading into exosomes are categorized into pre- and post-biogenesis methodologies [177,178]. Pre-production strategies encompass methods such as transfection, co-incubation, and electroporation, implemented before the biogenesis of exosomes [179]. Post-production approaches include freeze–thaw cycles, incubation, sonication, extrusion, and hypotonic dialysis, applied after exosome formation [31,179,180]. For instance, Kim et al. utilized incubation and sonication to incorporate paclitaxel into exosomes derived from RAW 264.7 cells, aiming to counteract multidrug resistance in cancer therapy [181]. Similarly, Ohno et al. employed transfection to load anti-cancer let-7a miRNA into exosomes from HEK293 cells for breast cancer treatment [182].

The surface modification of exosomes represents another critical area of interest, achieved through the genetic manipulation of the exosomal membrane or the parental cells, the chemical conjugation of targeted ligands, electrostatic interactions, and the incorporation of magnetic nanoparticles [183]. The primary objective of these modifications is to achieve the targeted delivery of exosomes to specific cell types for precise therapeutic intervention [93]. Alvarez-Erviti et al. demonstrated targeted delivery to the central nervous system (CNS) by genetically engineering dendritic cells (DCs) to express Lamp2b fused with rabies viral glycoprotein (RVG) peptides [184]. In a similar vein, Zhu et al. chemically integrated c(RGDyK) tumor-targeting peptides onto exosomal surfaces to home in on glioblastoma cells [185].

Further innovations include the integration of  $\alpha$ -EGFR,  $\alpha$ -mCherry, and  $\alpha$ -HER2 nanobodies onto the exosomal surface via phospholipid conjugation, effectively altering their targeting specificity in vitro [186]. This approach parallels strategies leveraging native exosomal membrane proteins, such as Lamp2b and platelet-derived growth factor receptors, as fusion partners for targeting ligands [186]. Additionally, chemical engineering methods such as click chemistry have been explored for surface modification, though the impact of these alterations on exosome–cell interaction dynamics and delivery efficacy remain under investigation [187]. Current research efforts are focused on elucidating the relationship between modified exosomal surface properties and their functional outcomes, with a view towards optimizing exosome-based therapeutic delivery systems [188,189].

Exosomes have pronounced membrane curvature as a consequence of their diminutive radius. This curvature arises through two principal mechanisms: first, ESCRT-mediated endosomal membrane deformation, leading to intraluminal vesicle budding within multivesicular endosomes [190]; second, neutral sphingomyelinase-induced ceramide production [191]. Based on these characteristics, an innovative approach to target lipid membranes effectively involves the design of peptides mimicking membrane-interacting proteins [82]. This approach could pioneer a new category of peptide sensors capable of simultaneous detection of phosphatidylserine (PS) and membrane curvature. Research findings indicate that the binding efficiency of the myristoylated alanine-rich C kinase substrate (MARCKS) effector domain (ED) is markedly reduced in the mutants MARCKSmut1 and MARCKSmut2, suggesting that MARCKS-ED can recognize PS-enriched, curved membranes [192]. This recognition is facilitated by phenylalanine (Phe) residues adapting to asymmetrically stretched bilayers and filling structural gaps in highly curved vesicles, thereby stabilizing membrane irregularities [193]. It was found that MARCKS-ED can differentiate between the sizes of lipid vesicles derived from both an animal model (rats)

and synthetic phospholipid models, binding preferentially to highly curved membrane surfaces [193]. The effectiveness of curvature sensing is primarily dependent on the integration of aromatic Phe residues from the ED region into the lipid bilayers, as well as on the electrostatic interactions between the cationic residues (e.g., lysine, arginine) in the ED region and the negatively charged PS [194]. These lipid-targeting strategies offer new possibilities for probing exosomes *in vivo* or *ex vivo*, enhancing our ability to study critical biological phenomena such as apoptosis and vesicular shedding. Furthermore, MARCKS-ED-conjugated therapeutics could potentially achieve targeted delivery by binding to specific exosomes, followed by endocytosis, although the mechanisms of exosome cellular uptake remain under exploration [195].

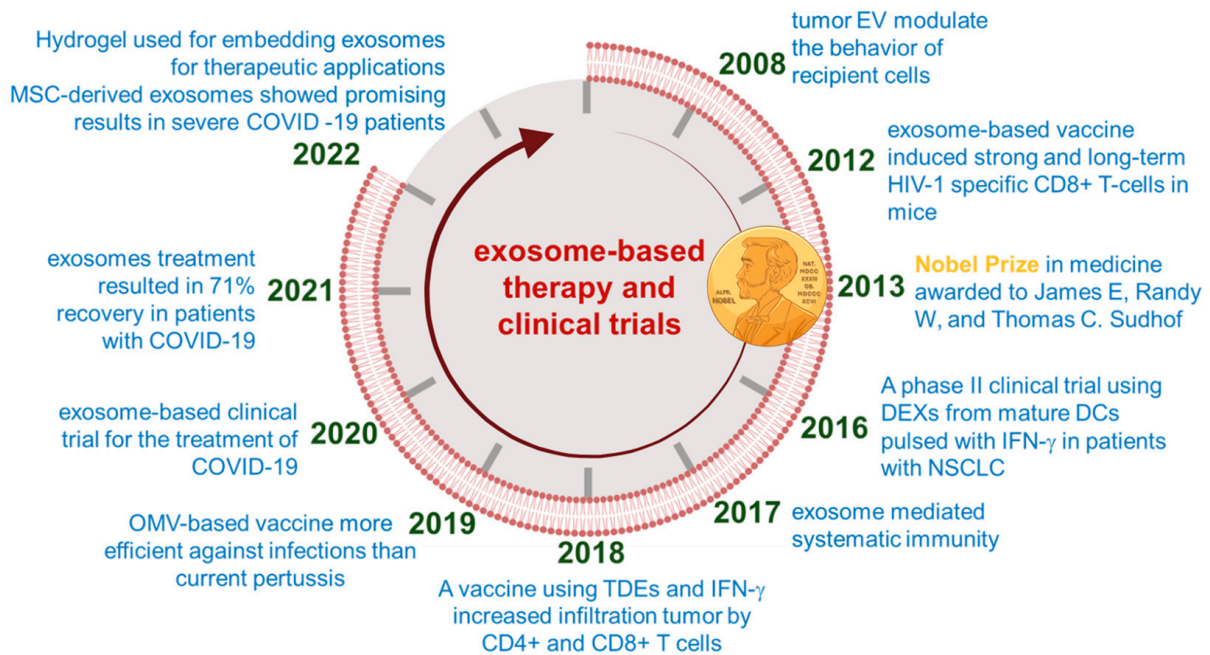
In the context of infectious diseases, exosome-sized vesicles interconnecting red blood cells infected with malaria could enhance parasite survival under stress conditions [196]. MARCKS-ED targeting could disrupt intercellular communication via exosome-like vesicles between red blood cells infected with malaria, potentially delaying the development of drug resistance [197]. Advancing our understanding of exosomal functions in pathological conditions necessitates systematic peptide truncation to identify the minimal active sequence and essential residues for curvature detection by MARCKS-ED [194]. Enhancements in peptide affinity or specificity for specific exosomal populations could be attained through further residue modification and chemical optimization, including deletion, alanine scanning, and cyclization [198]. Investigating the membrane-binding capabilities of peptides derived from MARCKS-related proteins, which share nearly identical effector domains but differ by minor residue substitutions, could provide insights into how such modifications impact curvature sensing [194].

Further research involving systematic truncation is essential to determine the smallest active sequence and the minimal residues necessary for curvature sensing by MARCKS-ED [195]. Enhancements in binding affinity or specificity of MARCKS-derived peptides for cell-specific exosomes could be achieved through residue analysis and chemical optimization, including deletion, alanine scanning, and cyclization [199]. Additionally, evaluating the membrane-binding capabilities of peptide derivatives from a MARCKS homolog, which features a serine-to-proline substitution in the ED, could clarify whether this residue's structural rigidity impacts curvature sensing [200]. An advanced method for visualizing the lipid composition and membrane curvature of exosomes would involve the application of bioorthogonal "click" chemistry to label the curvature probes with small molecule fluorophores, avoiding the use of large fluorescent proteins [201].

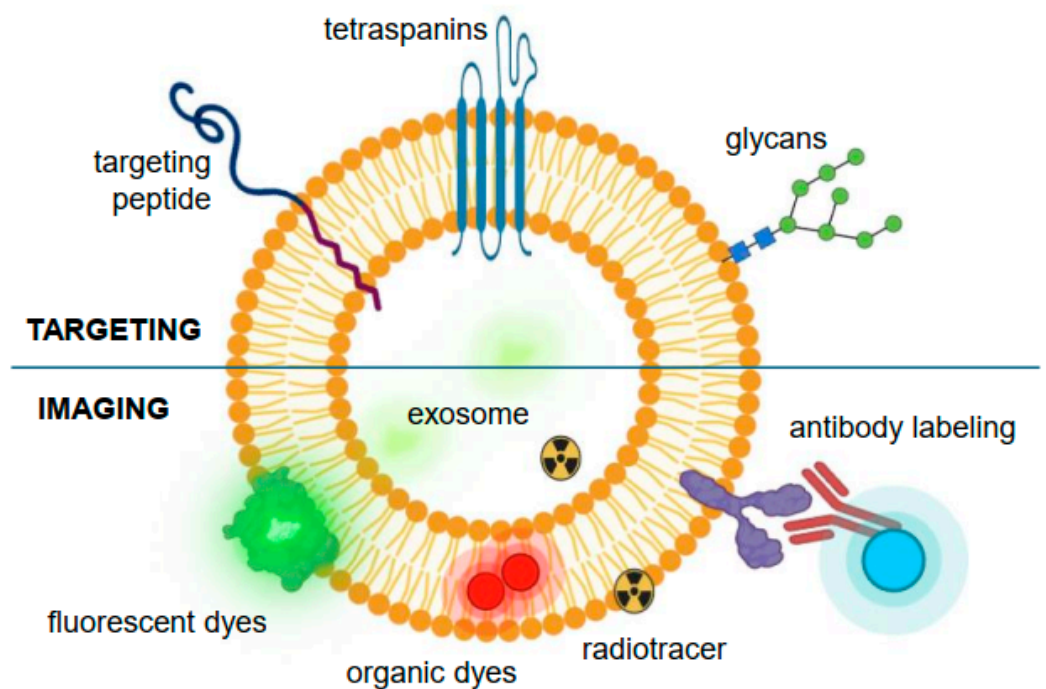
Elucidating the pharmacokinetic behavior of exosomes within biological systems and selecting an optimal delivery mechanism are paramount for determining the *in vivo* fate of these nanovesicles [202]. For the clinical advancement of exosome-based therapies (Figure 3) [26], it is imperative to consider several critical factors in the modification of exosome surfaces [203]. These include the selection of an appropriate targeting ligand or labeling agent, which requires a comprehensive understanding of the target site and the intended therapeutic application, all the while ensuring the absence of undesired immunogenic responses (Figure 4) [204]. Additionally, maintaining the physicochemical stability of exosomes necessitates the prevention of inadvertent alterations to their surface charge characteristics [176,204].

While covalent modification methods are frequently employed to affix labeling or targeting agents across the exosome surface, they present several challenges [205]. These techniques are limited to the attachment of genetically encodable proteins and peptides, excluding the possibility of targeting a broader range of molecules [206]. Moreover, the implementation of genetic modifications must not disrupt the intrinsic functions of proteins constituting the exosome membrane, given its complex nature [207].





**Figure 3.** Timeline outlining the advancement in exosome-based therapy.



**Figure 4.** A schematic representation of the different strategies used for modifying the exosomal surface for targeted therapy and imaging applications. These strategies include labelling with antibodies, fluorescent dyes, radiolabeling, and other engineering techniques to enhance delivery specificity.

The application of click chemistry for targeting specific proteins within the exosome membrane for modification is met with difficulties due to the membrane's complexity [89]. However, advancements in the metabolic engineering of exosome parental cells have offered partial solutions to these challenges. This innovative approach involves the incorporation of artificially modified lipids, amino acids, and glycans to facilitate the expression of modified proteins on the exosome surface [208,209]. After this expression, additional chemical treatments are required to attach the desired labeling or targeting moieties, thus

enabling the precision modification of exosomes for therapeutic purposes [209,210]. The utilization of click chemistry for exosome surface modification inherently poses a risk of chemical residue retention, which may elicit safety concerns regarding the modified exosomes and potentially complicate the regulatory approval process [211]. Additionally, the stability of aptamers under experimental conditions presents another significant challenge. Given these limitations, there is a growing preference for more efficient methods of exosome surface modification that circumvent these drawbacks [26,212].

An innovative approach emerging in this context involves the encapsulation of exosomes within phenol-metal-based nanofilms. This technique employs a coordination complex formed between tannic acid (TA) and  $\text{Fe}^{3+}$ , onto which a targeting ligand can be functionalized via Michael addition to free amine groups [213]. For instance, this method has been applied to fabricate glutathione-capped gold nanoparticles by anchoring them to FA-functionalized, DOX-loaded exosomes (Exos-DOX-TA- $\text{Fe}^{3+}$ -FA), demonstrating the versatility and efficacy of this approach [213,214].

The TA- $\text{Fe}^{3+}$  nanofilm offers commendable resistance against oxidants, heat, and ultraviolet (UV) light, presenting a robust platform for exosome modification [215]. However, it is crucial to assess the impact of such modifications on the structural integrity of the exosomes and the natural functions of their lipid, carbohydrate, and surface protein components [216]. Addressing these concerns meticulously is vital for harnessing the full potential of exosomes as an effective drug delivery system. Looking forward, the development of single-exosome analysis and the broader application of these novel technologies in a clinical setting will necessitate further clinical validation and the establishment of reproducibility [8]. This progression is essential for confirming the safety, efficacy, and reliability of exosome-based therapeutic delivery vehicles, paving the way for their successful integration into clinical practice [217].

## 6. Bio-Engineered Exosome Therapy in Precision Medicine

Exosome-based methodologies in precision medicine embody an integrative approach, combining molecular biology, nanotechnology, and individualized healthcare. They are poised to significantly enhance the prognosis, treatment, and monitoring of diseases in the future [218]. The foundational aspect of precision and personalized medicine involves the collection of patient-specific information, including lifestyle factors and genetic predispositions [219]. This information is crucial for devising tailored therapeutic strategies. Exosomes play a pivotal role in both the diagnosis of diseases and, potentially, preemptive therapeutic interventions, should early biomarkers be identified [220]. The advent of advanced “next generation” diagnostic techniques has facilitated the identification of myriad mutations and has enriched our understanding of the pathogenesis of various conditions, including cancers, neurological disorders, and infectious diseases [219,221]. These developments enable the formulation of individualized treatment regimens based on patient-specific biomarkers identified through exosomal analysis. A quantum leap in medical innovation is the concept of the “liquid biopsy,” a non-tissue-based biopsy method long-awaited for its minimal invasiveness [222,223]. This approach allows for the non-invasive screening, evaluation, monitoring, and diagnosis of diseases, garnering considerable interest due to its low-risk profile [219]. Recent advancements in liquid biopsy technologies have further streamlined the profiling of tumoral databases, encompassing methylation patterns and DNA/chromatin modifications [223]. Exosomes are integral to liquid biopsies, serving as carriers of biomarkers reflective of cellular conditions and being present across all body fluids [224]. The principal objective of precision medicine is the targeted treatment, or “ablation,” of pathological states with minimal collateral damage to healthy tissues, contingent upon the availability of comprehensive, personalized data sets [225]. Exosomes are particularly valuable in liquid biopsies for conditions such as pancreatic cancer, which are challenging to detect in their early, asymptomatic stages, and in circumstances where traditional tissue biopsies pose risks, such as during pregnancy [226].

Exosomes play a dual role in homeostatic processes; they can promote diseases, such as neuroinflammation and diabetes, and protect against them, facilitating immune homeostasis, tissue regeneration, and repair [227]. This dual functionality is pivotal as the utilization of exosomes as biomarkers and therapeutic agents in personalized medicine progresses [228]. Through interdisciplinary strategies, it is possible to load exosomes with specific cargoes, including DNA, RNA, oligonucleotides, proteins, and pharmaceuticals, drawing from extensive databases to maximize therapeutic efficacy. These modularized exosomes can be engineered with customizable surface molecules, enhancing their targeting capabilities, and minimizing damage to healthy tissues [224]. However, a significant challenge in the development of exosome-based personalized and precision medicine is the inherent heterogeneity of exosomes [8,229]. To address this complexity, a unified exosome profiling strategy that combines intradisciplinary expertise, advanced nanotechnology, and a multi-omics approach is essential. Additionally, comprehensive toxicological analysis is required to ensure the safety and effectiveness of exosome-based therapies. In this context, modularized exosomes, with their tailored compositions and targeting abilities, hold great promise for advancing the fields of precision and personalized medicine.

The bioengineering of exosomes is an excellent approach to develop proper production standards and quality, but it may impair the outcomes by inaccurate targeting due to over-modification and leads to unintended side effects [230]. In addition, these manipulations could trigger adverse immune reactions, causing inflammation. Complex modifications could complicate the regulatory approval process, delaying clinical application [231]. Scaling up the production of bioengineered EVs, including exosomes, for large-scale applications presents several challenges and requires optimizations [29]. The isolation method includes ultracentrifugation, the density-gradient method, immunoprecipitation, which are labor-intensive and not easily scalable. By developing high-throughput methods for rapid and accurate characterizations and ensuring batch-to-batch consistency in surface modifications, it is possible to scale up the production of bio-engineered exosomes for clinical and commercial applications. Furthermore, there is a concern related to safety, which arises from the co-isolation of proteins, lipids, and other cellular debris, which can contaminate exosome preparations [230]. This can be overcome by implementing multi-step purification processes, such as combining size-exclusion chromatography with affinity capture, which can enhance purity. Additionally, there are risks associated with the high shear forces during isolation and processing, which can damage exosomes. Therefore, using gentle processing techniques such as tangential flow filtration can minimize the damage and maintain exosome integrity by reducing shear stress and efficiently separating exosomes from other vesicles [230].

## 7. Future Perspective and Conclusions

As the field of exosome research continues to evolve, the horizon for their application in therapeutic contexts appears increasingly promising. The future of exosome-based therapies is poised at the confluence of technological innovation, deeper biological understanding, and regulatory refinement. Key areas of focus will likely include the enhancement of exosome isolation and purification techniques to achieve higher yield and purity, which is critical for clinical applications. Furthermore, the development of scalable manufacturing processes that can maintain the functional integrity of exosomes will be essential to meet the demands of clinical trials and subsequent therapeutic use. Advancements in the genetic and biochemical surface modification of exosomes will enable more precise targeting and cargo delivery, opening new avenues for the treatment of a wide range of diseases, from cancer to neurodegenerative disorders. The integration of cutting-edge technologies, such as CRISPR-Cas9 for genome editing within exosomes, could further enhance their therapeutic potential. Moreover, the exploration of synthetic and biomimetic exosomes presents an intriguing frontier that may overcome some of the limitations associated with natural exosomal systems. Regulatory considerations will remain at the forefront, with a need for standardized protocols and benchmarks that can streamline the approval process

for exosome-based therapies. Collaborative efforts between researchers, clinicians, and regulatory agencies will be crucial to establishing a framework that ensures safety and efficacy without stifling innovation. It is necessary to conduct a thorough and time-consuming safety examination. When evaluating the regulatory needs for exosome therapeutic uses, the purity of exosomes is crucial. For approval, the FDA and other international regulatory bodies look for safety, effectiveness, potency, and purity. For therapeutic application, a pure product, devoid of impurities such as proteins, peptides, cell-free DNA, and other cell detritus, is necessary [55]. Large animals or primates are now being used by more researchers to examine the safety and effectiveness of exosome products. Human safety regarding exosomes is becoming more widely acknowledged. Although the FDA has not yet authorized any exosome products for therapeutic use [22,23], they have provided specific guidelines to sponsors on how to supply the necessary chemistry, manufacturing, and control (CMC) data to ensure the safety of their products [232]. Furthermore, biologics intended for the mitigation, treatment, cure, or prevention of illness must meet strict regulations, set out by the FDA and other international regulatory bodies. Exosomes must be isolated and purified with strict quality, purity, potency, and repeatability standards. More controls are needed for the exosome alterations that follow. Exosome standards are probably going to include both the contents and the cells from where they originated. There needs to be more standardization and review of exosome release criteria. The exosome-based product age is almost upon us, thanks to advancements in separation technology and our growing understanding of exosomes [233]. Furthermore, the integration of computational biology, machine learning, and artificial intelligence in exosome research holds the potential to unravel complex biological interactions, predict therapeutic outcomes, and optimize treatment regimens. This multidisciplinary approach will not only enhance our understanding of exosomal functions but also streamline the development of exosome-based diagnostics and therapeutics.

In summary, the exploration of exosomes as vehicles for therapeutic delivery embodies a significant leap toward the realization of precision medicine and pharmaceutical innovation. Exosome-based therapies from bench to bedside are fraught with challenges, but they are undeniably paved with significant potential [234]. The journey from conceptual understanding to clinical application of exosome-based therapies encapsulates the collaborative effort of researchers, clinicians, and regulatory authorities, underscored by the commitment to scientific rigor and patient safety. The future of exosome-based therapies is not without its uncertainties, yet the foundation laid by current research efforts provides a robust basis for optimism. Continued exploration and innovation within this domain hold the promise of revolutionizing the way we approach disease treatment, offering hope for patient-centric, precision medical solutions that could fundamentally alter the therapeutic landscape. As we stand on the cusp of this promising frontier, the path forward is complex and demands a multidisciplinary effort. The evolution of exosome research stands as a testament to the relentless pursuit of knowledge and the quest for therapeutic innovation. The collective efforts of the scientific community, clinicians, and regulatory bodies are crucial for navigating the complex regulatory landscape, ultimately facilitating the delivery of these innovative therapies to patients, and heralding new paradigms in medical treatment and patient care. As we peer into the future, exosomes may well be at the heart of the next wave of breakthroughs in pharmaceutical sciences and medicine, offering a beacon of hope for patients around the globe.

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## Abbreviations

|                |   |
|----------------|---|
| AFM            | atomic force microscopy                                   |
| BMP-2          | bone morphogenetic protein 2                              |
| CMC            | chemistry, manufacturing, and control                     |
| CNS            | central nervous system                                    |
| DNA            | deoxyribonucleic acid                                     |
| dSTORM         | direct stochastic optical reconstruction microscopy       |
| DCs            | dendritic cells   |
| DLS            | dynamic light scattering                                  |
| ED             | effector domain   |
| EM             | electron microscopy                                       |
| ELISA          | enzyme-linked immunosorbent assay                         |
| ECM            | extracellular matrix                                      |
| EV             | extracellular vesicle                                     |
| FCM            | flow cytometry  |
| FDA            | food and drug administration                              |
| GMP            | good manufacturing practice                               |
| HDL            | high density lipoproteins                                 |
| HIF-1 $\alpha$ | hypoxia-inducible factor 1-alpha                          |
| IFN- $\gamma$  | interferon-gamma  |
| ISEV           | international society for extracellular vesicles          |
| IND            | investigational new drug                                  |
| IDL            | intermediate-density lipoproteins                         |
| LDL            | low density lipoproteins                                  |
| LPS            | lipopolysaccharide  |
| miRNA          | microRNA  |
| MISEV          | minimal information for studies of extracellular vesicles |
| MARCKS         | myristoylated alanine-rich C kinase substrate             |
| NTA            | nanoparticle tracking analysis                            |
| nFCM           | nano-flow cytometry                                       |
| PCR            | polymerase chain reaction                                 |
| PBS-HAT        | phosphate buffered saline-human albumin and trehalose     |
| PS             | phosphatidylserine  |
| Phe            | phenylalanine   |
| qPCR           | quantitative polymerase chain reaction                    |
| RNA            | ribonucleic acid  |
| RVG            | rabies viral glycoprotein                                 |
| SEM            | scanning electron microscopy                              |
| TNF- $\alpha$  | tumor necrosis factor-alpha                               |
| TEM            | transmission electron microscopy                          |
| TRPS           | tunable resistive pulse sensing                           |
| TIRF           | total internal reflection fluorescence                    |
| TA             | tannic acid   |
| UV             | ultraviolet light   |
| VLDL           | very low-density lipoproteins                             |

## References

- Li, X.; Corbett, A.L.; Taatizadeh, E.; Tasnim, N.; Little, J.P.; Garnis, C.; Daugaard, M.; Guns, E.; Hoorfar, M.; Li, I.T.S. Challenges and opportunities in exosome research-Perspectives from biology, engineering, and cancer therapy. *APL Bioeng.* **2019**, *3*, 011503. [[CrossRef](#)] [[PubMed](#)]
- Zou, Z.; Li, H.; Xu, G.; Hu, Y.; Zhang, W.; Tian, K. Current Knowledge and Future Perspectives of Exosomes as Nanocarriers in Diagnosis and Treatment of Diseases. *Int. J. Nanomed.* **2023**, *18*, 4751–4778. [[CrossRef](#)] [[PubMed](#)]
- Rajput, A.; Varshney, A.; Bajaj, R.; Pokharkar, V. Exosomes as New Generation Vehicles for Drug Delivery: Biomedical Applications and Future Perspectives. *Molecules* **2022**, *27*, 7289. [[CrossRef](#)] [[PubMed](#)]
- Butreddy, A.; Kommineni, N.; Dudhipala, N. Exosomes as Naturally Occurring Vehicles for Delivery of Biopharmaceuticals: Insights from Drug Delivery to Clinical Perspectives. *Nanomaterials* **2021**, *11*, 1481. [[CrossRef](#)]
- Herrmann, I.K.; Wood, M.J.A.; Fuhrmann, G. Extracellular vesicles as a next-generation drug delivery platform. *Nat. Nanotechnol.* **2021**, *16*, 748–759. [[CrossRef](#)] [[PubMed](#)]

6. Yang, L.; Yang, Y.; Chen, Y.; Xu, Y.; Peng, J. Cell-based drug delivery systems and their in vivo fate. *Adv. Drug Deliv. Rev.* **2022**, *187*, 114394. [CrossRef] [PubMed]
7. Rezaie, J.; Feghhi, M.; Etemadi, T. A review on exosomes application in clinical trials: Perspective, questions, and challenges. *Cell Commun. Signal.* **2022**, *20*, 145. [CrossRef] [PubMed]
8. Koh, H.; Kim, H.; Kang, S.; Yoo, T.-H. Exosome-Based Drug Delivery: Translation from Bench to Clinic. *Pharmaceutics* **2023**, *15*, 2042. [CrossRef] [PubMed]
9. Sousa, P.; Lopes, B.; Sousa, A.C.; Moreira, A.; Coelho, A.; Alvites, R.; Alves, N.; Geuna, S.; Maurício, A.C. Advancements and Insights in Exosome-Based Therapies for Wound Healing: A Comprehensive Systematic Review (2018-June 2023). *Biomedicines* **2023**, *11*, 2099. [CrossRef]
10. 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. [CrossRef]
11. Kwon, Y.; Park, J. Methods to analyze extracellular vesicles at single particle level. *Micro Nano Syst. Lett.* **2022**, *10*, 14. [CrossRef]
12. Song, Y.; Kim, Y.; Ha, S.; Sheller-Miller, S.; Yoo, J.; Choi, C.; Park, C.H. The emerging role of exosomes as novel therapeutics: Biology, technologies, clinical applications, and the next. *Am. J. Reprod. Immunol.* **2021**, *85*, e13329. [CrossRef] [PubMed]
13. Wang, X.; Xia, J.; Yang, L.; Dai, J.; He, L. Recent progress in exosome research: Isolation, characterization and clinical applications. *Cancer Gene Ther.* **2023**, *30*, 1051–1065. [CrossRef] [PubMed]
14. Han, C.; Sun, X.; Liu, L.; Jiang, H.; Shen, Y.; Xu, X.; Li, J.; Zhang, G.; Huang, J.; Lin, Z.; et al. Exosomes and Their Therapeutic Potentials of Stem Cells. *Stem Cells Int.* **2016**, *2016*, 7653489. [CrossRef] [PubMed]
15. Hong, P.; Yang, H.; Wu, Y.; Li, K.; Tang, Z. The functions and clinical application potential of exosomes derived from adipose mesenchymal stem cells: A comprehensive review. *Stem Cell Res. Ther.* **2019**, *10*, 242. [CrossRef] [PubMed]
16. 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*, 17. [CrossRef]
17. Asadpour, A.; Yahaya, B.H.; Bicknell, K.; Cottrell, G.S.; Widera, D. Uncovering the gray zone: Mapping the global landscape of direct-to-consumer businesses offering interventions based on secretomes, extracellular vesicles, and exosomes. *Stem Cell Res. Ther.* **2023**, *14*, 111. [CrossRef] [PubMed]
18. Khan, A.Q.; Akhtar, S.; Prabhu, K.S.; Zarif, L.; Khan, R.; Alam, M.; Buddenkotte, J.; Ahmad, A.; Steinhoff, M.; Uddin, S. Exosomes: Emerging Diagnostic and Therapeutic Targets in Cutaneous Diseases. *Int. J. Mol. Sci.* **2020**, *21*, 9264. [CrossRef] [PubMed]
19. Colao, I.L.; Corteling, R.; Bracewell, D.; Wall, I. Manufacturing Exosomes: A Promising Therapeutic Platform. *Trends Mol. Med.* **2018**, *24*, 242–256. [CrossRef]
20. Li, M.; Fang, F.; Sun, M.; Zhang, Y.; Hu, M.; Zhang, J. Extracellular vesicles as bioactive nanotherapeutics: An emerging paradigm for regenerative medicine. *Theranostics* **2022**, *12*, 4879–4903. [CrossRef]
21. Wang, Z.; Wang, X.; Xu, W.; Li, Y.; Lai, R.; Qiu, X.; Chen, X.; Chen, Z.; Mi, B.; Wu, M.; et al. Translational Challenges and Prospective Solutions in the Implementation of Biomimetic Delivery Systems. *Pharmaceutics* **2023**, *15*, 2623. [CrossRef] [PubMed]
22. FDA. Public Safety Notification on Exosome Products. 2019. Available online: <https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/public-safety-notification-exosome-products> (accessed on 20 April 2024).
23. FDA. Consumer Alert on Regenerative Medicine Products Including Stem Cells and Exosomes. 2020. Available online: <https://www.fda.gov/vaccines-blood-biologics/consumers-biologics/consumer-alert-regenerative-medicine-products-including-stem-cells-and-exosomes> (accessed on 20 April 2024).
24. Ahn, S.H.; Ryu, S.W.; Choi, H.; You, S.; Park, J.; Choi, C. Manufacturing Therapeutic Exosomes: From Bench to Industry. *Mol. Cells* **2022**, *45*, 284–290. [CrossRef] [PubMed]
25. Thomas, S.C.; Kim, J.W.; Pauletti, G.M.; Hassett, D.J.; Kotagiri, N. Exosomes: Biological Pharmaceutical Nanovectors for Theranostics. *Front. Bioeng. Biotechnol.* **2021**, *9*, 808614. [CrossRef] [PubMed]
26. Hussien, B.M.; Faraj, G.S.H.; Rasul, M.F.; Hidayat, H.J.; Salihi, A.; Baniahmad, A.; Taheri, M.; Ghafouri-Frad, S. Strategies to overcome the main challenges of the use of exosomes as drug carrier for cancer therapy. *Cancer Cell Int.* **2022**, *22*, 323. [CrossRef] [PubMed]
27. Vyas, K.S.; Kaufman, J.; Munavalli, G.S.; Robertson, K.; Behfar, A.; Wyles, S.P. Exosomes: The latest in regenerative aesthetics. *Regen. Med.* **2023**, *18*, 181–194. [CrossRef] [PubMed]
28. Chen, Y.-S.; Lin, E.-Y.; Chiou, T.-W.; Harn, H.-J. Exosomes in clinical trial and their production in compliance with good manufacturing practice. *Tzu Chi Med. J.* **2020**, *32*, 113–120. [CrossRef]
29. Syromiatnikova, V.; Prokopenko, A.; Gomzikova, M. Methods of the Large-Scale Production of Extracellular Vesicles. *Int. J. Mol. Sci.* **2022**, *23*, 10522. [CrossRef] [PubMed]
30. Qu, Q.; Fu, B.; Long, Y.; Liu, Z.Y.; Tian, X.H. Current Strategies for Promoting the Large-scale Production of Exosomes. *Curr. Neuropharmacol.* **2023**, *21*, 1964–1979. [CrossRef]
31. Huang, G.; Lin, G.; Zhu, Y.; Duan, W.; Jin, D. Emerging technologies for profiling extracellular vesicle heterogeneity. *Lab Chip* **2020**, *20*, 2423–2437. [CrossRef]
32. Yang, B.; Lin, Y.; Huang, Y.; Zhu, N.; Shen, Y.-Q. Extracellular vesicles modulate key signalling pathways in refractory wound healing. *Burns Trauma* **2023**, *11*, tkad039. [CrossRef]
33. Willms, E.; Cabañas, C.; Mäger, I.; Wood, M.J.A.; Vader, P. Extracellular Vesicle Heterogeneity: Subpopulations, Isolation Techniques, and Diverse Functions in Cancer Progression. *Front. Immunol.* **2018**, *9*, 738. [CrossRef] [PubMed]

34. Meggiolaro, A.; Moccia, V.; Brun, P.; Pierno, M.; Mistura, G.; Zappulli, V.; Ferraro, D. Microfluidic Strategies for Extracellular Vesicle Isolation: Towards Clinical Applications. *Biosensors* **2022**, *13*, 50. [[CrossRef](#)] [[PubMed](#)]
35. Konoshenko, M.Y.; Lekchnov, E.A.; Vlassov, A.V.; Laktionov, P.P. Isolation of Extracellular Vesicles: General Methodologies and Latest Trends. *Biomed. Res. Int.* **2018**, *2018*, 8545347. [[CrossRef](#)] [[PubMed](#)]
36. Coughlan, C.; Bruce, K.D.; Burgy, O.; Boyd, T.D.; Michel, C.R.; Garcia-Perez, J.E.; Adame, V.; Anton, P.; Bettcher, B.M.; Chial, H.J.; et al. Exosome Isolation by Ultracentrifugation and Precipitation and Techniques for Downstream Analyses. *Curr. Protoc. Cell Biol.* **2020**, *88*, e110. [[CrossRef](#)] [[PubMed](#)]
37. Chen, J.; Li, P.; Zhang, T.; Xu, Z.; Huang, X.; Wang, R.; Du, L. Review on Strategies and Technologies for Exosome Isolation and Purification. *Front. Bioeng. Biotechnol.* **2022**, *9*, 811971. [[CrossRef](#)]
38. Sidhom, K.; Obi, P.O.; Saleem, A. A Review of Exosomal Isolation Methods: Is Size Exclusion Chromatography the Best Option? *Int. J. Mol. Sci.* **2020**, *21*, 6466. [[CrossRef](#)] [[PubMed](#)]
39. Yang, D.; Zhang, W.; Zhang, H.; Zhang, F.; Chen, L.; Ma, L.; Larcher, L.M.; Chen, S.; Liu, N.; Zhao, Q.; et al. Progress, opportunity, and perspective on exosome isolation—Efforts for efficient exosome-based theranostics. *Theranostics* **2020**, *10*, 3684–3707. [[CrossRef](#)] [[PubMed](#)]
40. Liangsupree, T.; Multia, E.; Riekkola, M.-L. Modern isolation and separation techniques for extracellular vesicles. *J. Chromatogr. A* **2021**, *1636*, 461773. [[CrossRef](#)]
41. Gholizadeh, S.; Shehata Draz, M.; Zarghooni, M.; Sanati-Nezhad, A.; Ghavami, S.; Shafiee, H.; Akbari, M. Microfluidic approaches for isolation, detection, and characterization of extracellular vesicles: Current status and future directions. *Biosens. Bioelectron.* **2017**, *91*, 588–605. [[CrossRef](#)]
42. Zhou, X.; Brown, B.A.; Siegel, A.P.; El Masry, M.S.; Zeng, X.; Song, W.; Das, A.; Khandelwal, P.; Clark, A.; Singh, K.; et al. Exosome-Mediated Crosstalk between Keratinocytes and Macrophages in Cutaneous Wound Healing. *ACS Nano* **2020**, *14*, 12732–12748. [[CrossRef](#)]
43. Guda, P.R.; Sharma, A.; Anthony, A.J.; El Masry, M.S.; Couse, A.D.; Ghatak, P.D.; Das, A.; Timsina, L.; Trinidad, J.C.; Roy, S.; et al. Nanoscopic and functional characterization of keratinocyte-originating exosomes in the wound fluid of non-diabetic and diabetic chronic wound patients. *Nano Today* **2023**, *52*, 101954. [[CrossRef](#)] [[PubMed](#)]
44. Clayton, A.; Boilard, E.; Buzas, E.I.; Cheng, L.; Falcón-Perez, J.M.; Gardiner, C.; Gustafson, D.; Gualerzi, A.; Hendrix, A.; Hoffman, A.; et al. Considerations towards a roadmap for collection, handling and storage of blood extracellular vesicles. *J. Extracell. Vesicles* **2019**, *8*, 1647027. [[CrossRef](#)] [[PubMed](#)]
45. Venturella, M.; Carpi, F.M.; Zocco, D. Standardization of Blood Collection and Processing for the Diagnostic Use of Extracellular Vesicles. *Curr. Pathobiol. Rep.* **2019**, *7*, 1–8. [[CrossRef](#)]
46. Coumans, F.A.W.; Brisson, A.R.; Buzas, E.I.; Dignat-George, F.; Drees, E.E.E.; El-Andalousi, S.; Emanuelli, C.; Gasecka, A.; Hendrix, A.; Hill, A.F.; et al. Methodological Guidelines to Study Extracellular Vesicles. *Circ. Res.* **2017**, *120*, 1632–1648. [[CrossRef](#)] [[PubMed](#)]
47. Lacroix, R.; Judicone, C.; Poncelet, P.; Robert, S.; Arnaud, L.; Sampol, J.; Dignat-George, F. Impact of pre-analytical parameters on the measurement of circulating microparticles: Towards standardization of protocol. *J. Thromb. Haemost.* **2012**, *10*, 437–446. [[CrossRef](#)] [[PubMed](#)]
48. Buntsma, N.C.; Gasecka, A.; Roos, Y.; van Leeuwen, T.G.; van der Pol, E.; Nieuwland, R. EDTA stabilizes the concentration of platelet-derived extracellular vesicles during blood collection and handling. *Platelets* **2022**, *33*, 764–771. [[CrossRef](#)] [[PubMed](#)]
49. Lacroix, R.; Judicone, C.; Mooberry, M.; Boucekine, M.; Key, N.S.; Dignat-George, F. Standardization of pre-analytical variables in plasma microparticle determination: Results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop. *J. Thromb. Haemost.* **2013**, *11*, 1190–1193. [[CrossRef](#)] [[PubMed](#)]
50. Holcar, M.; Kanduđer, M.; Lenassi, M. Blood Nanoparticles—Influence on Extracellular Vesicle Isolation and Characterization. *Front. Pharmacol.* **2021**, *12*, 773844. [[CrossRef](#)] [[PubMed](#)]
51. Vila-Liante, V.; Sánchez-López, V.; Martínez-Sales, V.; Ramón-Nuñez, L.A.; Arellano-Orden, E.; Cano-Ruiz, A.; Rodríguez-Martorell, F.J.; Gao, L.; Otero-Candelera, R. Impact of sample processing on the measurement of circulating microparticles: Storage and centrifugation parameters. *Clin. Chem. Lab. Med.* **2016**, *54*, 1759–1767.
52. Aguilera-Rojas, M.; Sharbati, S.; Stein, T.; Candela Andrade, M.; Kohn, B.; Einspanier, R. Systematic analysis of different degrees of haemolysis on miRNA levels in serum and serum-derived extracellular vesicles from dogs. *BMC Vet. Res.* **2022**, *18*, 355. [[CrossRef](#)]
53. Gurunathan, S.; Kang, M.H.; Jeyaraj, M.; Qasim, M.; Kim, J.H. Review of the Isolation, Characterization, Biological Function, and Multifarious Therapeutic Approaches of Exosomes. *Cells* **2019**, *8*, 307. [[CrossRef](#)] [[PubMed](#)]
54. Mulcahy, L.A.; Pink, R.C.; Carter, D.R. Routes and mechanisms of extracellular vesicle uptake. *J. Extracell. Vesicles* **2014**, *3*, 24641. [[CrossRef](#)] [[PubMed](#)]
55. Klyachko, N.L.; Arzt, C.J.; Li, S.M.; Gololobova, O.A.; Batrakova, E.V. Extracellular Vesicle-Based Therapeutics: Preclinical and Clinical Investigations. *Pharmaceutics* **2020**, *12*, 1171. [[CrossRef](#)] [[PubMed](#)]
56. O'Brien, K.; Ughetto, S.; Mahjoun, S.; Nair, A.V.; Breakefield, X.O. Uptake, functionality, and re-release of extracellular vesicle-encapsulated cargo. *Cell Rep.* **2022**, *39*, 110651. [[CrossRef](#)] [[PubMed](#)]
57. Mathieu, M.; Martin-Jaular, L.; Lavieu, G.; Théry, C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat. Cell Biol.* **2019**, *21*, 9–17. [[CrossRef](#)] [[PubMed](#)]

58. Heidarzadeh, M.; Zarebkohan, A.; Rahbarghazi, R.; Sokullu, E. Protein corona and exosomes: New challenges and prospects. *Cell Commun. Signal.* **2023**, *21*, 64. [[CrossRef](#)]
59. Capolla, S.; Colombo, F.; De Maso, L.; Mauro, P.; Bertocin, P.; Kähne, T.; Engler, A.; Núñez, L.; Spretz, R.; Larsen, G.; et al. Surface antibody changes protein corona both in human and mouse serum but not final opsonization and elimination of targeted polymeric nanoparticles. *J. Nanobiotechnol.* **2023**, *21*, 376. [[CrossRef](#)] [[PubMed](#)]
60. Tóth, E.; Turiák, L.; Visnovitz, T.; Cserép, C.; Mázló, A.; Sódar, B.W.; Försönits, A.I.; Petővári, G.; Sebestyén, A.; Komlósi, Z.; et al. Formation of a protein corona on the surface of extracellular vesicles in blood plasma. *J. Extracell. Vesicles* **2021**, *10*, e12140. [[CrossRef](#)]
61. Wolfram, J.; Yang, Y.; Shen, J.; Moten, A.; Chen, C.; Shen, H.; Ferrari, M.; Zhao, Y. The nano-plasma interface: Implications of the protein corona. *Colloids Surf B Biointerfaces* **2014**, *124*, 17–24. [[CrossRef](#)]
62. Ghebosu, R.E.; Pendiuk Goncalves, J.; Wolfram, J. Extracellular Vesicle and Lipoprotein Interactions. *Nano Lett.* **2024**, *24*, 1–8. [[CrossRef](#)]
63. Nguyen, V.H.; Lee, B.J. Protein corona: A new approach for nanomedicine design. *Int. J. Nanomed.* **2017**, *12*, 3137–3151. [[CrossRef](#)] [[PubMed](#)]
64. Tenzer, S.; Docter, D.; Rosfa, S.; Wlodarski, A.; Kuharev, J.; Rekik, A.; Knauer, S.K.; Bantz, C.; Nawroth, T.; Bier, C.; et al. Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: A comprehensive quantitative proteomic analysis. *ACS Nano* **2011**, *5*, 7155–7167. [[CrossRef](#)] [[PubMed](#)]
65. Shang, L.; Wang, Y.; Jiang, J.; Dong, S. pH-dependent protein conformational changes in albumin:gold nanoparticle bioconjugates: A spectroscopic study. *Langmuir* **2007**, *23*, 2714–2721. [[CrossRef](#)] [[PubMed](#)]
66. Malonga, H.; Neault, J.F.; Tajmir-Riahi, H.A. Transfer RNA binding to human serum albumin: A model for protein-RNA interaction. *DNA and Cell Biol.* **2006**, *25*, 393–398. [[CrossRef](#)] [[PubMed](#)]
67. Bertrand, N.; Grenier, P.; Mahmoudi, M.; Lima, E.M.; Appel, E.A.; Dormont, F.; Lim, J.M.; Karnik, R.; Langer, R.; Farokhzad, O.C. Mechanistic understanding of in vivo protein corona formation on polymeric nanoparticles and impact on pharmacokinetics. *Nat Commun.* **2017**, *8*, 777. [[CrossRef](#)]
68. Carrillo-Conde, B.R.; Ramer-Tait, A.E.; Wannemuehler, M.J.; Narasimhan, B. Chemistry-dependent adsorption of serum proteins onto polyanhydride microparticles differentially influences dendritic cell uptake and activation. *Acta Biomater* **2012**, *8*, 3618–3628. [[CrossRef](#)]
69. Hiltbrunner, S.; Larssen, P.; Eldh, M.; Martinez-Bravo, M.-J.; Wagner, A.K.; Karlsson, M.C.; Gabrielsson, S. Exosomal cancer immunotherapy is independent of MHC molecules on exosomes. *Oncotarget* **2016**, *7*, 387. [[CrossRef](#)] [[PubMed](#)]
70. Buzás, E.I.; Tóth, E.Á.; Sódar, B.W.; Szabó-Taylor, K.É. Molecular interactions at the surface of extracellular vesicles. *Semin Immunopathol* **2018**, *40*, 453–464. [[CrossRef](#)] [[PubMed](#)]
71. Dietz, L.; Oberländer, J.; Mateos-Maroto, A.; Schunke, J.; Fichter, M.; Krämer-Albers, E.M.; Landfester, K.; Mailänder, V. Uptake of extracellular vesicles into immune cells is enhanced by the protein corona. *J. Extracell. Vesicles* **2023**, *12*, e12399. [[CrossRef](#)]
72. Botha, J.; Handberg, A.; Simonsen, J.B. Lipid-based strategies used to identify extracellular vesicles in flow cytometry can be confounded by lipoproteins: Evaluations of annexin V, lactadherin, and detergent lysis. *J. Extracell. Vesicles* **2022**, *11*, e12200. [[CrossRef](#)]
73. Busatto, S.; Walker, S.A.; Grayson, W.; Pham, A.; Tian, M.; Nesto, N.; Barklund, J.; Wolfram, J. Lipoprotein-based drug delivery. *Adv. Drug Deliv. Rev.* **2020**, *159*, 377–390. [[CrossRef](#)] [[PubMed](#)]
74. Wu, M.; Chen, C.; Wang, Z.; Bachman, H.; Ouyang, Y.; Huang, P.H.; Sadovsky, Y.; Huang, T.J. Separating extracellular vesicles and lipoproteins via acoustofluidics. *Lab Chip* **2019**, *19*, 1174–1182. [[CrossRef](#)] [[PubMed](#)]
75. Karimi, N.; Cvjetkovic, A.; Jang, S.C.; Crescitelli, R.; Feizi, M.; Nieuwland, R.; Lotvall, J.; Lässer, C. Detailed analysis of the plasma extracellular vesicle proteome after separation from lipoproteins. *Cell. Mol. Life Sci.* **2018**, *75*, 2873–2886. [[CrossRef](#)]
76. Mo, Z.-C.; Ren, K.; Liu, X.; Tang, Z.-L.; Yi, G.-H. A high-density lipoprotein-mediated drug delivery system. *Adv. Drug Deliv. Rev.* **2016**, *106*, 132–147. [[CrossRef](#)] [[PubMed](#)]
77. Park, H.J.; Kuai, R.; Jeon, E.J.; Seo, Y.; Jung, Y.; Moon, J.J.; Schwendeman, A.; Cho, S.W. High-density lipoprotein-mimicking nanodiscs carrying peptide for enhanced therapeutic angiogenesis in diabetic hindlimb ischemia. *Biomaterials* **2018**, *161*, 69–80. [[CrossRef](#)] [[PubMed](#)]
78. Lozano-Andrés, E.; Enciso-Martinez, A.; Gijbers, A.; Ridolfi, A.; Van Niel, G.; Libregts, S.; Pinheiro, C.; van Herwijnen, M.J.C.; Hendrix, A.; Brucale, M.; et al. Physical association of low density lipoprotein particles and extracellular vesicles unveiled by single particle analysis. *J. Extracell. Vesicles* **2023**, *12*, e12376. [[CrossRef](#)] [[PubMed](#)]
79. Nguyen, P.; Le, A.; Pek, J.; Pham, T.; Jayasinghe, M.; Do, D.; Cao Dai, P.; Le, M. Extracellular vesicles and lipoproteins—Smart messengers of blood cells in the circulation. *J. Extracell. Biol.* **2022**, *1*, e49. [[CrossRef](#)]
80. Ridolfi, A.; Conti, L.; Brucale, M.; Frigerio, R.; Cardellini, J.; Musicò, A.; Romano, M.; Zendrini, A.; Polito, L.; Bergamaschi, G.; et al. Particle profiling of EV-lipoprotein mixtures by AFM nanomechanical imaging. *J. Extracell. Vesicles* **2023**, *12*, e12349. [[CrossRef](#)] [[PubMed](#)]
81. Donoso-Quezada, J.; Ayala-Mar, S.; González-Valdez, J. The role of lipids in exosome biology and intercellular communication: Function, analytics and applications. *Traffic* **2021**, *22*, 204–220. [[CrossRef](#)]
82. Salatin, S.; Maleki Dizaj, S.; Yari Khosroushahi, A. Effect of the surface modification, size, and shape on cellular uptake of nanoparticles. *Cell Biol. Int.* **2015**, *39*, 881–890. [[CrossRef](#)]



83. Caponnetto, F.; Manini, I.; Skrap, M.; Palmari-Pallag, T.; Di Loreto, C.; Beltrami, A.P.; Cesselli, D.; Ferrari, E. Size-dependent cellular uptake of exosomes. *Nanomed. Nanotechnol. Biol. Med.* **2017**, *13*, 1011–1020. [[CrossRef](#)] [[PubMed](#)]
84. Gurung, S.; Perocheau, D.; Touramanidou, L.; Baruteau, J. The exosome journey: From biogenesis to uptake and intracellular signalling. *Cell Commun. Signal.* **2021**, *19*, 47. [[CrossRef](#)] [[PubMed](#)]
85. Kim, H.I.; Park, J.; Zhu, Y.; Wang, X.; Han, Y.; Zhang, D. Recent advances in extracellular vesicles for therapeutic cargo delivery. *Exp. Mol. Med.* **2024**, *56*, 836–849. [[CrossRef](#)] [[PubMed](#)]
86. van der Pol, E.; Coumans, F.A.; Grootemaat, A.E.; Gardiner, C.; Sargent, I.L.; Harrison, P.; Sturk, A.; van Leeuwen, T.G.; Nieuwland, R. Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. *J. Thromb. Haemost.* **2014**, *12*, 1182–1192. [[CrossRef](#)] [[PubMed](#)]
87. Gong, J.; Wang, H.X.; Leong, K.W. Determination of Cellular Uptake and Endocytic Pathways. *Bio-protocol* **2019**, *9*, e3169. [[CrossRef](#)] [[PubMed](#)]
88. Di Bella, M.A. Overview and Update on Extracellular Vesicles: Considerations on Exosomes and Their Application in Modern Medicine. *Biology* **2022**, *11*, 804. [[CrossRef](#)] [[PubMed](#)]
89. Raposo, G.; Stoorvogel, W. Extracellular vesicles: Exosomes, microvesicles, and friends. *J. Cell Biol.* **2013**, *200*, 373–383. [[CrossRef](#)] [[PubMed](#)]
90. Karmacharya, M.; Kumar, S.; Cho, Y.K. Tuning the Extracellular Vesicles Membrane through Fusion for Biomedical Applications. *J. Funct. Biomater.* **2023**, *14*, 117. [[CrossRef](#)] [[PubMed](#)]
91. Chen, H.; Wang, L.; Zeng, X.; Schwarz, H.; Nanda, H.S.; Peng, X.; Zhou, Y. Exosomes, a New Star for Targeted Delivery. *Front. Cell Dev. Biol.* **2021**, *9*, 751079. [[CrossRef](#)]
92. Salunkhe, S.; Dheeraj; Basak, M.; Chitkara, D.; Mittal, A. Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: Strategies and significance. *J. Control Release* **2020**, *326*, 599–614.
93. Tian, T.; Zhang, H.X.; He, C.P.; Fan, S.; Zhu, Y.L.; Qi, C.; Huang, N.P.; Xiao, Z.D.; Lu, Z.H.; Tannous, B.A.; et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials* **2018**, *150*, 137–149. [[CrossRef](#)] [[PubMed](#)]
94. Liang, Y.; Xu, X.; Li, X.; Xiong, J.; Li, B.; Duan, L.; Wang, D.; Xia, J. Chondrocyte-Targeted MicroRNA Delivery by Engineered Exosomes toward a Cell-Free Osteoarthritis Therapy. *ACS Appl. Mater. Interfaces* **2020**, *12*, 36938–36947. [[CrossRef](#)] [[PubMed](#)]
95. Li, Q.; Hu, W.; Huang, Q.; Yang, J.; Li, B.; Ma, K.; Wei, Q.; Wang, Y.; Su, J.; Sun, M.; et al. MiR146a-loaded engineered exosomes released from silk fibroin patch promote diabetic wound healing by targeting IRAK1. *Signal Transduct. Target. Ther.* **2023**, *8*, 62. [[CrossRef](#)] [[PubMed](#)]
96. Shi, L.; Song, D.; Meng, C.; Cheng, Y.; Wang, B.; Yang, Z. Opportunities and challenges of engineered exosomes for diabetic wound healing. *Giant* **2024**, *18*, 100251. [[CrossRef](#)]
97. Gartz, M.; Strande, J.L. Examining the Paracrine Effects of Exosomes in Cardiovascular Disease and Repair. *J. Am. Heart Assoc.* **2018**, *7*, e007954. [[CrossRef](#)]
98. Yue, B. Biology of the extracellular matrix: An overview. *J. Glaucoma* **2014**, *23* (Suppl. 1), S20–S23. [[CrossRef](#)] [[PubMed](#)]
99. Wu, B.; Liu, D.A.; Guan, L.; Myint, P.K.; Chin, L.; Dang, H.; Xu, Y.; Ren, J.; Li, T.; Yu, Z.; et al. Stiff matrix induces exosome secretion to promote tumour growth. *Nat. Cell Biol.* **2023**, *25*, 415–424. [[CrossRef](#)] [[PubMed](#)]
100. Logozzi, M.; Mizzoni, D.; Angelini, D.F.; Di Raimo, R.; Falchi, M.; Battistini, L.; Fais, S. Microenvironmental pH and Exosome Levels Interplay in Human Cancer Cell Lines of Different Histotypes. *Cancers* **2018**, *10*, 370. [[CrossRef](#)] [[PubMed](#)]
101. Palomar-Alonso, N.; Lee, M.; Kim, M. Exosomes: Membrane-associated proteins, challenges and perspectives. *Biochem. Biophys. Rep.* **2024**, *37*, 101599. [[CrossRef](#)]
102. Nakase, I.; Ueno, N.; Matsuzawa, M.; Noguchi, K.; Hirano, M.; Omura, M.; Takenaka, T.; Sugiyama, A.; Bailey Kobayashi, N.; Hashimoto, T.; et al. Environmental pH stress influences cellular secretion and uptake of extracellular vesicles. *FEBS Open Bio.* **2021**, *11*, 753–767. [[CrossRef](#)]
103. Mahmood, A.; Otruba, Z.; Weisgerber, A.W.; Palay, M.D.; Nguyen, M.T.; Bills, B.L.; Knowles, M.K. Exosome secretion kinetics are controlled by temperature. *Biophys. J.* **2023**, *122*, 1301–1314. [[CrossRef](#)]
104. Wang, Y.; Xiao, T.; Zhao, C.; Li, G. The Regulation of Exosome Generation and Function in Physiological and Pathological Processes. *Int. J. Mol. Sci.* **2023**, *25*, 255. [[CrossRef](#)]
105. Whiteside, T.L. Tumor-Derived Exosomes and Their Role in Cancer Progression. *Adv. Clin. Chem.* **2016**, *74*, 103–141. [[PubMed](#)]
106. Chiaradia, E.; Tancini, B.; Emiliani, C.; Delo, F.; Pellegrino, R.M.; Tognoloni, A.; Urbanelli, L.; Buratta, S. Extracellular Vesicles under Oxidative Stress Conditions: Biological Properties and Physiological Roles. *Cells* **2021**, *10*, 1763. [[CrossRef](#)] [[PubMed](#)]
107. Kolesnikoff, N.; Chen, C.H.; Samuel, M.S. Interrelationships between the extracellular matrix and the immune microenvironment that govern epithelial tumour progression. *Clin. Sci.* **2022**, *136*, 361–377. [[CrossRef](#)]
108. Jabbari, N.; Akbariazar, E.; Feqhhi, M.; Rahbarghazi, R.; Rezaie, J. Breast cancer-derived exosomes: Tumor progression and therapeutic agents. *J. Cell Physiol.* **2020**, *235*, 6345–6356. [[CrossRef](#)]
109. Greening, D.W.; Gopal, S.K.; Xu, R.; Simpson, R.J.; Chen, W. Exosomes and their roles in immune regulation and cancer. *Semin. Cell Dev. Biol.* **2015**, *40*, 72–81. [[CrossRef](#)] [[PubMed](#)]
110. Chaput, N.; Théry, C. Exosomes: Immune properties and potential clinical implementations. *Semin. Immunopathol.* **2011**, *33*, 419–440. [[CrossRef](#)] [[PubMed](#)]

111. Shenoda, B.B.; Ajit, S.K. Modulation of Immune Responses by Exosomes Derived from Antigen-Presenting Cells. *Clin. Med. Insights Pathol.* **2016**, *9* (Suppl. 1), 1–8. [[CrossRef](#)]
112. Théry, C.; Amigorena, S. The cell biology of antigen presentation in dendritic cells. *Curr. Opin. Immunol.* **2001**, *13*, 45–51. [[CrossRef](#)]
113. Théry, C.; Ostrowski, M.; Segura, E. Membrane vesicles as conveyors of immune responses. *Nat. Rev. Immunol.* **2009**, *9*, 581–593. [[CrossRef](#)] [[PubMed](#)]
114. Iannotta, D.; Kijas, A.W.; Rowan, A.E.; Wolfram, J. Entry and exit of extracellular vesicles to and from the blood circulation. *Nat. Nanotechnol.* **2024**, *19*, 13–20. [[CrossRef](#)] [[PubMed](#)]
115. Wang, Z.; Zhao, Z.; Gao, B.; Zhang, L. Exosome mediated biological functions within skeletal microenvironment. *Front. Bioeng. Biotechnol.* **2022**, *10*, 953916. [[CrossRef](#)] [[PubMed](#)]
116. Wolfram, J.; Ferrari, M. Clinical Cancer Nanomedicine. *Nano Today* **2019**, *25*, 85–98. [[CrossRef](#)] [[PubMed](#)]
117. Rana, S.; Yue, S.; Stadel, D.; Zöller, M. Toward tailored exosomes: The exosomal tetraspanin web contributes to target cell selection. *Int. J. Biochem. Cell Biol.* **2012**, *44*, 1574–1584. [[CrossRef](#)]
118. Rudt, S.; Müller, R.H. In vitro phagocytosis assay of nano- and microparticles by chemiluminescence. III. Uptake of differently sized surface-modified particles, and its correlation to particle properties and in vivo distribution. *Eur. J. Pharm. Sci.* **1993**, *1*, 31–39.
119. Kalluri, R. The biology and function of exosomes in cancer. *J. Clin. Investig.* **2016**, *126*, 1208–1215. [[CrossRef](#)]
120. Dai, J.; Su, Y.; Zhong, S.; Cong, L.; Liu, B.; Yang, J.; Tao, Y.; He, Z.; Chen, C.; Jiang, Y. Exosomes: Key players in cancer and potential therapeutic strategy. *Signal Transduct. Target. Ther.* **2020**, *5*, 145. [[CrossRef](#)]
121. Wei, H.; Chen, Q.; Lin, L.; Sha, C.; Li, T.; Liu, Y.; Yin, X.; Xu, Y.; Chen, L.; Gao, W.; et al. Regulation of exosome production and cargo sorting. *Int. J. Biol. Sci.* **2021**, *17*, 163–177. [[CrossRef](#)]
122. Essola, J.M.; Zhang, M.; Yang, H.; Li, F.; Xia, B.; Mavoungou, J.F.; Hussain, A.; Huang, Y. Exosome regulation of immune response mechanism: Pros and cons in immunotherapy. *Bioact. Mater.* **2024**, *32*, 124–146. [[CrossRef](#)]
123. Ha, D.; Yang, N.; Nadihe, V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: Current perspectives and future challenges. *Acta Pharm. Sin. B* **2016**, *6*, 287–296. [[CrossRef](#)]
124. Kobayashi, M.; Sawada, K.; Miyamoto, M.; Shimizu, A.; Yamamoto, M.; Kinose, Y.; Nakamura, K.; Kawano, M.; Kodama, M.; Hashimoto, K.; et al. Exploring the potential of engineered exosomes as delivery systems for tumor-suppressor microRNA replacement therapy in ovarian cancer. *Biochem. Biophys. Res. Commun.* **2020**, *527*, 153–161. [[CrossRef](#)] [[PubMed](#)]
125. Raguraman, R.; Bhavsar, D.; Kim, D.; Ren, X.; Sikavitsas, V.; Munshi, A.; Ramesh, R. Tumor-targeted exosomes for delivery of anticancer drugs. *Cancer Lett.* **2023**, *558*, 216093. [[CrossRef](#)] [[PubMed](#)]
126. Kase, Y.; Uzawa, K.; Wagai, S.; Yoshimura, S.; Yamamoto, J.-I.; Toeda, Y.; Okubo, M.; Eizuka, K.; Ando, T.; Nobuchi, T.; et al. Engineered exosomes delivering specific tumor-suppressive RNAi attenuate oral cancer progression. *Sci. Rep.* **2021**, *11*, 5897. [[CrossRef](#)]
127. Perocheau, D.; Touramanidou, L.; Gurung, S.; Gissen, P.; Baruteau, J. Clinical applications for exosomes: Are we there yet? *Br. J. Pharmacol.* **2021**, *178*, 2375–2392. [[CrossRef](#)] [[PubMed](#)]
128. Agrawal, L.; Engel, K.B.; Greytak, S.R.; Moore, H.M. Understanding preanalytical variables and their effects on clinical biomarkers of oncology and immunotherapy. *Semin. Cancer Biol.* **2018**, *52 Pt 2*, 26–38. [[CrossRef](#)]
129. Nieuwland, R.; Siljander, P.R.M.; Falcón-Pérez, J.M.; Witwer, K.W. Reproducibility of extracellular vesicle research. *Eur. J. Cell Biol.* **2022**, *101*, 151226. [[CrossRef](#)]
130. Yakubovich, E.I.; Polischouk, A.G.; Evtushenko, V.I. Principles and Problems of Exosome Isolation from Biological Fluids. *Biochem. Suppl. Ser. A Membr. Cell Biol.* **2022**, *16*, 115–126. [[CrossRef](#)]
131. Helwa, I.; Cai, J.; Drewry, M.D.; Zimmerman, A.; Dinkins, M.B.; Khaled, M.L.; Seremwe, M.; Dismuke, W.M.; Bieberich, E.; Stamer, W.D.; et al. A Comparative Study of Serum Exosome Isolation Using Differential Ultracentrifugation and Three Commercial Reagents. *PLoS ONE* **2017**, *12*, e0170628. [[CrossRef](#)]
132. Soares Martins, T.; Catita, J.; Martins Rosa, I.; AB da Cruz e Silva, O.; Henriques, A.G. Exosome isolation from distinct biofluids using precipitation and column-based approaches. *PLoS ONE* **2018**, *13*, e0198820. [[CrossRef](#)]
133. Chitti, S.V.; Gummadi, S.; Kang, T.; Shahi, S.; Marzan, A.L.; Nedeva, C.; Sanwlani, R.; Bramich, K.; Stewart, S.; Petrovska, M.; et al. Vesiclepedia 2024: An extracellular vesicles and extracellular particles repository. *Nucleic Acids Res.* **2023**, *52*, D1694–D1698. [[CrossRef](#)] [[PubMed](#)]
134. Kalra, H.; Simpson, R.J.; Ji, H.; Aikawa, E.; Altevogt, P.; Askenase, P.; Bond, V.C.; Borràs, F.E.; Breakefield, X.; Budnik, V.; et al. Vesiclepedia: A compendium for extracellular vesicles with continuous community annotation. *PLoS Biol.* **2012**, *10*, e1001450. [[CrossRef](#)] [[PubMed](#)]
135. Witwer, K.W.; Buzás, E.I.; Bemis, L.T.; Bora, A.; Lässer, C.; Lötvall, J.; Nolte-’t Hoen, E.N.; Piper, M.G.; Sivaraman, S.; Skog, J.; et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J. Extracell. Vesicles* **2013**, *2*, 20360. [[CrossRef](#)]
136. Welsh, J.A.; Goberdhan, D.C.I.; O’Driscoll, L.; Buzas, E.I.; Blenkiron, C.; Bussolati, B.; Cai, H.; Di Vizio, D.; Driedonks, T.A.P.; Erdbrügger, U.; et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J. Extracell. Vesicles* **2024**, *13*, e12404. [[CrossRef](#)] [[PubMed](#)]
137. Lai, J.J.; Chau, Z.L.; Chen, S.Y.; Hill, J.J.; Korpany, K.V.; Liang, N.W.; Lin, L.H.; Lin, Y.H.; Liu, J.K.; Liu, Y.C.; et al. Exosome Processing and Characterization Approaches for Research and Technology Development. *Adv. Sci.* **2022**, *9*, e2103222. [[CrossRef](#)]

138. Hartjes, T.A.; Mytnyk, S.; Jenster, G.W.; van Steijn, V.; van Royen, M.E. Extracellular Vesicle Quantification and Characterization: Common Methods and Emerging Approaches. *Bioengineering* **2019**, *6*, 7. [[CrossRef](#)] [[PubMed](#)]
139. Corona, M.L.; Hurbain, I.; Raposo, G.; van Niel, G. Characterization of Extracellular Vesicles by Transmission Electron Microscopy and Immunolabeling Electron Microscopy. *Methods Mol. Biol.* **2023**, *2668*, 33–43.
140. Tanasi, I.; Adamo, A.; Kamga, P.T.; Bazzoni, R.; Krampera, M. High-throughput analysis and functional interpretation of extracellular vesicle content in hematological malignancies. *Comput. Struct. Biotechnol. J.* **2020**, *18*, 2670–2677. [[CrossRef](#)]
141. Jiang, C.; Fu, Y.; Liu, G.; Shu, B.; Davis, J.; Tofaris, G.K. Multiplexed Profiling of Extracellular Vesicles for Biomarker Development. *Nano-Micro Lett.* **2021**, *14*, 3. [[CrossRef](#)]
142. He, A.; Wang, M.; Li, X.; Chen, H.; Lim, K.; Lu, L.; Zhang, C. Role of Exosomes in the Pathogenesis and Theranostic of Alzheimer's Disease and Parkinson's Disease. *Int. J. Mol. Sci.* **2023**, *24*, 11054. [[CrossRef](#)]
143. D'Argenio, V. The High-Throughput Analyses Era: Are We Ready for the Data Struggle? *High Throughput* **2018**, *7*, 8. [[CrossRef](#)] [[PubMed](#)]
144. Huber, M.J.; Ivleva, N.P.; Booth, A.M.; Beer, I.; Bianchi, I.; Drexel, R.; Geiss, O.; Mehn, D.; Meier, F.; Molska, A.; et al. Physicochemical characterization and quantification of nanoplastics: Applicability, limitations and complementarity of batch and fractionation methods. *Anal. Bioanal. Chem.* **2023**, *415*, 3007–3031. [[CrossRef](#)] [[PubMed](#)]
145. Omrani, M.; Beyrampour-Basmenj, H.; Jahanban-Esfahlan, R.; Talebi, M.; Raesi, M.; Serej, Z.A.; Akbar-Gharalari, N.; Khodakarimi, S.; Wu, J.; Ebrahimi-Kalan, A. Global trend in exosome isolation and application: An update concept in management of diseases. *Mol. Cell Biochem.* **2024**, *479*, 679–691. [[CrossRef](#)] [[PubMed](#)]
146. Allelein, S.; Medina-Perez, P.; Lopes, A.L.H.; Rau, S.; Hause, G.; Kölsch, A.; Kuhlmeier, D. Potential and challenges of specifically isolating extracellular vesicles from heterogeneous populations. *Sci. Rep.* **2021**, *11*, 11585. [[CrossRef](#)] [[PubMed](#)]
147. Kurian, T.K.; Banik, S.; Gopal, D.; Chakrabarti, S.; Mazumder, N. Elucidating Methods for Isolation and Quantification of Exosomes: A Review. *Mol. Biotechnol.* **2021**, *63*, 249–266. [[CrossRef](#)] [[PubMed](#)]
148. Rastogi, S.; Sharma, V.; Bharti, P.S.; Rani, K.; Modi, G.P.; Nikolajeff, F.; Kumar, S. The Evolving Landscape of Exosomes in Neurodegenerative Diseases: Exosomes Characteristics and a Promising Role in Early Diagnosis. *Int. J. Mol. Sci.* **2021**, *22*, 440. [[CrossRef](#)] [[PubMed](#)]
149. Hilton, S.H.; White, I.M. Advances in the analysis of single extracellular vesicles: A critical review. *Sens. Actuators Rep.* **2021**, *3*, 100052. [[CrossRef](#)]
150. Zhu, J.; Wu, F.; Li, C.; Mao, J.; Wang, Y.; Zhou, X.; Xie, H.; Wen, C. Application of Single Extracellular Vesicle Analysis Techniques. *Int. J. Nanomed.* **2023**, *18*, 5365–5376. [[CrossRef](#)]
151. Groot Kormelink, T.; Arkesteijn, G.J.; Nauwelaers, F.A.; van den Engh, G.; Nolte-'t Hoen, E.N.; Wauben, M.H. Prerequisites for the analysis and sorting of extracellular vesicle subpopulations by high-resolution flow cytometry. *Cytometry A* **2016**, *89*, 135–147. [[CrossRef](#)]
152. McNamara, R.P.; Zhou, Y.; Eason, A.B.; Landis, J.T.; Chambers, M.G.; Willcox, S.; Peterson, T.A.; Schouest, B.; Maness, N.J.; MacLean, A.G.; et al. Imaging of surface microdomains on individual extracellular vesicles in 3-D. *J. Extracell. Vesicles* **2022**, *11*, e12191. [[CrossRef](#)]
153. Nizamudeen, Z.; Markus, R.; Lodge, R.; Parmenter, C.; Platt, M.; Chakrabarti, L.; Sottile, V. Rapid and accurate analysis of stem cell-derived extracellular vesicles with super resolution microscopy and live imaging. *Biochim. Biophys. Acta Mol. Cell Res.* **2018**, *1865*, 1891–1900. [[CrossRef](#)]
154. Bond, C.; Santiago-Ruiz, A.N.; Tang, Q.; Lakadamyali, M. Technological advances in super-resolution microscopy to study cellular processes. *Mol. Cell* **2022**, *82*, 315–332. [[CrossRef](#)]
155. Endesfelder, U.; Heilemann, M. Direct Stochastic Optical Reconstruction Microscopy (dSTORM). *Methods Mol. Biol.* **2015**, *1251*, 263–276.
156. Lehmann, M.; Gottschalk, B.; Puchkov, D.; Schmieder, P.; Schwagerus, S.; Hackenberger, C.P.; Haucke, V.; Schmoranzler, J. Multicolor Caged dSTORM Resolves the Ultrastructure of Synaptic Vesicles in the Brain. *Angew. Chem. Int. Ed.* **2015**, *54*, 13230–13235. [[CrossRef](#)]
157. Kudalkar, E.M.; Davis, T.N.; Asbury, C.L. Single-Molecule Total Internal Reflection Fluorescence Microscopy. *Cold Spring Harb. Protoc.* **2016**, *2016*, pdb.top077800. [[CrossRef](#)]
158. Ter-Ovanesyan, D.; Kowal, E.J.K.; Regev, A.; Church, G.M.; Cocucci, E. Imaging of Isolated Extracellular Vesicles Using Fluorescence Microscopy. *Methods Mol. Biol.* **2017**, *1660*, 233–241.
159. Skotland, T.; Hessvik, N.P.; Sandvig, K.; Llorente, A. Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. *J. Lipid Res.* **2019**, *60*, 9–18. [[CrossRef](#)]
160. Gardiner, C.; Shaw, M.; Hole, P.; Smith, J.; Tannetta, D.; Redman, C.W.; Sargent, I.L. Measurement of refractive index by nanoparticle tracking analysis reveals heterogeneity in extracellular vesicles. *J. Extracell. Vesicles* **2014**, *3*, 25361. [[CrossRef](#)]
161. Hickey, S.M.; Ung, B.; Bader, C.; Brooks, R.; Lazniewska, J.; Johnson, I.R.D.; Sorvina, A.; Logan, J.; Martini, C.; Moore, C.R.; et al. Fluorescence Microscopy—An Outline of Hardware, Biological Handling, and Fluorophore Considerations. *Cells* **2021**, *11*, 35. [[CrossRef](#)] [[PubMed](#)]
162. Zhu, S.; Ma, L.; Wang, S.; Chen, C.; Zhang, W.; Yang, L.; Hang, W.; Nolan, J.P.; Wu, L.; Yan, X. Light-scattering detection below the level of single fluorescent molecules for high-resolution characterization of functional nanoparticles. *ACS Nano* **2014**, *8*, 10998–11006. [[CrossRef](#)] [[PubMed](#)]



163. Sharma, S.; LeClaire, M.; Gimzewski, J.K. Ascent of atomic force microscopy as a nanoanalytical tool for exosomes and other extracellular vesicles. *Nanotechnology* **2018**, *29*, 132001. [[CrossRef](#)]
164. Shao, H.; Im, H.; Castro, C.M.; Breakefield, X.; Weissleder, R.; Lee, H. New Technologies for Analysis of Extracellular Vesicles. *Chem. Rev.* **2018**, *118*, 1917–1950. [[CrossRef](#)]
165. Li, X.H.; Zhang, J.; Li, D.F.; Wu, W.; Xie, Z.W.; Liu, Q. Physiological and pathological insights into exosomes in the brain. *Zool. Res.* **2020**, *41*, 365–372. [[CrossRef](#)]
166. Liu, S.-L.; Sun, P.; Li, Y.; Liu, S.-S.; Lu, Y. Exosomes as critical mediators of cell-to-cell communication in cancer pathogenesis and their potential clinical application. *Transl. Cancer Res.* **2019**, *8*, 298–311. [[CrossRef](#)]
167. Bahr, M.M.; Amer, M.S.; Abo-El-Sooud, K.; Abdallah, A.N.; El-Tookhy, O.S. Preservation techniques of stem cells extracellular vesicles: A gate for manufacturing of clinical grade therapeutic extracellular vesicles and long-term clinical trials. *Int. J. Vet. Sci. Med.* **2020**, *8*, 1–8. [[CrossRef](#)]
168. Wu, J.Y.; Li, Y.J.; Hu, X.B.; Huang, S.; Xiang, D.X. Preservation of small extracellular vesicles for functional analysis and therapeutic applications: A comparative evaluation of storage conditions. *Drug Deliv.* **2021**, *28*, 162–170. [[CrossRef](#)]
169. Jeyaram, A.; Jay, S.M. Preservation and Storage Stability of Extracellular Vesicles for Therapeutic Applications. *AAPS J.* **2017**, *20*, 1–7. [[CrossRef](#)]
170. Qazi, R.E.M.; Sajid, Z.; Zhao, C.; Hussain, I.; Iftikhar, F.; Jameel, M.; Rehman, F.U.; Mian, A.A. Lyophilization Based Isolation of Exosomes. *Int. J. Mol. Sci.* **2023**, *24*, 10477. [[CrossRef](#)]
171. Cottle, C.; Porter, A.P.; Lipat, A.; Turner-Lyles, C.; Nguyen, J.; Moll, G.; Chinnadurai, R. Impact of Cryopreservation and Freeze-Thawing on Therapeutic Properties of Mesenchymal Stromal/Stem Cells and Other Common Cellular Therapeutics. *Curr. Stem Cell Rep.* **2022**, *8*, 72–92. [[CrossRef](#)] [[PubMed](#)]
172. Görgens, A.; Corso, G.; Hagey, D.W.; Jawad Wiklander, R.; Gustafsson, M.O.; Felldin, U.; Lee, Y.; Bostancioglu, R.B.; Sork, H.; Liang, X.; et al. Identification of storage conditions stabilizing extracellular vesicles preparations. *J. Extracell. Vesicles* **2022**, *11*, e12238. [[CrossRef](#)] [[PubMed](#)]
173. Kusuma, G.D.; Barabadi, M.; Tan, J.L.; Morton, D.A.V.; Frith, J.E.; Lim, R. To Protect and to Preserve: Novel Preservation Strategies for Extracellular Vesicles. *Front. Pharmacol.* **2018**, *9*, 417722. [[CrossRef](#)]
174. Gelibter, S.; Marostica, G.; Mandelli, A.; Siciliani, S.; Podini, P.; Finardi, A.; Furlan, R. The impact of storage on extracellular vesicles: A systematic study. *J. Extracell. Vesicles* **2022**, *11*, e12162. [[CrossRef](#)]
175. Sivanantham, A.; Jin, Y. Impact of Storage Conditions on EV Integrity/Surface Markers and Cargos. *Life* **2022**, *12*, 697. [[CrossRef](#)] [[PubMed](#)]
176. Sadeghi, S.; Tehrani, F.R.; Tahmasebi, S.; Shafiee, A.; Hashemi, S.M. Exosome engineering in cell therapy and drug delivery. *Inflammopharmacology* **2023**, *31*, 145–169. [[CrossRef](#)] [[PubMed](#)]
177. Zeng, H.; Guo, S.; Ren, X.; Wu, Z.; Liu, S.; Yao, X. Current Strategies for Exosome Cargo Loading and Targeting Delivery. *Cells* **2023**, *12*, 1416. [[CrossRef](#)]
178. Chen, H.; Yao, H.; Chi, J.; Li, C.; Liu, Y.; Yang, J.; Yu, J.; Wang, J.; Ruan, Y.; Pi, J.; et al. Engineered exosomes as drug and RNA co-delivery system: New hope for enhanced therapeutics? *Front. Bioeng. Biotechnol.* **2023**, *11*, 1254356. [[CrossRef](#)] [[PubMed](#)]
179. Patel, G.; Agnihotri, T.G.; Gitte, M.; Shinde, T.; Gomte, S.S.; Goswami, R.; Jain, A. Exosomes: A potential diagnostic and treatment modality in the quest for counteracting cancer. *Cell Oncol.* **2023**, *46*, 1159–1179. [[CrossRef](#)] [[PubMed](#)]
180. Xu, M.; Yang, Q.; Sun, X.; Wang, Y. Recent Advancements in the Loading and Modification of Therapeutic Exosomes. *Front. Bioeng. Biotechnol.* **2020**, *8*, 586130. [[CrossRef](#)]
181. Kim, M.S.; Haney, M.J.; Zhao, Y.; Mahajan, V.; Deygen, I.; Klyachko, N.L.; Inskoe, E.; Piroyan, A.; Sokolsky, M.; Okolie, O.; et al. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. *Nanomedicine* **2016**, *12*, 655–664. [[CrossRef](#)]
182. Ohno, S.; Takanashi, M.; Sudo, K.; Ueda, S.; Ishikawa, A.; Matsuyama, N.; Fujita, K.; Mizutani, T.; Ohgi, T.; Ochiya, T.; et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Mol. Ther.* **2013**, *21*, 185–191. [[CrossRef](#)]
183. Kučuk, N.; Primožič, M.; Knez, Ž.; Leitgeb, M. Exosomes Engineering and Their Roles as Therapy Delivery Tools, Therapeutic Targets, and Biomarkers. *Int. J. Mol. Sci.* **2021**, *22*, 9543. [[CrossRef](#)]
184. Xu, M.; Feng, T.; Liu, B.; Qiu, F.; Xu, Y.; Zhao, Y.; Zheng, Y. Engineered exosomes: Desirable target-tracking characteristics for cerebrovascular and neurodegenerative disease therapies. *Theranostics* **2021**, *11*, 8926–8944. [[CrossRef](#)]
185. Zhu, Q.; Ling, X.; Yang, Y.; Zhang, J.; Li, Q.; Niu, X.; Hu, G.; Chen, B.; Li, H.; Wang, Y.; et al. Embryonic Stem Cells-Derived Exosomes Endowed with Targeting Properties as Chemotherapeutics Delivery Vehicles for Glioblastoma Therapy. *Adv. Sci.* **2019**, *6*, 1801899. [[CrossRef](#)]
186. Pham, T.C.; Jayasinghe, M.K.; Pham, T.T.; Yang, Y.; Wei, L.; Usman, W.M.; Chen, H.; Pisisinu, M.; Gong, J.; Kim, S.; et al. Covalent conjugation of extracellular vesicles with peptides and nanobodies for targeted therapeutic delivery. *J. Extracell. Vesicles* **2021**, *10*, e12057. [[CrossRef](#)]
187. Wang, J.; Wang, M.; Jiang, N.; Ding, S.; Peng, Q.; Zheng, L. Emerging chemical engineering of exosomes as “bioscaffolds” in diagnostics and therapeutics. *Genes Dis.* **2023**, *10*, 1494–1512. [[CrossRef](#)]
188. Sharma, V.; Mukhopadhyay, C.D. Exosome as drug delivery system: Current advancements. *Extracell. Vesicle* **2024**, *3*, 100032. [[CrossRef](#)]



189. Li, T.; Li, X.; Han, G.; Liang, M.; Yang, Z.; Zhang, C.; Huang, S.; Tai, S.; Yu, S. The Therapeutic Potential and Clinical Significance of Exosomes as Carriers of Drug Delivery System. *Pharmaceutics* **2022**, *15*, 21. [\[CrossRef\]](#)
190. Krylova, S.V.; Feng, D. The Machinery of Exosomes: Biogenesis, Release, and Uptake. *Int. J. Mol. Sci.* **2023**, *24*, 1337. [\[CrossRef\]](#)
191. Ghadami, S.; Dellinger, K. The lipid composition of extracellular vesicles: Applications in diagnostics and therapeutic delivery. *Front. Mol. Biosci.* **2023**, *10*, 1198044. [\[CrossRef\]](#) [\[PubMed\]](#)
192. Morton, L.A.; Yang, H.; Saludes, J.P.; Fiorini, Z.; Beninson, L.; Chapman, E.R.; Fleshner, M.; Xue, D.; Yin, H. MARCKS-ED peptide as a curvature and lipid sensor. *ACS Chem. Biol.* **2013**, *8*, 218–225. [\[CrossRef\]](#) [\[PubMed\]](#)
193. Unterreitmeier, S.; Fuchs, A.; Schäffler, T.; Heym, R.G.; Frishman, D.; Langosch, D. Phenylalanine promotes interaction of transmembrane domains via GxxxG motifs. *J. Mol. Biol.* **2007**, *374*, 705–718. [\[CrossRef\]](#)
194. Yan, L.; de Jesus, A.J.; Tamura, R.; Li, V.; Cheng, K.; Yin, H. Curvature sensing MARCKS-ED peptides bind to membranes in a stereo-independent manner. *J. Pept. Sci.* **2015**, *21*, 577–585. [\[CrossRef\]](#) [\[PubMed\]](#)
195. Flynn, A.D.; Yin, H. Lipid-Targeting Peptide Probes for Extracellular Vesicles. *J. Cell Physiol.* **2016**, *231*, 2327–2332. [\[CrossRef\]](#) [\[PubMed\]](#)
196. Babatunde, K.A.; Yesodha Subramanian, B.; Ahouidi, A.D.; Martinez Murillo, P.; Walch, M.; Mantel, P.Y. Role of Extracellular Vesicles in Cellular Cross Talk in Malaria. *Front. Immunol.* **2020**, *11*, 22. [\[CrossRef\]](#) [\[PubMed\]](#)
197. Regev-Rudzki, N.; Wilson, D.W.; Carvalho, T.G.; Sisquella, X.; Coleman, B.M.; Rug, M.; Bursac, D.; Angrisano, F.; Gee, M.; Hill, A.F.; et al. Cell-cell communication between malaria-infected red blood cells via exosome-like vesicles. *Cell* **2013**, *153*, 1120–1133. [\[CrossRef\]](#) [\[PubMed\]](#)
198. Ngambenjawong, C.; Pineda, J.M.; Pun, S.H. Engineering an Affinity-Enhanced Peptide through Optimization of Cyclization Chemistry. *Bioconjug. Chem.* **2016**, *27*, 2854–2862. [\[CrossRef\]](#) [\[PubMed\]](#)
199. Xu, K.; Jin, Y.; Li, Y.; Huang, Y.; Zhao, R. Recent Progress of Exosome Isolation and Peptide Recognition-Guided Strategies for Exosome Research. *Front. Chem.* **2022**, *10*, 844124. [\[CrossRef\]](#) [\[PubMed\]](#)
200. Morton, L.A.; Tamura, R.; de Jesus, A.J.; Espinoza, A.; Yin, H. Biophysical investigations with MARCKS-ED: Dissecting the molecular mechanism of its curvature sensing behaviors. *Biochim. Biophys. Acta* **2014**, *1838*, 3137–3144. [\[CrossRef\]](#) [\[PubMed\]](#)
201. Quadri, Z.; Elsherbini, A.; Bieberich, E. Extracellular vesicles in pharmacology: Novel approaches in diagnostics and therapy. *Pharmacol. Res.* **2022**, *175*, 105980. [\[CrossRef\]](#)
202. Morishita, M.; Takahashi, Y.; Nishikawa, M.; Takakura, Y. Pharmacokinetics of Exosomes-An Important Factor for Elucidating the Biological Roles of Exosomes and for the Development of Exosome-Based Therapeutics. *J. Pharm. Sci.* **2017**, *106*, 2265–2269. [\[CrossRef\]](#)
203. Zhang, Y.; Liu, Q.; Zhang, X.; Huang, H.; Tang, S.; Chai, Y.; Xu, Z.; Li, M.; Chen, X.; Liu, J.; et al. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy. *J. Nanobiotechnol.* **2022**, *20*, 279. [\[CrossRef\]](#)
204. Bao, C.; Xiang, H.; Chen, Q.; Zhao, Y.; Gao, Q.; Huang, F.; Mao, L. A Review of Labeling Approaches Used in Small Extracellular Vesicles Tracing and Imaging. *Int. J. Nanomed.* **2023**, *18*, 4567–4588. [\[CrossRef\]](#) [\[PubMed\]](#)
205. Choi, H.; Choi, Y.; Yim, H.Y.; Mirzaaghasi, A.; Yoo, J.K.; Choi, C. Biodistribution of Exosomes and Engineering Strategies for Targeted Delivery of Therapeutic Exosomes. *Tissue Eng. Regen. Med.* **2021**, *18*, 499–511. [\[CrossRef\]](#)
206. Bruno, B.J.; Miller, G.D.; Lim, C.S. Basics and recent advances in peptide and protein drug delivery. *Ther. Deliv.* **2013**, *4*, 1443–1467. [\[CrossRef\]](#)
207. Samanta, S.; Rajasingh, S.; Drosos, N.; Zhou, Z.; Dawn, B.; Rajasingh, J. Exosomes: New molecular targets of diseases. *Acta Pharmacol. Sin.* **2018**, *39*, 501–513. [\[CrossRef\]](#) [\[PubMed\]](#)
208. Susa, F.; Limongi, T.; Dumontel, B.; Vighetto, V.; Cauda, V. Engineered Extracellular Vesicles as a Reliable Tool in Cancer Nanomedicine. *Cancers* **2019**, *11*, 1979. [\[CrossRef\]](#)
209. Kooijmans, S.A.A.; de Jong, O.G.; Schiffelers, R.M. Exploring interactions between extracellular vesicles and cells for innovative drug delivery system design. *Adv. Drug Deliv. Rev.* **2021**, *173*, 252–278. [\[CrossRef\]](#) [\[PubMed\]](#)
210. Santelices, J.; Ou, M.; Hui, W.W.; Maegawa, G.H.B.; Edelmann, M.J. Fluorescent Labeling of Small Extracellular Vesicles (EVs) Isolated from Conditioned Media. *Bio-protocol* **2022**, *12*, e4447. [\[CrossRef\]](#) [\[PubMed\]](#)
211. N'Diaye, E.R.; Orefice, N.S.; Ghezzi, C.; Boumendjel, A. Chemically Modified Extracellular Vesicles and Applications in Radiolabeling and Drug Delivery. *Pharmaceutics* **2022**, *14*, 653. [\[CrossRef\]](#)
212. Szymanowski, W.; Szymanowska, A.; Bielawska, A.; Lopez-Berestein, G.; Rodriguez-Aguayo, C.; Amero, P. Aptamers as Potential Therapeutic Tools for Ovarian Cancer: Advancements and Challenges. *Cancers* **2023**, *15*, 5300. [\[CrossRef\]](#)
213. Chen, Z.; Xiong, M.; Tian, J.; Song, D.; Duan, S.; Zhang, L. Encapsulation and assessment of therapeutic cargo in engineered exosomes: A systematic review. *J. Nanobiotechnol.* **2024**, *22*, 18. [\[CrossRef\]](#)
214. Zhang, Z.; Jia, J.; Lai, Y.; Ma, Y.; Weng, J.; Sun, L. Conjugating folic acid to gold nanoparticles through glutathione for targeting and detecting cancer cells. *Bioorganic Med. Chem.* **2010**, *18*, 5528–5534. [\[CrossRef\]](#) [\[PubMed\]](#)
215. Xu, Z.; Wang, X.; Liu, X.; Cui, Z.; Yang, X.; Yeung, K.W.K.; Chung, J.C.; Chu, P.K.; Wu, S. Tannic Acid/Fe<sup>3+</sup>/Ag Nanofilm Exhibiting Superior Photodynamic and Physical Antibacterial Activity. *ACS Appl. Mater. Interfaces* **2017**, *9*, 39657–39671. [\[CrossRef\]](#) [\[PubMed\]](#)
216. Zhang, Y.; Bi, J.; Huang, J.; Tang, Y.; Du, S.; Li, P. Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications. *Int. J. Nanomed.* **2020**, *15*, 6917–6934. [\[CrossRef\]](#)

217. Muthu, S.; Bapat, A.; Jain, R.; Jeyaraman, N.; Jeyaraman, M. Exosomal therapy—a new frontier in regenerative medicine. *Stem Cell Investig.* **2021**, *8*, 7. [[CrossRef](#)] [[PubMed](#)]
218. Huda, M.N.; Nafiujjaman, M.; Deaguero, I.G.; Okonkwo, J.; Hill, M.L.; Kim, T.; Nurunnabi, M. Potential Use of Exosomes as Diagnostic Biomarkers and in Targeted Drug Delivery: Progress in Clinical and Preclinical Applications. *ACS Biomater. Sci. Eng.* **2021**, *7*, 2106–2149. [[CrossRef](#)]
219. Beetler, D.J.; Di Florio, D.N.; Bruno, K.A.; Ikezu, T.; March, K.L.; Cooper, L.T.; Jr Wolfram, J.; Fairweather, D. Extracellular vesicles as personalized medicine. *Mol. Aspects Med.* **2023**, *91*, 101155. [[CrossRef](#)] [[PubMed](#)]
220. Mosquera-Heredia, M.I.; Morales, L.C.; Vidal, O.M.; Barceló, E.; Silvera-Redondo, C.; Vélez, J.I.; Garavito-Galofre, P. Exosomes: Potential Disease Biomarkers and New Therapeutic Targets. *Biomedicines* **2021**, *9*, 1061. [[CrossRef](#)]
221. Del Bene, M.; Osti, D.; Faletti, S.; Beznoussenko, G.V.; DiMeco, F.; Pelicci, G. Extracellular vesicles: The key for precision medicine in glioblastoma. *Neuro-Oncology* **2021**, *24*, 184–196. [[CrossRef](#)]
222. Shang, M.; Ji, J.S.; Song, C.; Gao, B.J.; Jin, J.G.; Kuo, W.P.; Kang, H. *Extracellular Vesicles: A Brief Overview and Its Role in Precision Medicine*, in *Extracellular Vesicles: Methods and Protocols*; Kuo, W.P., Jia, S., Eds.; Springer: New York, NY, USA, 2017; pp. 1–14.
223. Lone, S.N.; Nisar, S.; Masoodi, T.; Singh, M.; Rizwan, A.; Hashem, S.; El-Rifai, W.; Bedognetti, D.; Batra, S.K.; Haris, M.; et al. Liquid biopsy: A step closer to transform diagnosis, prognosis and future of cancer treatments. *Mol. Cancer* **2022**, *21*, 79. [[CrossRef](#)]
224. Areny-Balagueró, A.; Solé-Porta, A.; Camprubí-Rimblas, M.; Campaña-Duel, E.; Ceccato, A.; Roig, A.; Closa, D.; Artigas, A. Bioengineered extracellular vesicles: Future of precision medicine for sepsis. *Intensive Care Med. Exp.* **2023**, *11*, 11. [[CrossRef](#)] [[PubMed](#)]
225. Mora, E.M.; Álvarez-Cubela, S.; Oltra, E. Biobanking of Exosomes in the Era of Precision Medicine: Are We There Yet? *Int. J. Mol. Sci.* **2015**, *17*, 13. [[CrossRef](#)] [[PubMed](#)]
226. Yang, J.; Shin, T.-S.; Kim, J.S.; Jee, Y.-K.; Kim, Y.-K. A new horizon of precision medicine: Combination of the microbiome and extracellular vesicles. *Exp. Mol. Med.* **2022**, *54*, 466–482. [[CrossRef](#)] [[PubMed](#)]
227. Desdín-Micó, G.; Mittelbrunn, M. Role of exosomes in the protection of cellular homeostasis. *Cell Adhes. Migr.* **2017**, *11*, 127–134. [[CrossRef](#)] [[PubMed](#)]
228. Chutipongtanate, S.; Pattanapanyasat, K.; Meller, J.; Serrano López, J. Editorial: Driving extracellular vesicles toward applications in precision medicine. *Front. Med.* **2022**, *9*, 1049697. [[CrossRef](#)]
229. Arraud, N.; Linares, R.; Tan, S.; Gounou, C.; Pasquet, J.M.; Mornet, S.; Brisson, A.R. Extracellular vesicles from blood plasma: Determination of their morphology, size, phenotype and concentration. *J. Thromb. Haemost.* **2014**, *12*, 614–627. [[CrossRef](#)] [[PubMed](#)]
230. Yang, G.; Waheed, S.; Wang, C.; Shekh, M.; Li, Z.; Wu, J. Exosomes and Their Bioengineering Strategies in the Cutaneous Wound Healing and Related Complications: Current Knowledge and Future Perspectives. *Int. J. Biol. Sci.* **2023**, *19*, 1430–1454. [[CrossRef](#)] [[PubMed](#)]
231. Cao, J.; Lv, G.; Wei, F. Engineering exosomes to reshape the immune microenvironment in breast cancer: Molecular insights and therapeutic opportunities. *Clin. Transl. Med.* **2024**, *14*, e1645. [[CrossRef](#)] [[PubMed](#)]
232. FDA. Public Safety Alert Due to Marketing of Unapproved Stem Cell and Exosome Products. 2019. Available online: <https://www.fda.gov/safety/medical-product-safety-information/public-safety-alert-due-marketing-unapproved-stem-cell-and-exosome-products> (accessed on 20 April 2024).
233. Cheng, K.; Kalluri, R. Guidelines for clinical translation and commercialization of extracellular vesicles and exosomes based therapeutics. *Extracell. Vesicle* **2023**, *2*, 100029. [[CrossRef](#)]
234. Tzng, E.; Bayardo, N.; Yang, P.C. Current challenges surrounding exosome treatments. *Extracell. Vesicle* **2023**, *2*, 100023. [[CrossRef](#)]

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