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Diagnostic and therapeutic applications of melanoma-derived exosomes in nanomedicine

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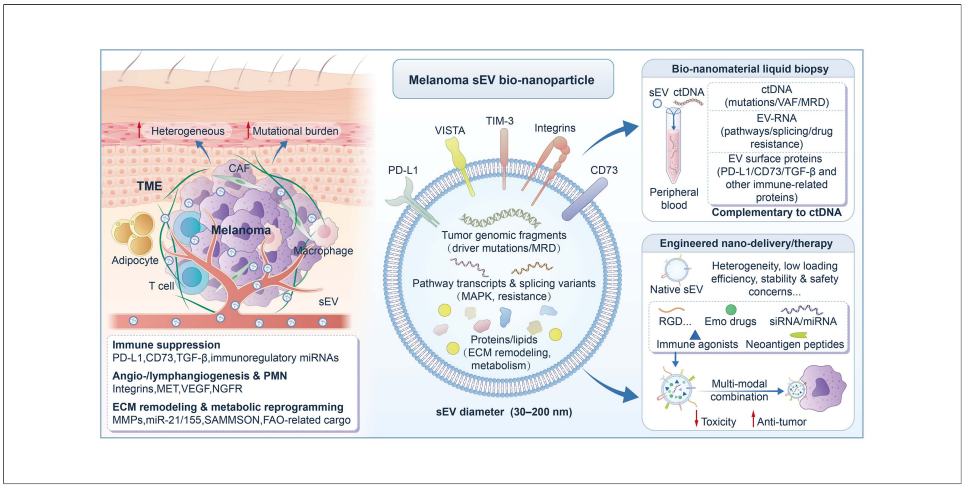

Abstract

Melanoma remains challenging due to pronounced heterogeneity, early metastasis, and dynamic treatment resistance. Conventional tissue biopsy and imaging provide essential information but are limited by invasiveness, sampling bias, and an inability to capture longitudinal tumor-immune evolution. As endogenous bio-nanomaterials, small extracellular vesicles/exosomes circulate stably in body fluids and carry multi-omic cargo (DNA, RNA, proteins, lipids), enabling minimally invasive liquid biopsy and real-time disease monitoring. Beyond diagnostics, engineered exosomal nanomaterials can be functionalized and loaded with therapeutic payloads to improve targeted delivery and support combination regimens, including immunotherapy. This review highlights mechanistic roles of exosomes in melanoma progression, summarizes key liquid-biopsy biomarkers (e.g., exosomal PD-L1 and EV-miRNA/protein signatures), and discusses translational barriers such as standardization, scalable manufacturing, safety, and clinical validation.

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INTRODUCTION

Melanoma, as a malignant tumor, is characterized by pronounced heterogeneity and a strong propensity for metastasis; particularly in the context of continuous advances in immunotherapy and targeted therapy, how to achieve more refined stratification and monitoring across different stages of the disease spectrum remains a key challenge in clinical management[1-3]. The high incidence and poor prognosis of melanoma, together with the difficulty of early diagnosis and the development of therapeutic resistance in advanced stages, make it one of the most challenging cancers in clinical practice[4-7]. Traditional pathological biopsy and imaging examinations continue to serve as the cornerstone for staging and treatment response evaluation; however, their limited repeatability and inability to capture, in real time, the dynamic temporal evolution of clonal architecture and the immune microenvironment impose substantial constraints on clinical decision-making. Therefore, the development of novel, non-invasive diagnostic approaches is of particular importance.

Extracellular vesicles (EVs) are a heterogeneous group of membrane-enclosed particles released by cells into the extracellular space and are now recognized as important mediators of intercellular communication[8]. Among them, exosomes are generally defined as nanoscale vesicles of endosomal origin, most commonly ranging from 30 to 150 nm in diameter[9]. However, in many melanoma-related studies, these vesicles are still identified mainly on the basis of size range, isolation method, and conventional surface markers, rather than by direct verification of their endosomal biogenesis[10]. Accordingly, the terms “EVs”, “small extracellular vesicles (sEVs)”, and “exosomes” are sometimes used interchangeably in the published literature, which complicates cross-study comparison and interpretation[11]. For the sake of consistency and readability throughout this review, we primarily use the term “exosomes” to refer to nanoscale vesicular fractions that operationally correspond to exosome-enriched components in melanoma research[12]. At the same time, we acknowledge that, from a strict biogenetic perspective, part of these vesicles may be more accurately classified within the broader category of sEVs[13]. Unless the original studies explicitly distinguish different EV subpopulations, the term “exosomes” is used consistently in the following sections[14].

Melanoma-derived exosomes lie at the intersection of tumor biology and translational nanomedicine[15]. On the one hand, they actively participate in extracellular matrix (ECM) remodeling, immunosuppression, angiogenesis, metabolic reprogramming, and pre-metastatic niche formation, thus serving as important functional mediators of melanoma progression[16]. On the other hand, precisely because these exosomes carry multidimensional molecular information from tumor cells and their microenvironment in the form of nanoscale, lipid-bilayer-enclosed particles, they can also be developed as functionally informative analytes for liquid biopsy[17]. Furthermore, the very features that enable exosomes to mediate efficient intercellular communication—their lipid bilayer structure, complex molecular cargo, and surface components capable of interacting with recipient cells—also make them highly promising candidates for engineered drug delivery[18]. Therefore, the biological roles of melanoma exosomes in tumor progression and their translational

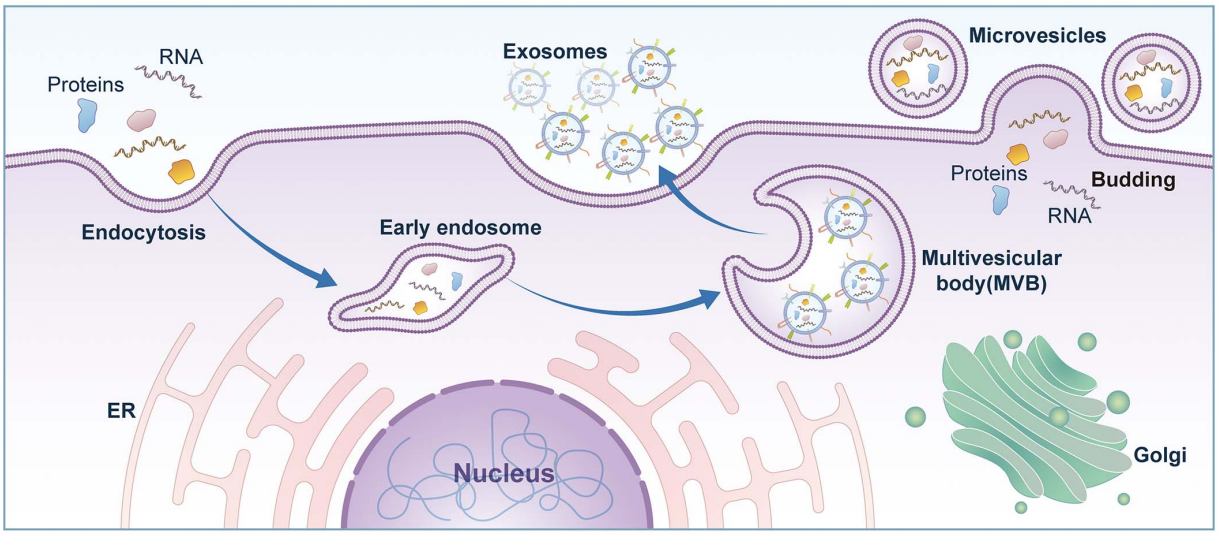

Figure 1. Biogenesis and release of exosomes. Exosome formation begins with plasma membrane invagination during endocytosis, leading to the formation of early endosomes. Early endosomes mature into late endosomes, whose limiting membranes invaginate to generate intraluminal vesicles (ILVs), ultimately forming multivesicular bodies (MVBs). Fusion of MVBs with the plasma membrane releases ILVs into the extracellular space as exosomes. Intracellular organelles, including the endoplasmic reticulum (ER) and Golgi apparatus, participate in the synthesis, sorting, and trafficking of exosome-associated components. ER: Endoplasmic reticulum; ILV: intraluminal vesicle; MVB: multivesicular body.

applications in liquid biopsy and therapeutic delivery should not be viewed as two disconnected themes, but rather as two interrelated dimensions of the same bio-nanomaterial system[19]. Based on this logic, the present review first summarizes how exosomes shape melanoma progression and the immune microenvironment, and then further discusses how these properties support emerging applications in liquid biopsy and engineered therapeutic delivery[20].

TRANSLATIONAL POTENTIAL AND APPLICATIONS OF EXOSOMES IN MELANOMA RESEARCH

Nanomaterial-based exosomes

In this review, we focus on the most frequently cited EV subtype, exosomes, which are generally considered to originate from the endosomal-multivesicular body pathway and be released via exocytosis, with a typical size range of approximately 30-150 nm[21,22] [Figure 1].

It is worth emphasizing that the significance of melanoma-associated exosomes lies not only in the rich biological information they carry, but also in the fact that they themselves constitute a class of bio-nanomaterials with a defined structural basis. Their lipid bilayer, surface membrane proteins and adhesion molecules, together with the nucleic acids, proteins, and lipids enclosed within them, collectively form the material basis through which exosomes exert their functions and determine their dual application potential in both liquid biopsy and therapeutic delivery[23]. Current engineering strategies applied to exosomes—whether donor-cell pretreatment, post-isolation surface modification, membrane fusion, or delivery-system construction—essentially involve nanoscale remodeling of their structure in order to further regulate biodistribution, cellular uptake, tissue penetration, and drug-release behavior *in vivo*[24]. Therefore, exosome design should not be understood merely as drug loading, but rather as a functional construction process centered on the coordinated relationships among membrane structure, surface molecules, and internal bioactive components[24].

It should be further noted that one of the major factors currently restricting progress in this field is the insufficient reproducibility and comparability across studies. First, differences in EV isolation methods directly affect the composition of the final vesicle population obtained. Ultracentrifugation, density-gradient

centrifugation, size-exclusion chromatography (SEC), polymer precipitation, immunoaffinity capture, and microfluidic platforms differ in recovery rate, purity, processing time, and impurity removal capacity, and the EV subpopulations they enrich, as well as the background of non-vesicular particle contamination, are not equivalent[25]. This means that even when different studies all use the term "exosomes" or "sEVs", their actual study objects may not be fully identical in structural composition and functional properties, thereby affecting cross-study comparisons of biomarker abundance, functional experimental outcomes, and delivery performance[26].

Second, source heterogeneity further increases the complexity of interpreting results. Melanoma-related EVs do not represent a single entity, but are influenced by multiple factors including donor-cell type, tumor stage, genetic background, hypoxic status, inflammatory stimulation, drug exposure, and culture conditions[26]. Differences in source may lead to significant variation in membrane molecular composition, internal bioactive content, and organ tropism, and consequently their biological effects and therapeutic performance are not directly comparable[23]. For liquid biopsy, this implies that EV signals detected in body fluids often represent composite readouts mixed from multiple origins; for drug-delivery studies, it underscores the need for more rigorous reporting of donor status, culture systems, and sample-processing workflows in order to improve interpretability and reproducibility[10].

In addition, differences among analytical platforms are another important source of limited comparability across studies. Common methods for EV characterization and detection currently include nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), Western blotting, flow cytometry, ExoView, ddPCR, sequencing, and mass spectrometry, and these platforms differ substantially in detection targets, sensitivity, quantification strategies, and normalization approaches[27]. Readouts for the same marker on different platforms are often difficult to directly align, meaning that even when studies show similar trends, they may still lack an integrated quantitative basis[28]. Therefore, future work should not only strengthen the description of EV structural features and design logic, but also reinforce the standardization of isolation, characterization, quantification, and reporting workflows in accordance with frameworks such as Minimal Information for Studies of Extracellular Vesicles (MISEV), so as to improve reproducibility, inter-study comparability, and the reliability of clinical translation[10].

Clinical applications of exosomes in liquid biopsy for melanoma

Liquid biopsy provides a noninvasive and repeatable assessment strategy for melanoma, making it particularly suitable for therapeutic response monitoring and recurrence surveillance[29,30]. Compared with circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs), exosomes offer distinct potential advantages: their molecular signals are not restricted to tumor cells alone but may also originate from immune cells, stromal cells, and other components of the tumor microenvironment (TME), thereby more closely reflecting functional tumor-host interactions[17,31,32]. In addition, the lipid bilayer membrane confers a degree of protection to encapsulated nucleic acids and proteins, rendering longitudinal trend analysis during follow-up more feasible and robust[33]. Overall, exosome-based liquid biopsy is better positioned as a complementary dimension to existing biomarker systems, strengthening the evidence base for stratified diagnosis, treatment selection, and dynamic clinical decision-making, rather than serving as a direct replacement for any single established indicator[34].

Diagnosis and risk stratification: from single biomarkers to composite molecular signatures

At the level of diagnosis and risk stratification, exosomes are capable of simultaneously providing multi-level information[35]. At the nucleic acid level (e.g., mRNA/miRNA), signatures often correlate more closely with pathway activity and functional states; at the protein level, immune checkpoints and profiles related to invasion, migration, and angiogenesis can indicate disease activity and immune context; meanwhile, lipid

composition and metabolism-related characteristics may reflect tumor metabolic reprogramming and structural differences in membranes[36]. Given the multi-omic nature of exosomes, single molecules are often insufficient to capture disease heterogeneity; therefore, a more rational approach involves constructing composite signatures and performing stratified validation across different stages or risk subgroups[37,38].

Prognostic assessment and monitoring of recurrence/metastasis

Given the high heterogeneity and significant risk of metastasis associated with melanoma, the clinical demand for continuous longitudinal monitoring is particularly acute[39,40]. The potential utility of exosomes in prognostic assessment is primarily manifested in their ability to: reflect fluctuations in tumor burden and systemic inflammatory/immune responses; indicate directional molecular changes prior to radiographically evident progression; and indirectly signal an increased risk of recurrence or metastasis through alterations in signaling pathways associated with matrix remodeling, immunosuppression, and angiogenesis[41-43].

Delivery potential of exosomes as nanocarriers

Material advantages of exosomes

Compared with synthetic nanomaterials, exosomes originate from the cellular membrane system, and their lipid bilayer structure and membrane protein composition generally confer superior biocompatibility and stability *in vivo*[44]. The receptors, adhesion molecules, and glycosylation patterns on the exosomal surface may influence target cell recognition, uptake, and intracellular trafficking pathways, thereby exhibiting delivery behaviors that more closely mimic physiological processes in certain contexts[45]. Furthermore, the ability of exosomes to encapsulate diverse cargos—including nucleic acids, proteins, and small molecules—provides a versatile platform for the development of multi-component intervention strategies[46-48]. It must be emphasized that exosomes are not entirely inert "empty shells"; their endogenous components may actively participate in signaling, which offers potential advantages while necessitating early evaluation of their safety profiles and application boundaries[49,50].

Synergistic points for combination therapy

In melanoma, insufficient response and acquired resistance remain the primary bottlenecks; consequently, the strategic positioning of exosomal delivery leans toward synergistic action[51]. When combined with immune checkpoint inhibitors, the delivery of immunomodulatory molecules to improve immune infiltration and effector T-cell function theoretically offers the potential to increase response rates or delay resistance. Within the context of BRAF/MEK targeted therapies, compensatory interventions can be designed to address resistance-related pathways and post-treatment microenvironmental rebound. Furthermore, when integrated with local modalities such as radiotherapy, ablation, or photothermal/photodynamic therapy, exosomes may facilitate the transition from "local control" to "systemic immune response" by enhancing immunogenic cell death and antigen presentation[52-54]. The commonality among these synergistic pathways is that exosomal delivery is not intended to replace existing therapies; instead, it seeks to amplify the systemic benefits of established regimens through more refined interventions at the local or cellular level, with efficacy validated by quantifiable incremental gains[55].

Therapeutic effects and applicability boundaries of exosome-mediated delivery

Whether exosome-mediated delivery is truly viable *in vivo* depends on the controllability of its biodistribution and clearance[56]. In practice, uptake by the reticuloendothelial system often limits the effective dose reaching the tumor; furthermore, variations in membrane composition and surface molecular profiles resulting from different sources and preparation protocols may lead to biased tissue distribution and undermine inter-study reproducibility. Simultaneously, the non-inert nature of exosomes implies that they may exert unintended regulatory effects in non-target tissues, necessitating vigilance regarding disturbances

to immune homeostasis, particularly in the context of immunotherapy[57-59]. Moreover, the route of administration (e.g., intravenous, local injection, or subcutaneous/lymphatic pathways) has a decisive impact on exposure and safety, directly shaping its application scenarios and development strategies[56,57]. Consequently, effective exosomal delivery is not an inherent property; it requires a systematic trade-off between source selection, administration strategy, and downstream optimization to achieve relatively predictable *in vivo* performance[58].

In summary, as natural membranous nanocarriers, exosomes provide a versatile platform for melanoma delivery and combination therapy, owing to their biocompatibility, functionalized surfaces, and capacity to carry diverse types of cargo[55]. However, their clinical feasibility is collectively determined by *in vivo* drug distribution and clearance, immunological safety, and the consistency of large-scale manufacturing and quality control[54]. Consequently, a more rational translational positioning is to view exosomal delivery as a complementary component of combination therapy.

EXOSOME-MEDIATED REMODELING OF THE MELANOMA MICROENVIRONMENT

Melanoma, characterized by its high somatic mutational burden and pervasive epigenetic dysregulation, drives malignant progression primarily through mechanisms of immune evasion and metastatic dissemination[60-63]. Within this highly heterogeneous context, sEVs, as endogenous biological nanocarriers, are increasingly recognized as key mediators in regulating the TME in melanoma. Based on their nanoscale biological architecture, sEVs possess natural membranous encapsulation, stable circulation properties, and highly precise targeting capabilities, and are enriched with diverse bioactive molecules—including proteins, lipids, miRNAs, and long non-coding RNAs (lncRNAs)—thereby functioning both as platforms for signal transduction and as direct participants in structural and metabolic remodeling[64-67]. Tumor-derived sEVs from melanoma establish an immunosuppressive barrier, promote angiogenesis/lymphangiogenesis and pre-metastatic niche (PMN) formation, and drive ECM remodeling and metabolic reprogramming, thereby constructing a tumor microenvironment that sustains continuous tumor growth and metastasis[68]. Figure 2 illustrates the pleiotropic effects of tumor EVs in shaping the tumor microenvironment in melanoma, including the establishment of an immunosuppressive barrier [Figure 2A], the promotion of angiogenesis and lymphangiogenesis [Figure 2B], and ECM remodeling through stromal reprogramming [Figure 2C].

Establishment of an immunosuppressive barrier

sEVs-PD-L1-mediated T cell suppression and systemic immune evasion

The PD-1/PD-L1 axis is central to immune evasion in melanoma[69]. In melanoma, PD-L1 is not only expressed on the surface of tumor cells and multiple immune cell populations, but is also closely associated with responses to immune checkpoint inhibitors and clinical prognosis[70-73]. Recent studies have demonstrated that melanoma cells can deliver biologically active PD-L1 into the circulation via sEVs, thereby establishing a systemic "circulating immune checkpoint" and challenging the traditional notion that PD-L1 activity is confined to the local TME[74-77]. In metastatic melanoma models, PD-L1 displayed on the surface of tumor-derived exosomes can directly bind to PD-1 on $CD8^+$ T cells, suppress TCR signaling, reduce cytokine secretion, and impair cytotoxic functions, thereby promoting tumor progression[74]. Blockade of sEVs-PD-L1 has been shown to restore T cell activity *in vivo*, induce systemic antitumor immune responses, and establish immunological memory, highlighting its independent and critical role in global immune regulation[30].

Myeloid immune cell reprogramming and indirect immunosuppression

At the bone marrow stage, melanoma-derived EVs can directly act on normal immature myeloid cells (IMCs)[78]. Studies based on murine models have shown that danger-associated molecular patterns (DAMPs) carried by tumor EVs activate TLR4 signaling in IMCs, driving their differentiation into immunosuppressive

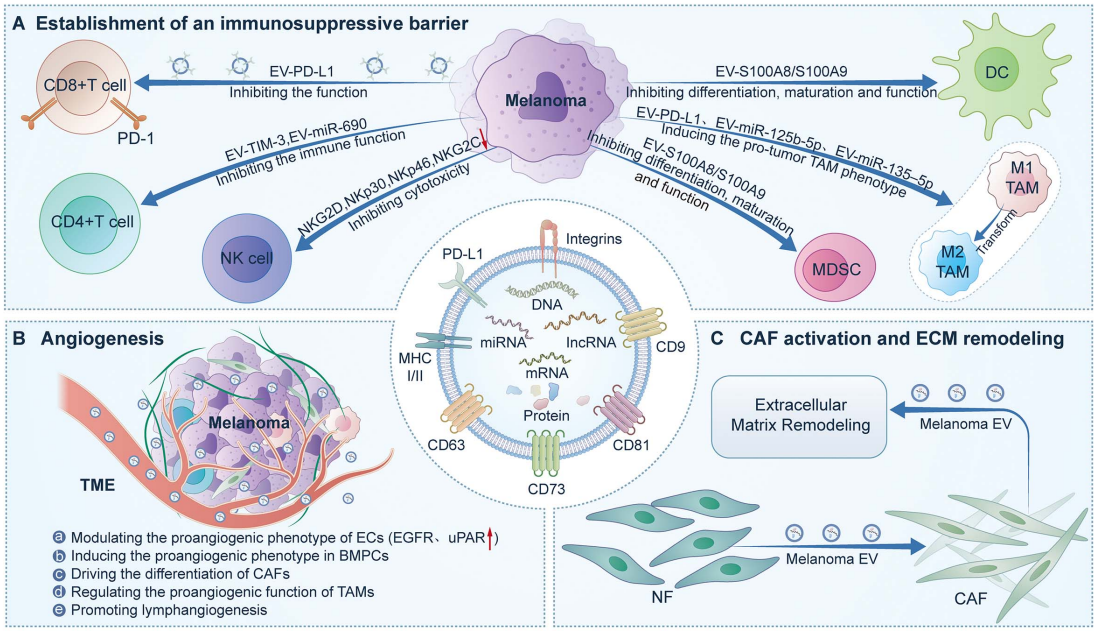

Figure 2. Pleiotropic roles of tumor-derived extracellular vesicles (EVs) in shaping the melanoma tumor microenvironment. (A) Tumor-derived EVs establish an immunosuppressive barrier through multiple mechanisms, including inhibition of CD8+ T-cell infiltration and function, impairment of CD4+ T-cell activity, suppression of natural killer (NK) cell cytotoxicity, induction of MDSCs, inhibition of DC maturation, and promotion of tumor-associated macrophage (TAM) polarization toward a pro-tumor M2-like phenotype. (B) Tumor-derived EVs promote angiogenesis and lymphangiogenesis by modulating endothelial cells (ECs), mobilizing bone marrow progenitor cells (BMPCs), driving CAF activation, and regulating pro-angiogenic TAM functions. (C) In melanoma, tumor-derived EVs contribute to CAF activation and ECM remodeling by promoting the transition of normal fibroblasts (NFs) into CAFs and by further enhancing matrix remodeling to support tumor progression. BMPC: Bone marrow progenitor cell; CAF: cancer-associated fibroblast; DC: dendritic cell; EC: endothelial cell; ECM: extracellular matrix; EV: extracellular vesicle; MDSC: myeloid-derived suppressor cell; NF: normal fibroblast; NK: natural killer; TAM: tumor-associated macrophage; TME: tumor microenvironment.

myeloid-derived suppressor cells (MDSCs) and markedly upregulating PD-L1 expression, thereby suppressing T cell activation and proliferation[78,79].

In addition, melanoma-derived EVs profoundly influence the differentiation and functional properties of antigen-presenting cells (APCs). Early studies have demonstrated that melanoma EVs inhibit the normal differentiation of monocytes into dendritic cells (DCs) and instead induce the formation of a $CD14^+$ immature myeloid cell population[80,81]. This process is partially dependent on the enrichment of TGF-β signaling within EVs, ultimately leading to impaired DC maturation, reduced antigen-presenting capacity, and compromised T cell proliferation and effector functions[81-83]. Tumor-derived exosomes isolated from the plasma of melanoma patients similarly drive APCs toward a suppressive phenotype, with their immunomodulatory effects closely associated with the enrichment of immunosuppressive factors such as TGF-β within exosomes[82,84-86].

In summary, melanoma-derived sEVs amplify and transform myeloid cell functional reprogramming that is initially confined to the local tumor site into a systemic, body-wide immunosuppressive effect through multilevel, cascade-like signal transmission and cellular reprogramming, thereby establishing a structurally stable and self-sustaining immunosuppressive barrier within the tumor microenvironment[87-89], as summarized in Table 1.

Promotion of angiogenesis and pre-metastatic niche (ecological niche) formation

For melanoma to achieve sustained proliferative expansion and distant metastasis, it must reconstruct its vascular and lymphatic networks and pre-establish a PMN in distant organs. Recent studies indicate that

Table 1. Cargo molecules in extracellular vesicles and their immunomodulatory functions

Cargo	Target cells	Mechanism of action	Citation
PD-L1	$CD8^+$ T cells	Inhibiting the function of $CD8^+$ T cells	[90]
PD-L1	$CD8^+$ T cells	Induce exhaustion of tumor-specific $CD8^+$ T cells	[91]
PD-L1	Macrophages	Leading to M2 macrophage polarization	[92]
miR-214	Macrophages	Inducing a proinflammatory activation of macrophages	[93]
miR-125b-5p	Macrophages	Inducing the pro-tumor TAMs phenotype	[94]
S100A8 and S100A9	DCs	Interfering with the process of DCs maturation	[80]
Multiple miRNAs	DCs	Inhibiting the function of DCs	[95,96]
miR-690	$CD4^+$ T cells	Induce apoptosis in $CD4^+$ T cells	[97]
TIM-3	$CD4^+$ T cells	Inhibiting the function of $CD4^+$ T cells	[98]
MICA	NK cells	Inhibiting the function of NK cells	[85]
Hsp86	MDSCs	Inducing the differentiation of MDSCs	[78,99]
Hsp70	MDSCs	Enhance the immunosuppressive function of MDSCs	[84]

TAMs: Tumor-associated macrophages; DCs: dendritic cells; NK cells: natural killer cells; MDSCs: myeloid-derived suppressor cells.

melanoma-derived sEVs systemically promote angiogenesis and lymphangiogenesis and participate in PMN remodeling by delivering pro-angiogenic factors, integrins, and diverse non-coding RNAs, thereby creating favorable conditions for metastatic dissemination.

Induction of angiogenesis

To meet the increased demand for oxygen and nutrients associated with rapid proliferation, metastatic cancer cells can induce angiogenesis, remodel pre-existing vasculature, and recruit bone marrow-derived cells (BMDCs). Peinado *et al.*[100,101] demonstrated that melanoma-derived exosomes confer a pro-angiogenic phenotype to BMDCs through horizontal transfer of the tyrosine kinase receptor MET, thereby promoting the formation of highly vascularized PMNs in organs prone to metastasis such as the lung[100,101]. Subsequent validation studies further confirmed that sEVs derived from B16F10 melanoma mildly promote the formation of lung and bone metastatic lesions; although no significant reduction in metastatic burden was observed following treatment with MET-downregulated sEVs, a decreasing trend was still evident, indicating an important regulatory role of MET in this process[102].

Induction of lymphangiogenesis and pre-metastatic microenvironment formation

In addition to the vascular system, melanoma relies heavily on lymphatic routes to achieve early metastatic dissemination. Clinical studies have demonstrated that lymphangiogenesis is significantly associated with sentinel lymph node metastasis and reduced disease-free survival (DFS) in melanoma patients[103]. Hood *et al.* were among the first to propose that melanoma-derived sEVs can be transported through lymphatic vessels to lymph nodes, where they remodel lymph node architecture prior to the arrival of tumor cells and guide melanoma cell migration toward "hotspot" regions enriched in tumor-derived exosomes[104].

García-Silva *et al.* further systematically elucidated the molecular mechanisms underlying sEV-mediated lymphangiogenesis, demonstrating that metastatic melanoma sEVs enriched in NGFR (p75NTR) are internalized by lymphatic endothelial cells, activating MAPK/ERK and NF-κB pathways to promote lymphangiogenesis[105]. Genetic ablation of NGFR or pharmacological inhibition of its signaling effectively suppresses lymph node metastasis and improves survival in murine models[105].

Driving extracellular matrix remodeling and metabolic reprogramming

Melanoma invasion and distant dissemination are highly dependent on the dynamic adaptation of the ECM and the metabolic microenvironment. By delivering ECM-modifying factors and metabolic regulators, sEVs enable bidirectional reprogramming of both stromal cells and tumor cells themselves.

ECM remodeling and the CAF network

Within the tumor microenvironment, sEVs have been demonstrated to induce the transdifferentiation of normal fibroblasts into cancer-associated fibroblasts (CAFs) and to extensively remodel the ECM[106,107]. Studies show that sEVs derived from B16F0 murine melanoma promote the upregulation of CAF markers, including α-smooth muscle actin (α-SMA) and fibroblast activation protein (FAP), in NIH/3T3 fibroblasts while significantly enhancing their migratory capacity[94,106]. In co-culture systems, fibroblasts conditioned by these sEVs further facilitate melanoma cell proliferation and migration[96,106].

Melanoma sEVs are enriched in multiple oncogenic microRNAs, including miR-21 and miR-155-5p. miR-21 suppresses the expression of tissue inhibitor of metalloproteinases-3 (TIMP3) while upregulating multiple matrix metalloproteinases (MMPs), thereby markedly enhancing ECM degradation[108,109]. In addition, membrane-type 1 matrix metalloproteinase (MT1-MMP), a key enzyme involved in matrix breakdown, has been identified in melanoma EVs released via a melanosome-associated secretory pathway, suggesting a melanoma-specific vesicle release mechanism tightly linked to ECM destruction[107,109,110].

Metabolic reprogramming

Metabolic reprogramming represents another hallmark of melanoma progression. sEVs induce a "reverse Warburg effect" in stromal cells: exosomal miR-155 and miR-210 reprogram fibroblasts toward aerobic glycolysis, producing metabolites like lactate to fuel tumor cell oxidative phosphorylation[111]. In this context, melanoma-derived sEVs are increasingly recognized as key mediators that induce and coordinate this “reverse” metabolic coupling between stromal and tumor compartments.

In reciprocal tumor-stroma communication, adipocyte-derived sEVs likewise exert profound effects on melanoma metabolism. Lazar *et al*. reported that adipocyte-derived sEVs enhance the migratory and invasive capacities of melanoma cells and are enriched in proteins associated with fatty acid oxidation (FAO)[112]. In the presence of adipocyte sEVs, intracellular FAO levels in melanoma cells are markedly increased, offering a metabolic explanation for the association between obesity and poor melanoma prognosis[112]. Subsequent studies further demonstrated that FAO-related proteins and their fatty acid substrates can be directly transferred to melanoma cells via sEVs, establishing a form of intercellular metabolic support[112,113]. Collectively, these findings indicate that melanoma not only remodels the tumor microenvironment through its own secreted sEVs but can also acquire and exploit sEVs derived from distant metabolic organs, such as adipose tissue, to obtain exogenous metabolic resources that fuel tumor progression.

EXOSOME-BASED BIO-NANOMATERIAL LIQUID BIOPSY PLATFORM

In recent years, exosomes have evolved from mere subjects of research into practical tools for liquid biopsy. Particularly in melanoma, various cell types—including tumor cells, cancer-associated fibroblasts, and immune cells—release exosomes in significant quantities. These vesicles carry enriched miRNA, proteins, and DNA fragments, which contribute to upregulating invasion and metastasis pathways, stromal remodeling, and immune suppression. As EV research advances, growing evidence suggests that the application of exosomes in melanoma extends beyond tumor monitoring to include the evaluation of immunotherapy efficacy and the tracking of minimal residual disease[114,115]. Representative EV isolation strategies and cross-tumor EV liquid-biopsy examples are summarized in Figure 3[116-118], including

commonly used EV isolation workflows from blood samples [Figure 3A], a representative cerebrospinal fluid-derived EV liquid-biopsy study in glioblastoma [Figure 3B], and a representative serum exosome-based biomarker analysis in lung cancer [Figure 3C].

From a nanomaterials perspective, melanoma-associated EVs represent a class of bio-nanoparticles actively released by tumor and immune cells. They typically range from 30 to 150 nm in diameter, are enveloped by a lipid bilayer, and express various surface molecules such as integrins, immune checkpoint molecules (e.g., PD-L1), and glycoproteins[108,119]. Their cargo includes encapsulated DNA fragments, mRNA, miRNA, lncRNA, as well as various functional proteins and lipids. Through intercellular communication, these EVs are involved in tumor invasion, metastasis, stromal remodeling, and the formation of an immunosuppressive microenvironment. They are recognized as key mediators in systemic tumor-host interactions[120,121]. An overview of the biological sources, sampling routes, downstream analytical workflow, proximal ocular EV sampling, nano-immune fingerprinting, ctDNA/ctEV complementarity, and clinical application continuum is shown in Figure 4A.

Nanoscale EV platforms and immunotherapy monitoring

Sample and methodological considerations for nanoscale liquid biopsy

As a class of bio-nanomaterials, exosomes are highly sensitive to sample processing conditions and detection platforms[23]. Regarding EV isolation techniques, while ultracentrifugation remains the standard method, approaches more commonly adopted in clinical settings include SEC, polymer-based precipitation, ultrafiltration, and immunoaffinity capture. Each method has its advantages and limitations, and the choice should be based on research objectives and clinical practicality[122].

In addition to peripheral blood-based sampling, ocular biofluids also provide clinically relevant proximal sources of tumor-derived vesicles in uveal melanoma, as illustrated in Figure 4B.

Dynamic readouts of exo-PD-L1

PD-L1, which is abundantly present in exosomes (exo-PD-L1), has been demonstrated to play a significant role in immunotherapy. PD-L1 is the target of immune checkpoint inhibitors, such as PD-1 inhibitors. Exo-PD-L1 can be regarded as an extension of the PD-1/PD-L1 axis, forming a systemic immunosuppressive layer in bodily fluids. This layer weakens the function of $CD8^+$ T cells and promotes tumor immune evasion[74].

The functional role of exo-PD-L1 as a decoy implies that PD-L1 molecules on exosomes can competitively bind to the PD-1 receptor, thereby inhibiting the immune system's attack on the tumor. This mechanism may explain why some melanoma patients with high plasma PD-L1 levels still exhibit poor responses to immunotherapy. Although the efficacy of immune checkpoint inhibitors theoretically relies on PD-1/PD-L1 binding, exo-PD-L1 may interfere with this effective immune response through its decoy function.

With ongoing clinical research, the dynamic changes in exo-PD-L1 levels are increasingly recognized as a real-time monitoring indicator that more accurately reflects the systemic immune state. Multiple cohort studies have found that plasma exo-PD-L1 levels are closely associated with tumor burden, degree of immunosuppression, and clinical outcomes. High baseline levels are often observed in patients with greater tumor burden and poorer prognosis, while a significant decline during early treatment (approximately 2-6 weeks) is commonly seen in patients who achieve objective responses or disease control[123].

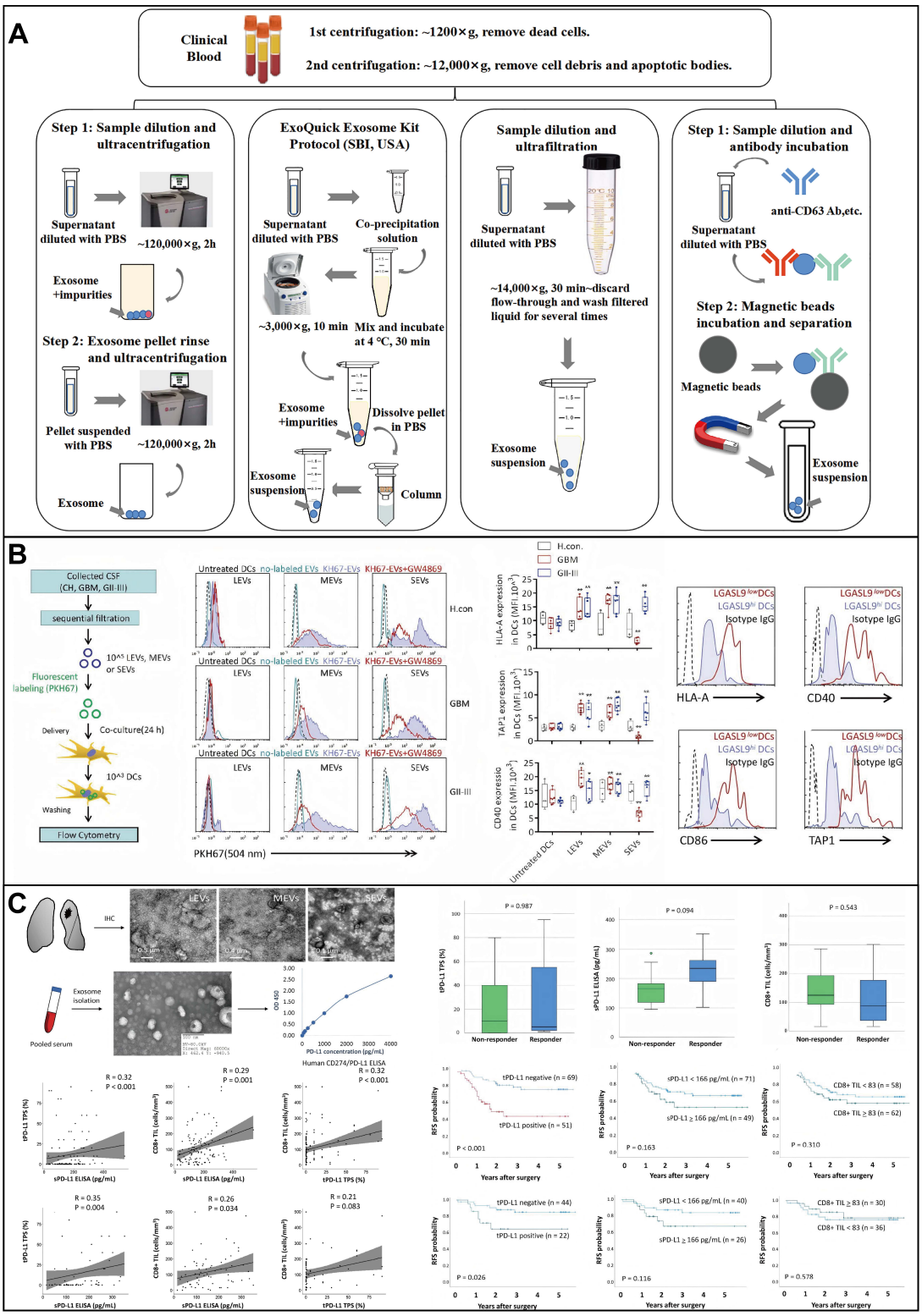

Figure 3. Liquid-biopsy sampling and isolation of exosomes/small extracellular vesicles (sEVs), with representative applications in glioblastoma and lung cancer. (A) Overview of commonly used EV isolation strategies from clinical blood samples, including differential ultracentrifugation, polymer-based precipitation, ultrafiltration, and immunoaffinity capture. Reprinted with permission[116]. Copyright 2022, Frontiers. (B) Representative glioblastoma study using cerebrospinal fluid (CSF)-derived EV subfractions for liquid biopsy and immunomodulatory analysis. Reprinted with permission[117]. Copyright 2020, Springer Nature. (C) Representative lung cancer study using serum-derived exosomes for morphological validation and immune-related biomarker analysis, including exosomal PD-L1 evaluation and correlation with tumor immune features and clinical outcomes. Reprinted with permission[118]. Copyright 2021, Springer Nature. CSF: Cerebrospinal fluid; EVs: extracellular vesicles; PD-L1: programmed death-ligand 1.

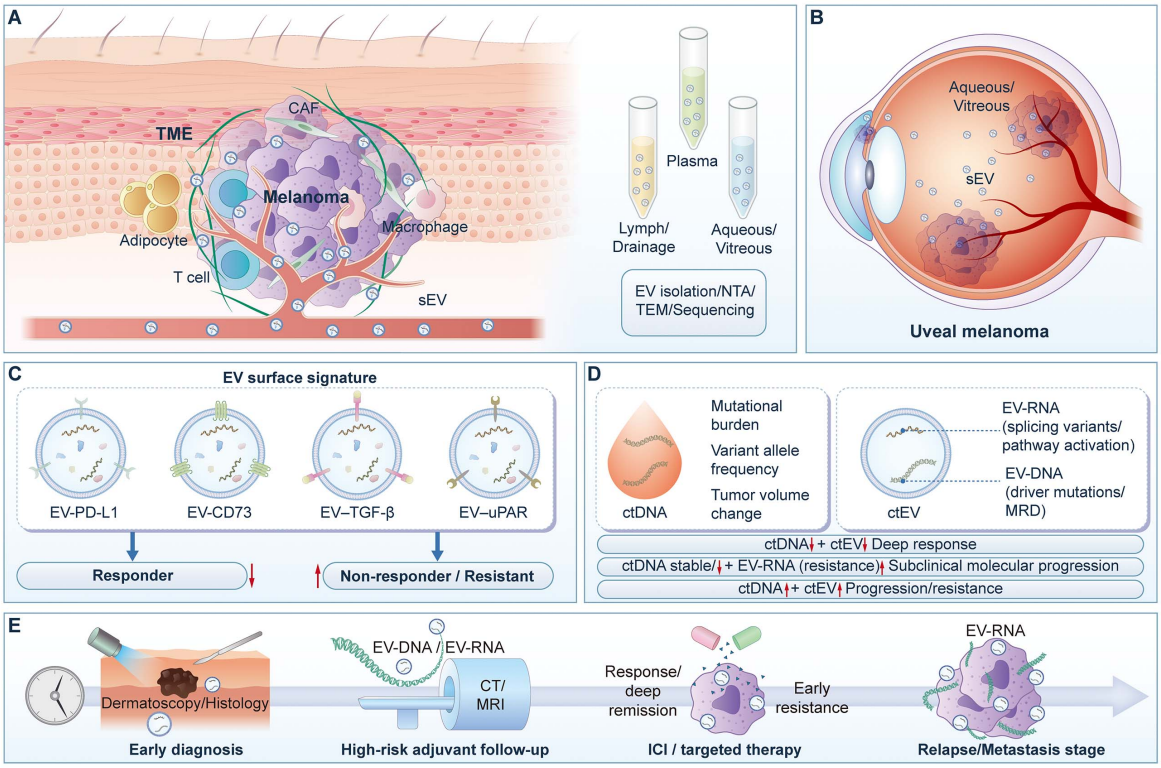

Figure 4. Structure and applications of melanoma EVs as bio-nanomaterial liquid-biopsy platforms. (A) Release of EVs from a heterogeneous melanoma TME into accessible biofluids, including plasma, lymphatic/drainage fluid, and ocular fluids, followed by downstream isolation and characterization workflows such as nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and sequencing. (B) Schematic illustration of uveal melanoma and tumor-derived small extracellular vesicles (sEVs) in aqueous and vitreous humor, supporting ocular fluid-based EV sampling. (C) Representative EV surface "nano-immune fingerprint" composed of immunoregulatory markers, including EV-programmed death-ligand 1 (EV-PD-L1), EV-CD73, EV-transforming growth factor-β (EV-TGF-β), and EV-urokinase-type plasminogen activator receptor (EV-uPAR), for stratification of responders and non-responders/resistant cases. (D) Complementary information framework comparing circulating tumor DNA (ctDNA) with circulating tumor EVs (ctEVs), including EV-DNA and EV-RNA, for evaluation of mutational burden, molecular residual disease, functional transcripts, pathway activation, treatment response, and molecular progression/resistance. (E) Clinical continuum of EV-based applications across melanoma management, including early diagnosis, adjuvant surveillance, on-treatment monitoring during immune checkpoint inhibitor or targeted therapy, and relapse/metastasis detection. ctDNA: Circulating tumor DNA; ctEV: circulating tumor extracellular vesicle; EVs: extracellular vesicles; NTA: nanoparticle tracking analysis; TEM: transmission electron microscopy; TME: tumor microenvironment; uPAR: urokinase-type plasminogen activator receptor.

Multiparameter analysis of nano-immunosuppressive axis: CD73, TGF-β, and uPAR

Beyond exo-PD-L1, several other immunosuppressive biomarkers detectable in exosomes provide valuable insights into the immune microenvironment. Taking EV-CD73 as an example, CD73 is a key molecule in the adenosinergic immunosuppressive pathway[124]. By catalyzing the conversion of AMP to adenosine, CD73 inhibits effector T cell function while promoting the activity of regulatory T cells (Tregs) and MDSCs[125]. This mechanism plays a significant role in shaping the immunosuppressive tumor microenvironment[126]. Studies have shown that the expression of $CD73^+$ EVs in the plasma of melanoma patients is significantly higher than in healthy controls, and their adenosine-generating capacity can suppress lymphocyte proliferation and IFN-γ secretion[127].

More importantly, clinical research has found that high or increasing levels of $CD73^+$ EVs before or during early treatment in patients receiving PD-1 inhibitors are associated with treatment resistance and poorer prognosis[127]. Therefore, $CD73^+$ EVs may serve as an early biomarker predictive of resistance and represent a potential therapeutic target for combination strategies, such as co-administration with A2A/A2B receptor antagonists, to enhance the efficacy of PD-1 inhibitors[128].

TGF-β-associated exosomes are another important marker in the immune microenvironment. TGF-β promotes fibrotic stromal remodeling, Treg recruitment, and exhaustion of effector T cells, mechanisms closely linked to immune escape and an immunosuppressive phenotype[129]. Multiple studies indicate that high levels of EV-TGF-β before or early in treatment suggest limited benefit from immunotherapy and poorer outcomes. Evidence supports including EV-TGF-β in risk stratification models for immunotherapy[87,130].

uPAR$^+$ EVs are strongly associated with tumor invasiveness and metastatic potential[131]. Research has found that in patients with metastatic melanoma, early increases in uPAR$^+$ EV levels during treatment are significantly correlated with drug resistance and shorter overall survival (OS). These exosomes, taken up by endothelial cells, can promote angiogenesis and increase vascular permeability, thereby facilitating conditions for tumor metastasis[132].

Therefore, combined assessment of biomarkers such as EV-CD73, EV-TGF-β, and EV-uPAR allows for a more precise characterization of the immunosuppressive microenvironment and identification of resistance mechanisms during immunotherapy[133]. Particularly in the course of treatment, when persistently high or rising exo-PD-L1 levels coincide with upregulation of EV-CD73 and/or EV-TGF-β, and ctDNA levels do not decrease or show signs of rebound, this pattern may indicate a high risk of immune escape and treatment resistance [Figure 4C][87,127,134].

EV-DNA and EV-RNA: driver mutation detection and pathway activity monitoring

EV-DNA

EV-DNA refers to tumor-derived DNA fragments encapsulated within nanoscale vesicles. Its primary advantages include high stability due to protective encapsulation and a relatively high tumor-origin fraction. Compared to cell-free DNA (cfDNA), EV-DNA can more effectively preserve tumor-specific genetic information[135,136]. This protection makes EV-DNA a more stable analyte for liquid biopsy applications, particularly advantageous for detecting low-frequency mutations[88].

Furthermore, EV-DNA can be used for confirming mutational profiles before and after targeted therapy and for tracking clonal evolution. Especially in patients where repeat tissue biopsies are challenging, EV-DNA serves as a complementary source to ctDNA, improving the success rate of mutation detection. During long-term targeted therapy, it can help identify newly emerging resistant clones. For example, in some patients undergoing targeted therapy, rapid changes in the mutational profile detected in EV-DNA may indicate the emergence of new resistance mechanisms, information which ctDNA alone might fail to capture in a timely manner[89].

EV-RNA

In contrast to EV-DNA, which focuses on the detection of structural mutations, EV-RNA primarily conveys functional information[137]. EV-RNA, including mRNA, miRNA, and lncRNA, reflects transcriptional activity within tumor cells and offers unique advantages in tracking non-mutational resistance mechanisms. Unlike ctDNA, which lacks functional readouts, EV-RNA can reveal tumor-associated changes such as transcription factor activation and gene splice variants, which are part of the adaptive evolution of cancer cells[138].

Through the analysis of EV-RNA, clinicians can identify key pathways involved in immune escape and detect gene splice variants at an early stage, enabling timely adjustment of treatment strategies to prevent the further development of resistance. Unlike ctDNA, EV-RNA captures more complex functional adaptations, illustrating how tumor cells may overcome therapeutic pressure through transcriptional regulation[138].

Table 2. Comparison of ctDNA and exosome-related biomarkers (EV-DNA, EV-RNA, and EV-proteins)

Category	ctDNA	EV-DNA	EV-RNA	EV-proteins	Representative references
Main biological information	Mutation spectrum, clonal evolution, tumor burden, copy-number changes	Partial genomic alterations, MRD, and tumor-derived DNA information	Transcriptional activity, splice variants, functional states, and resistance-related pathways	Phenotypic states, immune microenvironment, membrane-molecule expression, and intercellular communication features	Medhin *et al.*, 2023[114]
Information attribute	More structural information	Structural information combined with vesicle-protection features	More functional information	More phenotypic and microenvironmental information	Tsering *et al.*, 2024[141]
Stability	Susceptible to fragmentation and degradation	Relatively more stable because of vesicular membrane protection	More stable than free RNA because of vesicular protection	Membrane proteins and intravesicular proteins retain a certain degree of protection	Tsering *et al.*, 2024[141]
Detection methods	ddPCR, NGS, targeted sequencing	ddPCR, PCR, sequencing	qPCR, ddPCR, RNA-seq	ELISA, Western blot, flow cytometry, mass spectrometry, ExoView	Slusher *et al.*, 2024[17]
Advantages	Relatively mature clinical foundation; suitable for monitoring mutations and tumor-burden changes	May complement blind spots of ctDNA detection and may be advantageous in some low-abundance settings	More sensitive to immune escape, resistance-associated transcriptional regulation, and functional changes	Can directly reflect surface markers and microenvironmental status	Medhin *et al.*, 2023[114]
Limitations	Primarily reflects genomic-level information, with limited functional-state readouts	Insufficient standardization; isolation and quantification are strongly method-dependent	Large platform-related variation; preprocessing and normalization are complex	Quantitative consistency and cross-platform comparability remain limited	Slusher *et al.*, 2024[17]
Potential applications in melanoma	Monitoring BRAF/NRAS mutations, tumor burden, and recurrence	MRD assessment, supplementation of structural-variation information, and combined monitoring	Early resistance detection, immune-state assessment, and functional pathway changes	Diagnosis, staging, immunotherapy-response evaluation, and prognostic stratification	Medhin *et al.*, 2023[114]
Most suitable clinical scenarios	Treatment monitoring, recurrence warning, and tracking clonal evolution	Combined with ctDNA to improve capture of structural information	Monitoring functional resistance and immune escape	Assessing tumor phenotype and microenvironmental status	Slusher *et al.*, 2024[17]
Clinical positioning	A relatively mature component of liquid biopsy	Still in development	High research and translational potential	Suitable as a complementary functional biomarker	Shen *et al.*, 2023[142]
Overall assessment	Best suited to provide a structural map of the tumor	Complements the structural-information layer	Complements the functional-information layer	Complements the phenotypic/microenvironmental layer	

ctDNA and ctEV

ctDNA and circulating tumor-derived extracellular vesicles (ctEV, encompassing EV-DNA and EV-RNA) together form a multi-dimensional liquid biopsy framework with significant potential for tracking resistance mechanisms, monitoring minimal residual disease (MRD), and evaluating immunotherapy efficacy[139]. ctDNA primarily provides structural information such as tumor mutational burden, clonal expansion, and copy number variations. In contrast, ctEV, via its nanovesicular carriers, integrates DNA, RNA, and membrane protein-based functional biomarkers, thereby supplementing information on functional states such as immune evasion and metastatic potential [Figure 4D][108,140].

The major differences and complementary features of ctDNA and exosome-related biomarkers are summarized in Table 2.

Diagnosis, staging, and differentiation of borderline lesions

EV-miRNA in early diagnosis and staging

EV-miRNAs are recognized as biomarkers encapsulated within nanoscale vesicles, holding significant promise for early diagnosis and staging of melanoma[143]. Utilizing high-throughput screening and machine learning, multiple studies have identified EV-miRNA panels capable of distinguishing melanoma from benign pigmented lesions, as well as early-stage from late-stage disease[144]. For instance, one study reported that a plasma-based EV-miRNA panel comprising miR-200c-3p, miR-144-3p, and miR-221-3p demonstrated favorable AUC values for melanoma diagnosis and staging. Notably, it showed high sensitivity and specificity (AUC > 0.85) for early-stage detection[144].

EV-proteins: elevating traditional serum biomarkers to the nanoscale

EV-proteins represent a nanoscale extension of traditional serum biomarkers, offering significant diagnostic and prognostic value. For example, classical melanoma markers such as S100B and melanoma inhibitory activity (MIA) have been detected in circulating EVs from patients with advanced melanoma, suggesting their potential utility as EV-based biomarkers[108,145]. Studies indicate that EV-S100B achieves an AUC value of approximately 0.85 in melanoma diagnosis, highlighting the advantage of this nanoscale protein biomarker[146].

Furthermore, research on communication proteins like EV-Connexin-43 demonstrates that their expression levels are significantly correlated with DFS and OS in melanoma[142]. ROC analysis shows that EV-Connexin-43 yields an AUC of about 0.77-0.78, supporting its potential as a supplementary marker for prognostic stratification and treatment intensity decisions[142]. Through small EV proteomics, researchers continue to identify candidate signature panels associated with metastasis risk and immune evasion—including adhesion molecules, cytoskeletal proteins, and metabolic enzymes—providing features for multi-marker panels and machine learning models[124].

Special lineages and proximal biofluids: the signal-to-noise advantage of nanoscale readouts

In special lineages of melanoma, such as uveal melanoma, EVs isolated from tumor-proximal biofluids (e.g., aqueous humor, vitreous) often carry a high abundance of tumor-associated information[147]. While the proteomic and miRNA signatures of these EVs overlap with those in peripheral blood, their enrichment is significantly higher. This confers a considerable advantage for "proximal nanoscale readouts" of localized lesions[148]. Studies show that EV concentrations in tumor-proximal fluids are typically greater than in peripheral blood, substantially improving the tumor signal-to-background noise ratio. This makes them particularly suitable for diagnosis, post-operative assessment of residual disease, and recurrence monitoring[149].

Multimodal integration: complementarity of nanoscale liquid biopsy with ctDNA and imaging

In summary, nanoscale readouts such as EV-miRNA and EV-proteins primarily reflect the phenotypic characteristics and microenvironmental state of tumors, whereas ctDNA conveys information on mutational burden and clonal expansion. By integrating these with imaging and traditional serum biomarkers, a multimodal framework encompassing "structure + function + behavior" can be constructed, enabling more precise whole-course patient management [Figure 4E][150]. In practical clinical pathways, EV-based liquid biopsy is likely to be initially adopted as a complementary tool alongside ctDNA and conventional markers within high-level medical centers. Its incremental value at specific clinical decision points can then be progressively validated through real-world cohort studies[151].

The combined analysis of EV-miRNA, EV-proteins, and ctDNA offers significant advantages in addressing clinical challenges. For instance, EV-miRNA can aid in distinguishing melanoma from benign lesions,

EV-proteins enhance the diagnosis of advanced tumors, and ctDNA provides insights into mutational burden and clonal dynamics. Integrating these layers of information allows for more accurate early diagnosis, treatment stratification, and recurrence monitoring. Particularly in scenarios where imaging and traditional serum biomarkers yield ambiguous results, EVs liquid biopsy delivers additional clinical value, holding broad application prospects in early melanoma detection and the dynamic monitoring of immunotherapy response[152].

APPLICATIONS OF ENGINEERED EXOSOMES IN CUTANEOUS MELANOMA: CARGO LOADING, TARGETED DELIVERY, AND MULTIMODAL COMBINATION STRATEGIES

Properties and limitations of native exosomes

Melanoma is among the most aggressive cutaneous malignancies, and once it progresses to stage IV (metastatic disease), the 5-year survival rate drops to approximately 16%[153]. Native exosomes exhibit limited penetration into subcutaneous tumors, which is insufficient to meet therapeutic demands, and their practical application in melanoma therefore remains constrained by multiple challenges.

First, the purification and large-scale production of exosomes still face significant technical bottlenecks. Currently used isolation techniques—including ultracentrifugation, ultrafiltration, and immunoaffinity-based methods—are difficult to simultaneously satisfy pharmaceutical-grade requirements in terms of yield, purity, and preservation of biological activity[154]. Second, exosome stability severely limits their long-term storage and transportation. A study on exosomes derived from umbilical cord blood mesenchymal stem cells (UCB-MSCs) reported a 15%-20% loss of biological activity during production processes compliant with Good Manufacturing Practice (GMP) standards[155]. In addition, native exosomes generally exhibit low loading efficiency for gene therapeutics, RNA molecules, and conventional chemotherapeutic agents, highlighting the urgent need for more efficient and controllable cargo-loading strategies to improve delivery efficiency and therapeutic efficacy[156,157].

Moreover, surface chemical modification or the loading of exogenous bioactive agents may alter the native immunological properties of exosomes, potentially exposing immunosuppressive molecules or reshaping the local immune microenvironment, thereby eliciting unintended immunosuppressive or inflammatory responses[158-160].

Taken together, although native exosomes possess notable advantages as nanocarriers in terms of biocompatibility and functional versatility, their intrinsic limitations in purification efficiency, structural stability, cargo-loading capacity, and biosafety have driven increasing interest in engineering strategies aimed at overcoming key barriers to their application in tumor therapy and related fields.

Core objectives of engineering strategies

The central objective of engineered exosomes is to overcome the intrinsic limitations of native exosomes in practical applications by systematically enhancing targeting specificity, cargo-loading efficiency, and biosafety, thereby maximizing their value in disease diagnosis and therapy. As summarized in Figure 5, this includes representative cargo-loading strategies [Figure 5A], surface functionalization approaches for improving targeting specificity [Figure 5B], induction of tumor cell apoptosis through engineered exosomal delivery [Figure 5C], remodeling of the tumor immune microenvironment [Figure 5D], and theranostic co-delivery of therapeutic agents and imaging tracers [Figure 5E].

Classification and representative examples of exosomal drug-loading technologies

Exosomal drug-loading technologies can be broadly categorized into two main approaches: passive loading and active loading. These two strategies differ in terms of the types of therapeutics they are suitable for, operational complexity, and their impact on exosomal structural integrity and biological function[161].

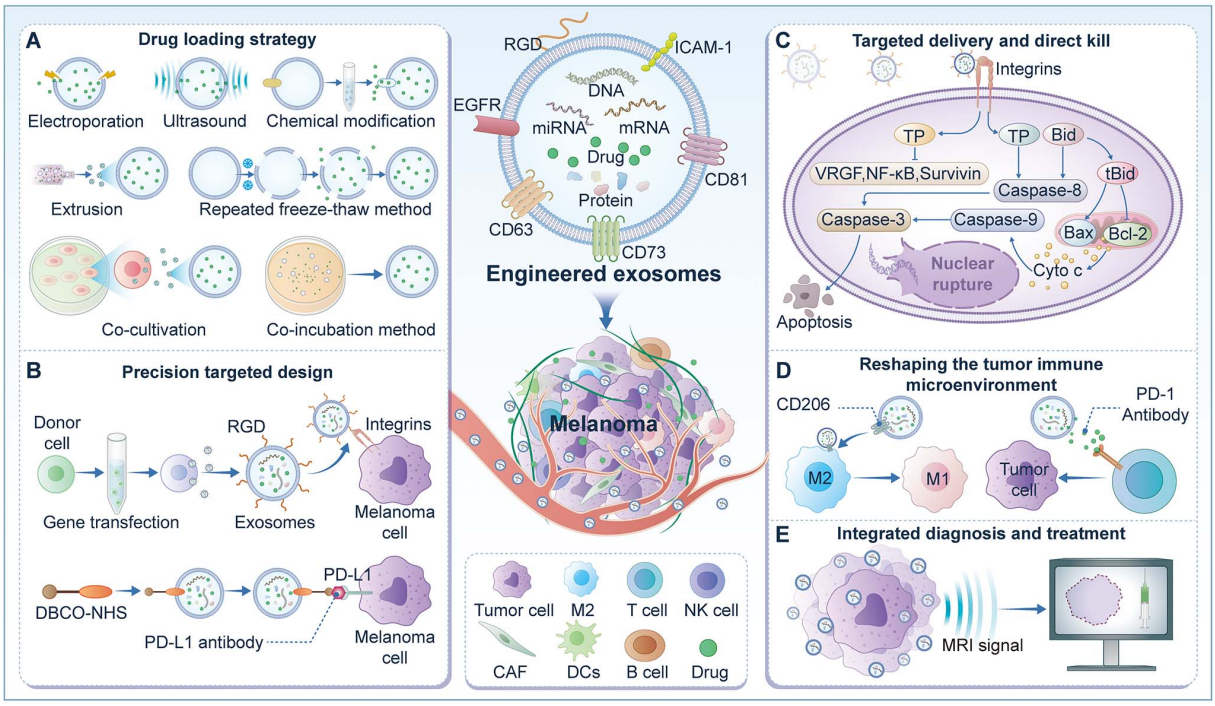

Figure 5. Engineering strategies for enhancing exosome drug loading, targeting, and therapeutic function. (A) Representative drug-loading strategies for engineered exosomes. (B) Surface functionalization approaches for improving targeting specificity. (C) One mechanism by which engineered exosomes induce tumor cell death, in which triptolide-loaded exosomes activate the intracellular caspase-3 pathway and trigger apoptosis. (D) Exosome-mediated remodeling of the tumor microenvironment through modulation of immune cells and immune-related molecules, including macrophages and T cells. (E) Co-delivery of therapeutic agents and imaging tracers by engineered exosomes for tumor-targeted theranostics. TP: Triptolide.

Passive loading strategies

Passive loading strategies rely on the natural interactions between drug molecules and exosomes or endogenous cellular pathways, offering operational simplicity but generally lower loading efficiency, and mainly include co-incubation and co-culture approaches. Representative examples are shown in Figure 6A-C (tumor-derived EV-based doxorubicin delivery and antitumor evaluation), Figure 6D and E (engineered blood exosome-based gene/chemo co-delivery characterization), and Figure 6F-M (producer-cell engineering/co-culture-associated loading strategies using TRAIL-expressing and triptolide-loaded engineered exosomes)[162-164].

Co-incubation is one of the simplest passive loading methods, in which exosomes are incubated with therapeutic agents under defined conditions to enable drug encapsulation via diffusion, lipid membrane fusion, or transmembrane transport mechanisms[165]. In melanoma therapy, doxorubicin-loaded exosomes generated by co-incubation exhibit favorable biocompatibility and low immunogenicity, thereby reducing systemic toxicity while enhancing drug accumulation at tumor sites[166]. Nevertheless, the drug-loading capacity of this method is limited and remains inefficient for macromolecular or hydrophilic therapeutics. For instance, when doxorubicin was loaded into HBc virus-like particle nanoparticles, incubation at 70 °C for 90 min achieved high loading efficiency, highlighting temperature as a critical determinant of encapsulation efficiency[167].

Technological advances in targeted delivery strategies

The innate tropism of exosomes primarily depends on the characteristics of the donor cells and generally lacks high efficiency and specificity toward diseased tissues. In the context of cutaneous melanoma therapy, this limitation significantly constrains both therapeutic efficacy and clinical applicability. Consequently,

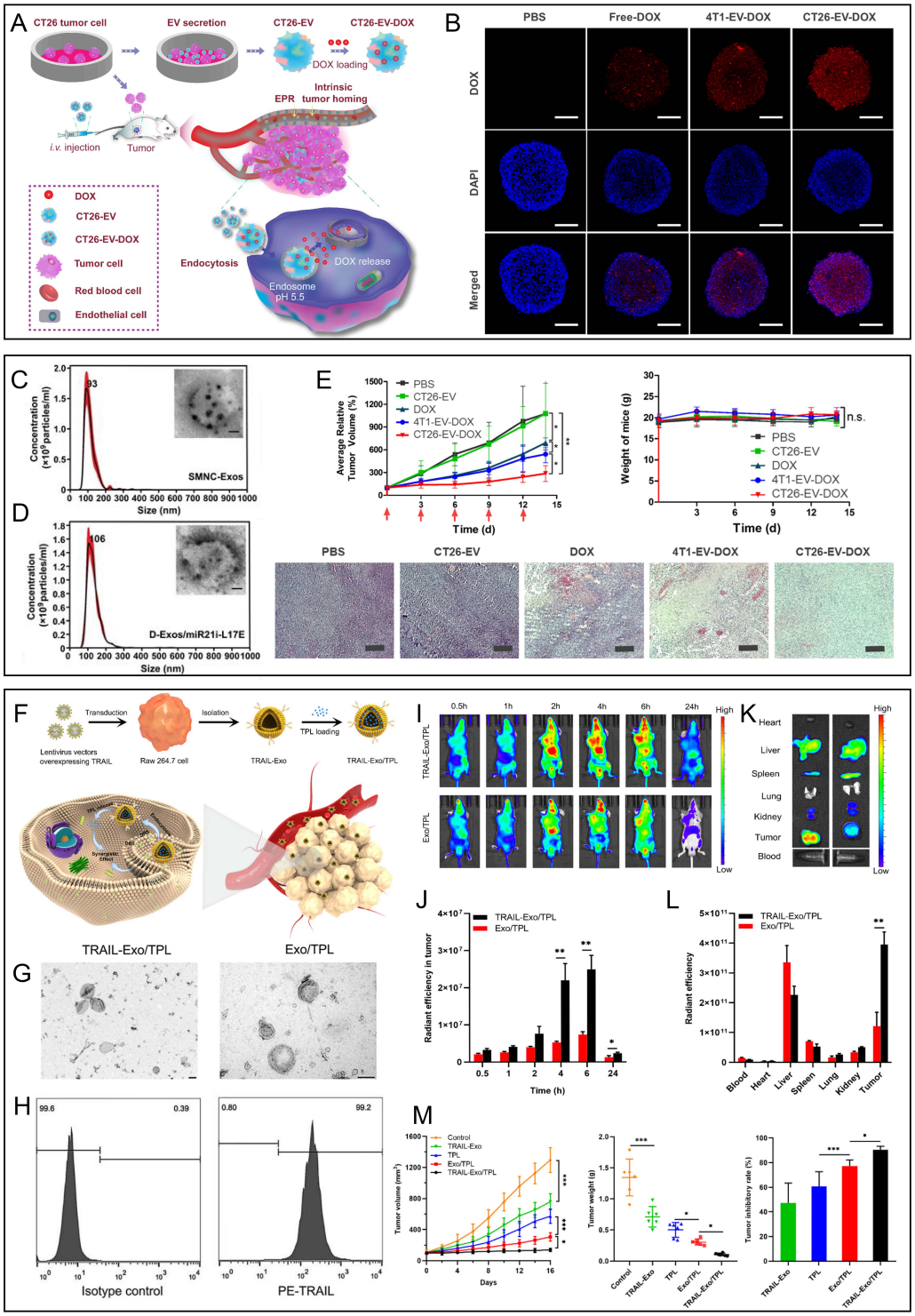

Figure 6. Representative studies of engineered exosome-based drug delivery, cellular uptake, biodistribution, and antitumor efficacy. (A, B, E) Tumor-derived extracellular vesicles loaded with doxorubicin for systemic delivery, tumor accumulation, three-dimensional tumor-spheroid penetration, and *in vivo* therapeutic evaluation. Scale bars: 200 μm in (B, E). Reprinted with permission[162]. Copyright 2022, Taylor & Francis. (C and D) Characterization of engineered blood exosomes and D-Exos/miR21i-L17E for tumor-targeting gene/chemo combination therapy. Reprinted with permission[163]. Copyright 2020, Ivyspring International Publisher. (F-M) Generation of TRAIL-expressing exosomes and triptolide-loaded engineered exosomes, together with ultrastructural characterization, surface-marker validation, *in vivo* imaging, biodistribution, and antitumor efficacy assessment. (G) scale bar: 100 nm. (J-M) $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Reprinted with permission[164]. Copyright 2021, American Chemical Society. DOX: Doxorubicin; TEM: transmission electron microscopy; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; TPL: triptolide.

engineering strategies have focused on endowing exosomes with the ability to actively recognize and accumulate in melanoma cells or within the tumor microenvironment, which has become a major research focus in recent years. Current technological progress mainly concentrates on three directions: engineering of donor cells, direct functional modification of exosomes, and the design of intelligent targeting systems.

Donor cell engineering-based targeting strategies

This strategy involves genetically modifying donor cells (e.g., MSCs, HEK-293T cells) to stably express fusion proteins composed of targeting elements and exosomal membrane proteins such as Lamp2b or CD63, thereby enabling the continuous production of targeted exosomes at the source[168-170]. Its advantages include uniform targeting functionality and avoidance of potential carrier damage during subsequent purification.

To target integrin αvβ3, which is overexpressed on melanoma cell surfaces, researchers fused the high-affinity cyclic RGD (cRGD) peptide with the extracellular domain of lysosome-associated membrane protein 2B (Lamp2b), successfully generating targeted engineered exosomes [Figure 7A-F][171]. In B16-F10 melanoma-bearing mouse models, iRGD-modified exosomes loaded with doxorubicin exhibited over threefold higher fluorescence intensity at tumor sites compared to unmodified exosomes, accompanied by significant tumor growth inhibition, prolonged survival, and markedly reduced systemic toxicity[169,172,173].

Direct functionalization of exosomes

Direct functionalization of exosomes refers to a class of engineering strategies in which targeting molecules or functional moieties are anchored onto the exosomal membrane after isolation and purification through chemical or physical approaches. This method does not require genetic modification of donor cells, offering high modularity, operational flexibility, and broad applicability, making it widely adopted for constructing precise exosome delivery systems.

Chemical conjugation is one of the most established direct modification methods. It primarily exploits reactive functional groups exposed on exosomal membrane proteins or lipids, such as amino, carboxyl, or thiol groups, to covalently link targeting ligands or functional molecules[174]. Bioorthogonal click chemistry (BCC), such as azide-alkyne cycloaddition, is considered an ideal tool for exosome surface modification due to its mild reaction conditions, high selectivity, and excellent biocompatibility[175]. For example, using copper-free BCC, researchers metabolically engineered donor cells to produce surface-modified EVs, which demonstrated effective tissue targeting and accumulation in rheumatoid arthritis and various tumor models, including breast, colon, and prostate cancers[176]. In melanoma, exosomes can potentially be functionalized via DBCO-NHS chemistry on their surface and subsequently conjugated with azide-modified PD-L1 antibodies, generating exosomes that target PD-L1-overexpressing melanoma cells. This strategy blocks the PD-1/PD-L1 immune checkpoint while enabling specific drug accumulation within tumor cells, enhancing the synergistic efficacy of immune checkpoint inhibitors and targeted therapeutics[177-180]. Importantly, this approach preserves the native targeting properties and bioactivity of exosomes while avoiding structural or functional damage to the exosomal membrane, making it suitable for *in vivo* imaging and precise therapy[122].

Additionally, Bolaños *et al*. conjugated gold nanorods to B16F-derived exosomes and chemically modified them with methotrexate (MTX). Leveraging MTX's high affinity for folate receptors overexpressed in melanoma cells, this engineered system achieved a gold accumulation in tumor tissue of 5.5% of the injected dose, compared with ~1% using conventional nanodelivery systems[181]. Consistent with the updated Figure 7G-J, Duan *et al*. developed a pYEEIE peptide-functionalized Rhodiola rosea-derived exosome-like nanovesicle system for targeted doxorubicin delivery, in which the pYEEIE ligand and RELN carrier were used to enhance melanoma-targeted accumulation and antitumor efficacy [Figure 7G-J]. Similarly,

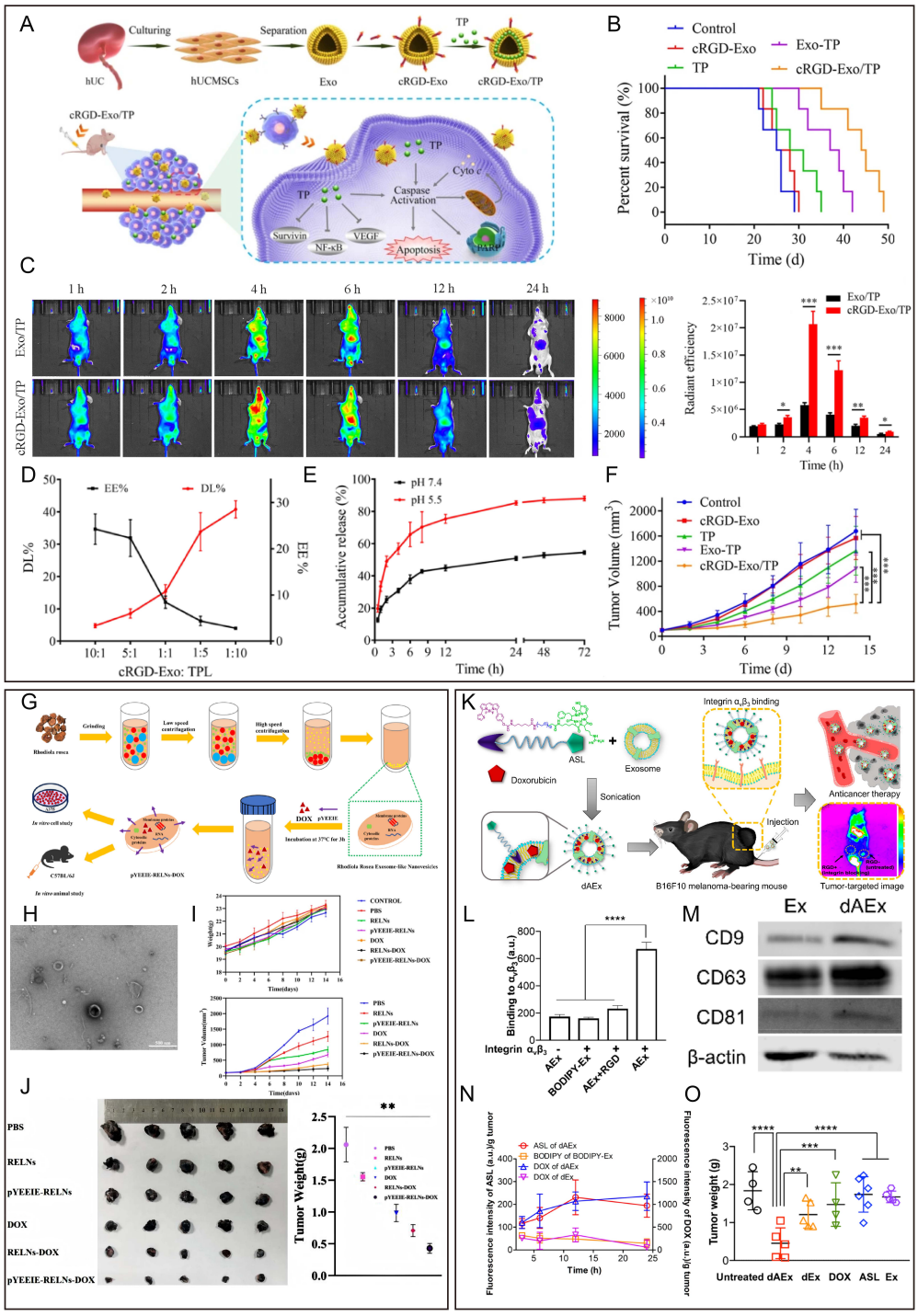

Figure 7. Strategies for achieving exosome-targeted delivery in melanoma therapy. (A-F) Engineering of human umbilical cord mesenchymal stem cell-derived exosomes with cyclic arginine-glycine-aspartic acid (cRGD) peptide to encapsulate triptolide, thereby constructing a biomimetic targeted drug delivery system (cRGD-Exo/TP), with evaluation of survival, biodistribution, drug loading, encapsulation efficiency, drug release, and *in vivo* antitumor efficacy. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Reprinted with permission[171]. Copyright 2022, Springer Nature. (G-J) Construction of pYEEIE peptide-functionalized Rhodiola rosea-derived exosome-like nanovesicles loaded with doxorubicin (pYEEIE-RELNs-DOX), including preparation workflow, ultrastructural characterization, *in vivo* imaging/tumor-targeting assessment, and antitumor efficacy evaluation in melanoma models. ***P* < 0.01, Reprinted with permission[192]. Copyright 2025, Frontiers. (K-O) Construction of an active-targeting exosome platform using a membrane-anchoring BODIPY group, polyethylene glycol (PEG) spacer, and cRGD targeting ligand, followed by assessment of integrin αvβ3 binding, exosome-marker expression, time-dependent tumor accumulation, and tumor suppression *in vivo*. ****P* < 0.01, ****P* < 0.0001, Reprinted with permission[172]. Copyright 2020, American Chemical Society. DL: Drug loading; DOX: doxorubicin; EE: encapsulation efficiency; RELN: Rhodiola rosea-derived exosome-like nanovesicle; TP: triptolide.

Chen *et al*. developed anti-CD20 antibody-conjugated, doxorubicin-loaded exosomes, which demonstrated therapeutic potential in targeting CD20+ A375 and WM266 melanoma models[182].

Membrane fusion is another important direct functionalization technique. By co-processing exosomes with synthetic liposomes or engineered cell membrane vesicles under extrusion conditions, physical fusion and functional integration of distinct membrane components can be achieved[183-185]. For example, dendritic cell-derived exosomes fused with B16-F10 melanoma cell membranes via micro-extrusion produced hybrid vesicles with biomimetic properties. This hybrid system retained MHC molecules from dendritic cell exosomes and transmembrane proteins from melanoma cell membranes, enabling efficient homing to subcutaneous melanoma and lung metastases, and acting as an antigen presentation platform to enhance $CD8^+$ T cell activation and proliferation, effectively inhibiting tumor growth and metastasis[186]. Furthermore, Gao *et al*. designed T cell-derived photoreactive hybrid γδ-T cell exosomes for targeted photodynamic therapy. Upon light irradiation, these exosomes induced melanoma cell apoptosis and generated reactive oxygen species (ROS) to achieve synergistic antitumor effects[187].

Design of multistage and smart targeting systems

To further enhance exosome penetration and targeting specificity in tumor tissues, researchers have developed multistage targeting systems and stimulus-responsive smart targeting systems, simulating programmed *in vivo* delivery and enabling precise recognition of the tumor microenvironment.

Multistage targeting systems activate different functional modules sequentially, mimicking hierarchical drug delivery mechanisms *in vivo*. For example, in the first targeting stage, the broad affinity of cRGD peptides for integrin αvβ3 allows exosomes to initially anchor on the endothelium of tumor neovasculature. In the second targeting stage, a matrix metalloproteinase-2 (MMP-2) sensitive peptide linker connects a cell-penetrating peptide to the exosome surface. Upon entering the tumor microenvironment, highly expressed MMP-2 specifically cleaves this linker, exposing and activating the cell-penetrating peptide, thereby facilitating exosome transvascular and dense ECM penetration and subsequent tumor parenchyma entry. Studies show that the tumor penetration depth of such multistage systems is over 2.5-fold greater than that of single-targeting systems[163,188]. Similarly, Kang *et al*. constructed an active targeting system (ASL) by combining a membrane-anchoring group (BODIPY)-spacer (PEG)-targeting ligand (cRGD peptide)[172]. In a B16-F10 melanoma mouse model, the system enabled precise tumor localization via specific cRGD-integrin interactions, integrating *in vivo* imaging, active targeting, and drug delivery functions [Figure 7K-O][172].

By precisely regulating delivery routes and the tumor immune microenvironment, multistage and stimulus-responsive smart exosome systems significantly enhance antitumor targeting and therapeutic efficacy, demonstrating broad clinical potential in immunotherapy and precision treatment of highly invasive tumors such as melanoma[189]. Representative examples of active loading and hybrid-engineering strategies include: electroporation-based exogenous loading of miR-195-5p into EVs for melanoma-targeted therapy sensitization [Figure 8A-F][190], ultrasound-assisted delivery in a murine melanoma model [Figure 8G-J][191], and fusion-based exosome-liposome hybrid nanoplatform co-delivering TP and miR497, included as a cross-tumor technical example of hybrid construction and evaluation [Figure 8K-N][183].

Multimodal exosome-based therapy and integrated theranostics

Rational design of combined chemotherapy and immunotherapy

To synergistically enhance cytotoxic tumor-cell killing and immune activation, engineered exosome-based systems may be designed either to improve targeted chemotherapy or to combine therapeutic delivery with immune microenvironment modulation. For example, pYEEIE-RELNs-DOX achieved targeted doxorubicin delivery and antitumor efficacy in melanoma-bearing mice[192], whereas exosome-mediated co-delivery of

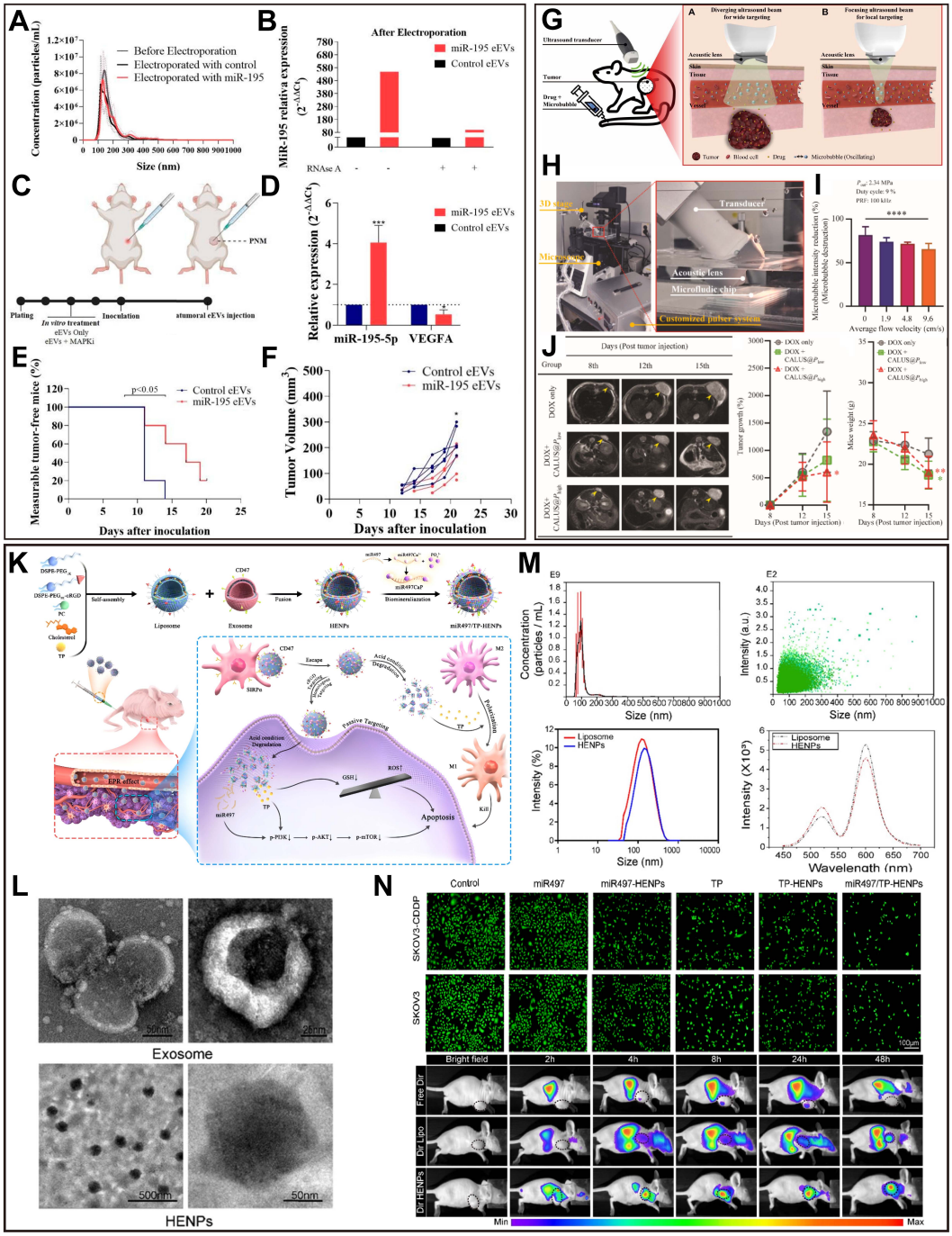

Figure 8. Application of active loading and hybrid-engineering strategies for exosome-mediated drug delivery. (A-F) Electroporation-based loading of miR-195-5p into extracellular vesicles (miR-195 eEVs) for melanoma treatment sensitization, including particle-size distribution, miRNA-loading validation, intratumoral administration, VEGFA modulation, measurable tumor-free rate, and tumor-volume evaluation. *P < 0.05, **P < 0.01, ***P < 0.001, Reprinted with permission[190]. Copyright 2023, MDPI. (G-J) Ultrasound-mediated drug delivery system using a customized convex acoustic lens-attached ultrasound (CALUS) platform for enhanced antitumor efficacy in a murine melanoma subcutaneous model, including *in vitro* system design, microbubble-intensity reduction, MRI assessment, tumor growth, and body-weight changes. ****P < 0.0001, Reprinted with permission[191]. Copyright 2023, Elsevier. (K-N) Cross-tumor example of exosome-liposome hybrid nanoparticles (HENPs) co-delivering triptolide (TP) and miR497, including hybrid-vesicle construction, ultrastructural characterization, particle-size/fluorescence characterization, and apoptosis assessment. Reprinted with permission[183]. Copyright 2022, Springer Nature. CALUS: Convex acoustic lens-attached ultrasound; eEVs: engineered extracellular vesicles; HENPs: hybrid exosome-liposome nanoparticles; MRI: magnetic resonance imaging; TP: triptolide; VEGFA: vascular endothelial growth factor A.

transforming growth factor-β receptor 1 kinase inhibitors and toll-like receptor 7/8 agonists provides a cross-tumor example of combining vesicle-based delivery with immunomodulatory therapy[193]. These examples support the rationale for exosome-assisted combination therapy, but melanoma-specific chemoimmunotherapy designs still require direct validation in appropriate preclinical models.

Integration of targeted therapy and gene-editing technologies

Exosomes are highly efficient carriers for the delivery of nucleic acid-based therapeutics, but the current cited evidence should be framed more cautiously. In melanoma, EVs loaded with miR-195-5p have been shown to sensitize melanoma cells to MAPK-targeted kinase inhibitors and to reduce tumor growth *in vivo*[190]. Beyond melanoma-specific EV data, engineered tumor-derived exosomes have also been explored in other tumor contexts to modulate chemoresistance and metastatic behavior[194], while non-EV RNAi nanoplatforms such as peptidomimetic lipid nanoparticles have demonstrated that STAT3 silencing can downregulate PD-L1 and inhibit melanoma growth[195]. Together, these findings support the conceptual feasibility of nucleic-acid-based nanodelivery, but they do not yet establish a mature exosome platform co-delivering BRAF V600E-specific siRNA and MEK inhibitors in melanoma.

Theranostics: the potential of exosomes in integrated imaging and therapy

By integrating imaging probes and therapeutic agents on the surface or within exosomes, real-time monitoring of treatment processes and quantitative evaluation of therapeutic efficacy can be achieved. In image-guided surgery, melanoma-targeting exosomes labeled with near-infrared (NIR) fluorescent dyes (such as Cy5.5 and DiR) can be directed to tumor tissues, enabling intraoperative, high-contrast visualization of micrometastatic lesions and metastatic lymph nodes. This approach assists surgeons in accurately delineating surgical margins and improves the completeness of tumor resection[196,197].

Challenges in precise targeted delivery of exosomes

Despite the substantial progress achieved in engineered exosome-based targeting strategies, their clinical translation still faces multiple challenges. As outlined in Figure 9, these include major translational goals, challenges, and potential solutions for melanoma liquid biopsy applications [Figure 9A-C], as well as corresponding issues in drug delivery and melanoma therapy [Figure 9D-F]. First, the heterogeneity of exosome sources leads to considerable variability in composition and biological function, compromising delivery stability and reproducibility, while standardized protocols for purification and quality control are still lacking[198]. Second, exosomes exhibit relatively short circulation half-lives *in vivo* and are prone to rapid clearance by the immune system, which markedly limits their delivery efficiency to target tissues[199]. Third, currently available loading techniques, such as electroporation and ultrasound-assisted loading, often suffer from limited drug encapsulation efficiency and may compromise exosomal membrane integrity. Fourth, targeting specificity remains insufficient, and achieving highly efficient and cell- or tissue-specific localization requires further systematic investigation. In addition, large-scale manufacturing and clinical translation are hindered by technical and regulatory barriers, including high production costs, batch-to-batch variability, and challenges in comprehensive safety evaluation. Finally, the lack of robust *in vivo* tracking and quantitative analytical technologies hampers accurate assessment of exosome biodistribution and therapeutic efficacy, thereby constraining the advancement of exosome-based strategies toward clinical application[200].

It should be noted that although surface chemical modification of exosomes can markedly enhance target recognition, drug-loading capacity, and the programmability of the system, it may also alter their native immunological properties and *in vivo* behavior. On the one hand, operations such as covalent conjugation may interfere with the conformation of native membrane proteins, lipid organization, and glycosylation patterns on the exosomal surface, thereby affecting their interactions with the mononuclear phagocyte

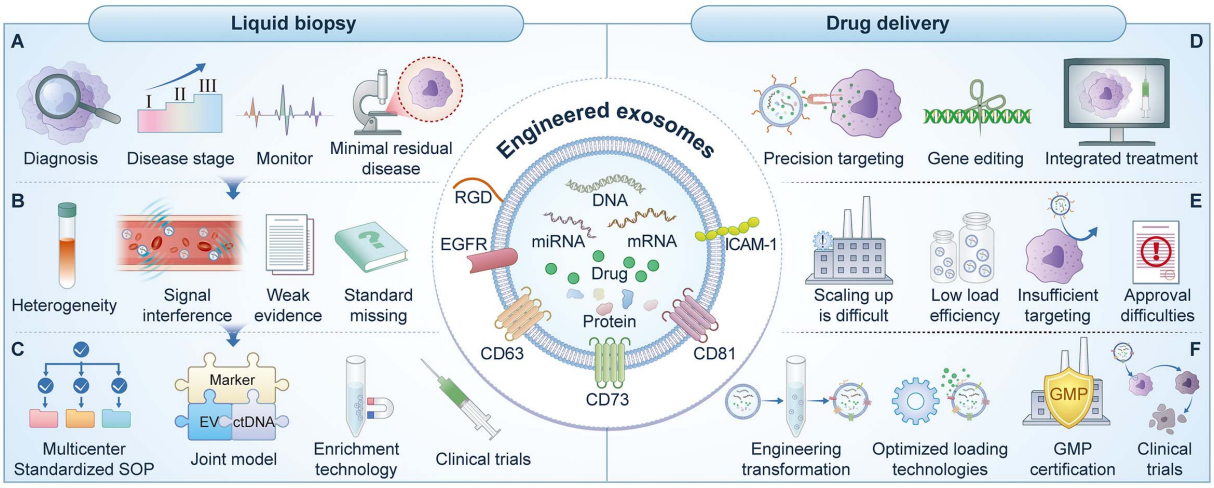

Figure 9. Goals and challenges in the translational application of engineered exosomes in melanoma. (A-C) Major goals, translational challenges, and potential solutions for the clinical application of engineered exosomes in melanoma liquid biopsy. (D-F) Major goals, translational challenges, and potential solutions for the clinical application of engineered exosomes in drug delivery and melanoma therapy.

system, the complement system, and peripheral immune cells. On the other hand, different modification strategies may exert divergent effects on immunological characteristics: certain targeting ligands may strengthen recognition of target cells, but may also increase the risk of nonspecific uptake by immune cells; whereas polymer coating or charge regulation may help prolong circulation time and reduce hepatic and splenic clearance, yet may simultaneously weaken membrane fusion and endocytic efficiency. Therefore, evaluation of engineered exosomes should not remain limited to targeting efficiency or loading capacity, but should also comprehensively assess immunogenicity, inflammatory responses, complement activation tendency, clearance patterns *in vivo*, and tolerability upon repeated administration, so as to achieve a balance between functional enhancement and maintenance of biocompatibility[45,201,202].

Despite the substantial progress achieved in engineered exosome-targeting strategies, their clinical translation still faces multiple challenges. First, the native targeting capability of exosomes is not determined by a single molecule, but rather by the combined influence of donor-cell origin, membrane adhesion molecules and integrin profiles, lipid composition, glycosylation patterns, and the recipient tissue microenvironment. Current studies suggest that surface integrins, ICAM-1- and VCAM-1-related interaction axes, and other membrane proteins may all participate, to some extent, in the recognition, adhesion, and uptake of exosomes by tumor cells, endothelial cells, or immune cells; in melanoma, these interactions are also closely associated with pre-metastatic niche formation in lymph nodes, local immunosuppression, and selective uptake by tumor-associated cells[203-205].

However, based on the currently available *in vivo* evidence, this native targeting behavior is more accurately described as relative enrichment rather than an absolute advantage sufficient to achieve highly specific tumor localization. After systemic administration, exosomes still commonly undergo preferential uptake by the liver, spleen, and mononuclear phagocyte system, display short circulation half-lives, and show substantial variability in biodistribution. Marked differences also exist among exosomes from different sources with respect to organ tropism, cellular uptake profiles, and *in vivo* stability. Therefore, native targeting alone is usually insufficient to ensure highly selective accumulation in melanoma tissue or to significantly reduce off-target effects, and further enhancement still requires surface ligand modification, multistage targeting design, immune-evasion optimization, and more precise *in vivo* tracing strategies[201,202,206]. A comparative overview of

Table 3. Comparison between engineered exosomes and clinically mature nanodelivery systems

Category	Engineered exosomes	Lipid nanoparticles (LNPs)	Representative references
Origin	Naturally derived membranous nanovesicles secreted by cells	Artificially formulated nanodelivery systems assembled chemically	Bader *et al*., 2024[207]
Structural basis	Natural lipid bilayer, membrane proteins, glycosylation, and endogenous bioactive components	Composed of ionizable lipids, helper lipids, cholesterol, PEG-lipids, and related components	Kim *et al*., 2024[46]
Particle-size range	Usually about 30-150 nm, but strongly influenced by source and isolation method	Generally more controllable, with a more uniform size distribution	Bader *et al*., 2024[207]
Cargo types	Can carry RNA, DNA, proteins, small molecules, lipids, etc., while also containing endogenous cargo	Mainly suitable for nucleic-acid delivery and some small molecules, with more controllable cargo design	Kim *et al*., 2024[46]
Loading/encapsulation capacity	Affected by membrane integrity, donor-cell status, and loading method; large batch-to-batch variation	More mature process, with clearer optimization pathways for encapsulation efficiency and formulation	Bader *et al*., 2024[207]
Targeting mechanism	May possess some degree of natural homing/cellular preference and can be further surface-modified	Mainly depends on passive distribution and artificial targeting-ligand modification	Zhao *et al*., 2024[201]
Biocompatibility	Generally good natural biocompatibility and cell-communication properties	Formulations can be standardized, but immune responses and toxicity still require attention	Kim *et al*., 2024[46]
In vivo distribution	Strongly influenced by source, membrane composition, glycosylation, and surface modification; hepatic and splenic accumulation remains common	PK is easier to regulate, but RES uptake and liver distribution are also common	Gandek *et al*., 2023[208]
Immunological features	May retain natural immunomodulatory properties, but can also introduce complex immune effects	Immunogenicity is relatively more controllable, though PEG and ionizable lipids may still trigger reactions	Zhao *et al*., 2024[201]
Stability and storage	Long-term storage, freeze-thaw stability, and preservation of activity remain challenging	Formulation stability and industrial storage/transport experience are more mature	Nieuwland *et al*., 2024[209]; György *et al*., 2024[210]
Scale-up production	Difficulties remain in yield, purification, standardization, and GMP scale-up	Process and CMC pathway are more mature, with clearer industrial advantages	Alter *et al*., 2025[211]
Quality control	High subpopulation heterogeneity makes quality-attribute definition more complex	Critical quality attributes and release standards are easier to establish	Welsh *et al*., 2024[10]; Xu *et al*., 2025[55]
Regulatory maturity	Clinical translation and regulatory pathway are still evolving	More mature clinical and regulatory experience already exists	Li *et al*., 2025[212]; Mizenko *et al*., 2024[213]
Main advantages	Natural membrane structure, biocompatibility, complex cargo, and potential natural targeting	Mature process, scalable manufacturing, and relatively better batch consistency	Kim *et al*., 2024[46]
Main limitations	Heterogeneity, low yield, difficult characterization and standardization, and variable *in vivo* behavior	Limited biological-information content; specificity and tissue penetration still need optimization in some settings	Bader *et al*., 2024[207]

engineered exosomes and clinically mature nanodelivery systems is provided in Table 3. The advantages, limitations, and representative applications of the major cargo-loading and surface-modification strategies are summarized in Table 4.

CLINICAL TRANSLATION AND REGULATORY CONSIDERATIONS

Following over a decade of fundamental research and early-stage validation, work on melanoma-derived exosomes has generally shifted from descriptive studies to applied exploration[9]. Whether employed as liquid biopsy readouts or as delivery vehicles, their successful translation into clinical practice hinges on addressing a consistent set of critical questions: Is the clinical need clearly defined? Can the detection and manufacturing processes be standardized? Can the evidence be reproduced in real-world settings? And can the final product fit into an actionable regulatory framework[212]. Accordingly, this section outlines the core

Table 4. Comparative summary of major cargo-loading and surface-modification strategies for engineered exosomes

Strategy	Category	Basic principle	Main advantages	Main limitations	Typical efficiency/practical performance	Best suited cargo or application	Representative references
Passive incubation	Cargo loading	Exosomes are co-incubated with cargo, and loading mainly occurs through passive diffusion or membrane partitioning	Simple, mild, easy to perform, and relatively preserves vesicle integrity	Usually low and variable loading efficiency; not ideal for large hydrophilic cargos	Low to moderate; generally more suitable for hydrophobic small molecules than for nucleic acids or proteins	Hydrophobic drugs, lipophilic probes, exploratory loading studies	Sun *et al.*, 2010[214]
Electroporation	Cargo loading	Electrical pulses transiently permeabilize the exosomal membrane, enabling charged cargos to enter	Widely used; suitable for siRNA, miRNA, and oligonucleotides; relatively accessible	May induce cargo aggregation and membrane damage; loading can be overestimated without rigorous purification	Moderate but highly variable; strongly affected by buffer composition and purification strategy	siRNA, miRNA, antisense oligonucleotides, some proteins	Wahlgren *et al.*, 2012[215]
Sonication	Cargo loading	Ultrasound-induced mechanical shear temporarily disrupts membrane organization and facilitates cargo entry	Often improves loading compared with passive incubation	May affect membrane integrity, endogenous cargo composition, and biological activity	Moderate to relatively high, but often with greater structural perturbation	Small molecules, hydrophobic cargos, applications prioritizing loading over strict preservation of native vesicle properties	Kim *et al.*, 2016[216]
Extrusion	Cargo loading/hybrid engineering	Exosomes and cargo are forced through membranes with defined pore sizes, promoting membrane disruption and reassembly	Can improve incorporation efficiency and generate relatively homogeneous engineered vesicles	Mechanically harsh; may disrupt membrane proteins and alter native EV identity	Moderate to relatively high, with increased risk of structural remodeling	Small molecules, membrane-associated cargos, EV-liposome hybrid preparation	Guo *et al.*, 2021[217]
Donor-cell engineering	Endogenous cargo loading/surface engineering	Parent cells are genetically or biologically engineered so that secreted exosomes carry desired cargos or display targeting motifs	Preserves membrane integrity; enables biologically integrated loading and stable ligand display	Labor-intensive; dependent on cell status and cargo sorting; lower process control and variable yield	Variable, often moderate; especially useful for biologically active nucleic acids and proteins	miRNA, mRNA, proteins, membrane-displayed peptides, receptors, targeting ligands	Ohno *et al.*, 2013[218]
Chemical conjugation	Surface modification	Covalent or affinity-based chemistry is used to attach ligands, dyes, polymers, or drugs to the exosomal surface	Stable and versatile surface functionalization; suitable for post-isolation engineering	May alter native membrane proteins, glycans, uptake behavior, and immunological properties	Generally efficient for surface decoration, but biological compatibility must be carefully evaluated	Targeting ligands, imaging probes, PEGylation, affinity tags	Pham *et al.*, 2021[219]
Click chemistry	Surface modification	Bioorthogonal reactions are used to install functional moieties on the exosomal membrane in a controlled manner	High specificity, modularity, and controllability	Requires reactive group installation and additional synthetic steps; over-modification may affect vesicle behavior	High controllability for surface engineering rather than bulk cargo loading	Targeting ligands, theranostic probes, multifunctional exosome design	Smyth *et al.*, 2014[220]
Membrane fusion	Surface modification/hybrid engineering	Exosomes are fused with liposomes or synthetic membranes using physical or chemical strategies	Combines native EV features with the tunability of synthetic nanocarriers	Generates hybrid vesicles rather than fully native exosomes; requires more complex characterization	Moderate to high engineering flexibility; useful for reformulation and surface remodeling	EV-liposome hybrids, combination delivery, membrane remodeling, synthetic lipid incorporation	Sato *et al.*, 2016[154]

challenges that must be overcome for exosome-based applications to achieve clinical adoption, focusing on four key dimensions: the level of evidence, standardization of the detection pipeline, manufacturing and safety considerations for therapeutic carriers, and regulatory pathways and trial design[213].

Level of evidence and clinical problem definition

Regarding liquid biopsy, a substantial body of research currently remains at the stage of retrospective or exploratory cohort studies. Significant variability in sample sizes, follow-up durations, and endpoint definitions across studies makes it difficult to directly compare conclusions and limits clinical applicability[9]. The key to advancing the evidence base lies not in identifying additional candidate molecules, but in first defining actionable clinical problems and aligning them with testable endpoints and clear decision-making scenarios[221]. Examples include: (1) Recurrence early warning and adjustment of follow-up frequency in high-risk post-operative patient cohorts. (2) Dynamic monitoring of early response/resistance trends during immunotherapy, informing decisions such as switching regimens early or adding combination therapies. (3) Aid in interpreting cases with "pseudoprogression/inflammatory response". (4) Evaluation of incremental value on top of existing risk models, i.e., whether exosome-based readouts can improve predictive performance or patient stratification[213].

Standardization and quality control of the detection pipeline

Uncertainty in exosome detection often originates first from pre-analytical variables and variations in isolation and enrichment strategies[209]. Blood collection and processing procedures (type of anticoagulant, hemolysis, centrifugation conditions, storage temperature, and freeze-thaw cycles, *etc*.) can significantly impact particle counts and nucleic acid/protein readings; different enrichment methods (ultracentrifugation, SEC, immunoaffinity capture, polymer-based precipitation, and their combinations) define the measurand, thereby affecting the comparability of studies[210,222]. Even on the same platform, quantification and normalization strategies (by volume, particle number, protein amount, or internal references) can alter the robustness of conclusions and the boundaries for extrapolation[10].

Regulatory pathways and trial design

For the clinical application of exosomes in liquid biopsy, the framework more closely aligns with that of In Vitro Diagnostics (IVD) or Laboratory-Developed Tests (LDTs). The core focus is on analytical performance (accuracy, precision, linear range, limit of detection, interference resistance, inter-batch consistency) and clinical performance (demonstrating application value within a defined population and with clear endpoints)[223]. The key is to pre-specify and standardize the sampling time points, threshold/model rules, and clinical decision-making pathways[213].

CONCLUSION

Based on the above discussion, a more feasible path forward can be summarized along three tracks: First, Problem-Driven Implementation of Liquid Biopsy: Prioritize clinical scenarios with well-defined decision-making pathways. Pre-specify sampling time points, thresholds, and model rules, with incremental value serving as the core evaluation metric[221]. Second, Delivery-Based Enhancement within a Combination Therapy Framework: Pair mechanistic pharmacodynamic readouts with clinical endpoints. Early focus should be placed on defining risk boundaries and manufacturability, moving beyond proof-of-concept demonstrations solely *in vitro* or in single animal models[59]. Third, Platform-Level Standardization: Establish actionable quality control systems and cross-center consistency evaluations for both detection and manufacturing. This is essential to advance exosomes from research entities to clinical products[10].

In summary, exosomes represent a nanoscale platform for melanoma, offering both biological information and material properties. The success of their clinical translation will depend less on technical feasibility alone and more on the establishment of standardized, reproducible, and regulatory-compliant systemic frameworks, as well as the demonstration of incremental benefit in well-defined clinical contexts[114]. In the future, the clinical value of exosomes in melanoma is expected to materialize along a "Standardization-Validation-Integration-Decision-making" pathway.

DECLARATIONS

Authors' contributions

Conceptualization and writing - original draft: Li, L.; Zheng, X.; Shi, J.; Zhao, F.
Literature investigation and Figure arrangement: Chen, Y.; Jia, H.; Chen, J.
Writing-review & editing: Wang, X.; Luo, P.; Zhang, L.
Supervision and funding acquisition: Zhao, Z.; Lin, L.; Sun, Q.; Luo, Z.

Availability of data and materials

Not applicable.

AI and AI-assisted tools statement

Not applicable.

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Conflicts of interest

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Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Keilholz, U.; Ascierto, P.; Dummer, R.; et al. ESMO consensus conference recommendations on the management of metastatic melanoma: under the auspices of the ESMO Guidelines Committee. *Ann. Oncol.* **2020**, *31*, 1435-48. DOI
2. Atkins, M. B.; Curiel-Lewandrowski, C.; Fisher, D. E.; et al. The state of melanoma: emergent challenges and opportunities. *Clin. Cancer. Res.* **2021**, *27*, 2678-97. DOI PubMed PMC
3. Li, H.; Bian, Y.; Xiahou, Z.; Zhao, Z.; Zhao, F.; Zhang, Q. The cellular signaling crosstalk between memory B cells and tumor cells in nasopharyngeal carcinoma cannot be overlooked: Their involvement in tumor progression and treatment strategy is significant. *J. Cancer.* **2025**, *16*, 288-314. DOI PubMed PMC
4. Huang, S. K.; Hoon, D. S. Liquid biopsy utility for the surveillance of cutaneous malignant melanoma patients. *Mol. Oncol.* **2015**, *10*, 450-63. DOI PubMed PMC
5. Sivapalan, L.; Murray, J. C.; Canzoniero, J. V.; et al. Liquid biopsy approaches to capture tumor evolution and clinical outcomes during cancer immunotherapy. *J. Immunother. Cancer.* **2023**, *11*, e005924. DOI PubMed PMC
6. Patel, R. P.; Somasundram, P. M.; Smith, L. K.; Sheppard, K. E.; Mcarthur, G. A. The therapeutic potential of targeting minimal residual disease in melanoma. *Clin. Transl. Med.* **2023**, *13*, e1197. DOI PubMed PMC
7. Tirosh, I.; Izar, B.; Prakadan, S. M.; et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **2016**, *352*, 189-96. DOI
8. Whiteside, T. L. Biology of extracellular vesicles and the potential of tumor-derived vesicles for subverting immunotherapy of cancer. *J. Immunother. Cancer.* **2025**, *13*, e010376. DOI PubMed PMC
9. Zou, Y.; Zhou, Y.; Li, G.; Dong, Y.; Hu, S. Clinical applications of extracellular vesicles: recent advances and emerging trends. *Front. Bioeng. Biotechnol.* **2025**, *13*, 1671963. DOI PubMed PMC

10. Welsh, J. A.; Goberdhan, D. C. I.; O'driscoll, L.; et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J. Extracell. Vesicle.* **2024**, *13*, e12404. DOI
11. Ragni, E. Extracellular vesicles: recent advances and perspectives. *Front. Biosci.* **2025**, *30*, 36405. DOI PubMed
12. Tang, H.; Yu, D.; Zhang, J.; et al. The new advance of exosome-based liquid biopsy for cancer diagnosis. *J. Nanobiotechnol.* **2024**, *22*, 610. DOI PubMed PMC
13. Ghanam, J.; Lichá, K.; Chetty, V. K.; et al. Unravelling the significance of extracellular vesicle-associated DNA in cancer biology and its potential clinical applications. *J. Extracell. Vesicle.* **2025**, *14*, e70047. DOI PubMed PMC
14. Andre, M.; Caobi, A.; Miles, J. S.; Vashist, A.; Ruiz, M. A.; Raymond, A. D. Diagnostic potential of exosomal extracellular vesicles in oncology. *BMC. Cancer.* **2024**, *24*, 322. DOI PubMed PMC
15. Li, Z.; Gao, Y.; Cao, Y.; et al. Extracellular RNA in melanoma: advances, challenges, and opportunities. *Front. Cell. Dev. Biol.* **2023**, *11*, 1141543. DOI PubMed PMC
16. Zhan, Y.; Yang, Z.; Zeng, H.; Yu, J.; Chen, X.; Wu, Y. Extracellular vesicle-derived non-coding RNAs in remodeling melanoma. *Biomed. Pharmacother.* **2024**, *172*, 116213. DOI
17. Slusher, N.; Jones, N.; Nonaka, T. Liquid biopsy for diagnostic and prognostic evaluation of melanoma. *Front. Cell. Dev. Biol.* **2024**, *12*, 1420360. DOI PubMed PMC
18. Liu, J. J. J.; Liu, D.; To, S. K. Y.; Wong, A. S. T. Exosomes in cancer nanomedicine: biotechnological advancements and innovations. *Mol. Cancer.* **2025**, *24*, 166. DOI PubMed PMC
19. Ovchinnikova, L. A.; Lomakin, Y. A. Extracellular vesicles as a source of biomarkers for cancer diagnosis. *Acta. Nat.* **2025**, *17*, 28-40. DOI PubMed PMC
20. Babaei, S.; Fadaee, M.; Abbasi-Kenarsari, H.; Shanehbandi, D.; Kazemi, T. Exosome-based immunotherapy as an innovative therapeutic approach in melanoma. *Cell. Commun. Signal.* **2024**, *22*, 527. DOI PubMed PMC
21. Di Bella, M. A. Overview and update on extracellular vesicles: considerations on exosomes and their application in modern medicine. *Biology* **2022**, *11*, 804. DOI PubMed PMC
22. Zhang, Y.; Lan, M.; Chen, Y. Minimal information for studies of extracellular vesicles (MISEV): ten-year evolution (2014-2023). *Pharmaceutics* **2024**, *16*, 1394. DOI PubMed PMC
23. Di Bella, M. A.; Taverna, S. Extracellular vesicles: diagnostic and therapeutic applications in cancer. *Biology* **2024**, *13*, 716. DOI PubMed PMC
24. Yang, C.; Xue, Y.; Duan, Y.; Mao, C.; Wan, M. Extracellular vesicles and their engineering strategies, delivery systems, and biomedical applications. *J. Controlled. Release.* **2024**, *365*, 1089-123. DOI
25. Zhang, Q.; Jeppesen, D. K.; Higginbotham, J. N.; Franklin, J. L.; Coffey, R. J. Comprehensive isolation of extracellular vesicles and nanoparticles. *Nat. Protoc.* **2023**, *18*, 1462-87. DOI PubMed PMC
26. Carney, R. P.; Mizenko, R. R.; Bozkurt, B. T.; et al. Harnessing extracellular vesicle heterogeneity for diagnostic and therapeutic applications. *Nat. Nanotechnol.* **2024**, *20*, 14-25. DOI PubMed PMC
27. Li, B.; Wu, W.; Xu, W.; Qian, H.; Ji, C. Advances of extracellular vesicles isolation and detection frontier technology: from heterogeneity analysis to clinical application. *J. Nanobiotechnol.* **2025**, *23*, 678. DOI PubMed PMC
28. Woo, H.; Nam, Y.; Park, H. G.; Lee, H. Bridging laboratory innovation to translational research and commercialization of extracellular vesicle isolation and detection. *Biosens. Bioelectron.* **2025**, *282*, 117475. DOI PubMed PMC
29. Poggio, M.; Hu, T.; Pai, C.; et al. Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. *Cell* **2019**, *177*, 414-27.e13. DOI PubMed PMC
30. Urzì, O.; Olofsson Bagge, R.; Crescitelli, R. Extracellular vesicles in uveal melanoma - Biological roles and diagnostic value. *Cancer. Lett.* **2025**, *615*, 217531. DOI PubMed
31. Dong, X.; Lin, Y.; Li, K.; et al. Consensus statement on extracellular vesicles in liquid biopsy for advancing laboratory medicine. *Clin. Chem. Lab. Med.* **2025**, *63*, 465-82. DOI
32. Asleh, K.; Dery, V.; Taylor, C.; Davey, M.; Djeungoue-Petga, M.; Ouellette, R. J. Extracellular vesicle-based liquid biopsy biomarkers and their application in precision immuno-oncology. *Biomark. Res.* **2023**, *11*, 99. DOI PubMed PMC
33. Liu, J.; Hu, X.; Xin, W.; Wang, X. Exosomal non-coding RNAs: a new approach to melanoma diagnosis and therapeutic strategy. *Curr. Med. Chem.* **2024**, *31*, 6084-109. DOI
34. Ricciardi, E.; Giordani, E.; Zicchedda, G.; et al. Metastatic melanoma: liquid biopsy as a new precision medicine approach. *Int. J. Mol. Sci.* **2023**, *24*, 4014. DOI PubMed PMC
35. Minami, S.; Chikazu, D.; Ochiya, T.; Yoshioka, Y. Extracellular vesicle-based liquid biopsies in cancer: Future biomarkers for oral cancer. *Transl. Oncol.* **2023**, *38*, 101786. DOI PubMed PMC
36. Mondal, S. K.; Whiteside, T. L. Proteomic profiles of melanoma cell-derived exosomes in plasma: discovery of potential biomarkers of melanoma progression. *Melanoma. Res.* **2021**, *31*, 472-5. DOI PubMed PMC

37. Lattmann, E.; Räss, L.; Tognetti, M.; et al. Size-exclusion chromatography combined with DIA-MS enables deep proteome profiling of extracellular vesicles from melanoma plasma and serum. *Cell. Mol. Life. Sci.* **2024**, *81*, 90. DOI PubMed PMC
38. Clancy, J. W.; D'souza-Schorey, C. Tumor-derived extracellular vesicles: multifunctional entities in the tumor microenvironment. *Annu. Rev. Pathol. Mech. Dis.* **2023**, *18*, 205-29. DOI PubMed PMC
39. Helvind, N. M.; Brinch-Møller Weitemeyer, M.; Chakera, A. H.; et al. Stage-specific risk of recurrence and death from melanoma in Denmark, 2008-2021: a national observational cohort study of 25 720 patients with stage IA to IV melanoma. *JAMA. Dermatol.* **2023**, *159*, 1213. DOI PubMed PMC
40. Spiliopoulou, P.; Holanda Lopes, C. D.; Spreafico, A. Promising and minimally invasive biomarkers: targeting melanoma. *Cells* **2023**, *13*, 19. DOI PubMed PMC
41. Serrati, S.; Guida, M.; Di Fonte, R.; et al. Circulating extracellular vesicles expressing PD1 and PD-L1 predict response and mediate resistance to checkpoint inhibitors immunotherapy in metastatic melanoma. *Mol. Cancer.* **2022**, *21*, 20. DOI PubMed PMC
42. Sun, L.; Wei, X.; Zhao, Q.; et al. Dynamic change of PD-L2 on circulating plasma extracellular vesicles as a predictor of treatment response in melanoma patients receiving anti-PD-1 therapy. *J. Extracell. Vesicle.* **2025**, *14*, e70054. DOI
43. Hsu, C.; Chandramoorthy, H. C.; Mohammed, J. S.; et al. Exosomes as key mediators in immune and cancer cell interactions: insights in melanoma progression and therapy. *Arch. Dermatol. Res.* **2025**, *317*, 729. DOI PubMed
44. Serrano, D. R.; Juste, F.; Anaya, B. J.; et al. Exosome-based drug delivery: a next-generation platform for cancer, infection, neurological and immunological diseases, gene therapy and regenerative medicine. *Pharmaceutics* **2025**, *17*, 1336. DOI PubMed PMC
45. Johnson, V.; Vasu, S.; Kumar, U. S.; Kumar, M. Surface-engineered extracellular vesicles in cancer immunotherapy. *Cancers* **2023**, *15*, 2838. DOI PubMed PMC
46. 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*, 836-49. DOI PubMed PMC
47. Chen, Y.; Luh, F.; Ho, Y.; Yen, Y. Exosomes: a review of biologic function, diagnostic and targeted therapy applications, and clinical trials. *J. Biomed. Sci.* **2024**, *31*, 67. DOI PubMed PMC
48. Brezgin, S.; Danilik, O.; Yudaeva, A.; et al. Basic guide for approaching drug delivery with extracellular vesicles. *Int. J. Mol. Sci.* **2024**, *25*, 10401. DOI PubMed PMC
49. Abbasi-malati, Z.; Azizi, S. G.; Milani, S. Z.; et al. Tumorigenic and tumoricidal properties of exosomes in cancers; a forward look. *Cell. Commun. Signal.* **2024**, *22*, 130. DOI PubMed PMC
50. Xia, Y.; Zhang, J.; Liu, G.; Wolfram, J. Immunogenicity of extracellular vesicles. *Adv. Mater.* **2024**, *36*, 2403199. DOI
51. Ziogas, D. C.; Theocharopoulos, C.; Koutouratsas, T.; Haanen, J.; Gogas, H. Mechanisms of resistance to immune checkpoint inhibitors in melanoma: what we have to overcome? *Cancer. Treat. Rev.* **2023**, *113*, 102499. DOI PubMed
52. Fernandez, M. F.; Choi, J.; Sosman, J. New approaches to targeted therapy in Melanoma. *Cancers* **2023**, *15*, 3224. DOI PubMed PMC
53. Yin, G.; Guo, W.; Huang, Z.; Chen, X. Efficacy of radiotherapy combined with immune checkpoint inhibitors in patients with melanoma: a systemic review and meta-analysis. *Melanoma. Res.* **2022**, *32*, 71-8. DOI
54. Van Delen, M.; Derdelinckx, J.; Wouters, K.; Nelissen, I.; Cools, N. A systematic review and meta-analysis of clinical trials assessing safety and efficacy of human extracellular vesicle-based therapy. *J. Extracell. Vesicle.* **2024**, *13*, e12458. DOI PubMed PMC
55. Xu, G.; Jin, J.; Fu, Z.; et al. Extracellular vesicle-based drug overview: research landscape, quality control and nonclinical evaluation strategies. *Sig. Transduct. Target. Ther.* **2025**, *10*, 255. DOI PubMed PMC
56. Su, X.; Wang, H.; Li, Q.; Chen, Z. Extracellular vesicles: a review of their therapeutic potentials, sources, biodistribution, and administration routes. *Int. J. Nanomed.* **2025**, *20*, 3175-99. DOI PubMed PMC
57. Kang, M.; Jordan, V.; Blenkiron, C.; Chamley, L. W. Biodistribution of extracellular vesicles following administration into animals: a systematic review. *J. Extracell. Vesicle.* **2021**, *10*, e12085. DOI PubMed PMC
58. Chen, T.; Chen, D.; Su, W.; Liang, J.; Liu, X.; Cai, M. Extracellular vesicles as vital players in drug delivery: a focus on clinical disease treatment. *Front. Bioeng. Biotechnol.* **2025**, *13*, 1600227. DOI PubMed PMC
59. Humbert, C.; Cordier, C.; Drut, I.; et al. GMP-compliant process for the manufacturing of an extracellular vesicles-enriched secretome product derived from cardiovascular progenitor cells suitable for a phase I clinical trial. *J. Extracell. Vesicle.* **2025**, *14*, e70145. DOI PubMed PMC
60. Akbani, R.; Akdemir, K. C.; Aksoy, B. A.; et al. Genomic classification of cutaneous melanoma. *Cell* **2015**, *161*, 1681-96. DOI
61. Moran, B.; Silva, R.; Perry, A. S.; Gallagher, W. M. Epigenetics of malignant melanoma. *Semin. Cancer. Biol.* **2018**, *51*, 80-8. DOI PubMed
62. Sarkar, D.; Leung, E. Y.; Baguley, B. C.; Finlay, G. J.; Askarian-Amiri, M. E. Epigenetic regulation in human melanoma: past and future. *Epigenetics* **2015**, *10*, 103-21. DOI PubMed PMC
63. Mannavola, F.; D'oronzo, S.; Cives, M.; et al. Extracellular vesicles and epigenetic modifications are hallmarks of melanoma progression. *Int. J. Mol. Sci.* **2019**, *21*, 52. DOI PubMed PMC

64. Kalluri, R.; Lebleu, V. S. The biology,function, and biomedical applications of exosomes. *Science* **2020**, *367*, eaau6977. DOI PubMed PMC
65. 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. DOI PubMed PMC
66. Zaborowski, M. P.; Balaj, L.; Breakefield, X. O.; Lai, C. P. Extracellular vesicles: composition, biological relevance, and methods of study. *Bioscience* **2015**, *65*, 783-97. DOI PubMed PMC
67. Yáñez-mó, M.; Siljander, P. R. M.; Andreu, Z.; et al. Biological properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicle.* **2015**, *4*, 27066. DOI
68. Lespagnol, A.; Duflaut, D.; Beekman, C.; et al. Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice. *Cell. Death. Differ.* **2008**, *15*, 1723-33. DOI
69. Payandeh, Z.; Khalili, S.; Somi, M. H.; et al. PD-1/PD-L1-dependent immune response in colorectal cancer. *J. Cell. Physiol.* **2020**, *235*, 5461-75. DOI
70. Jiang, Y.; Zhao, X.; Fu, J.; Wang, H. Progress and challenges in precise treatment of tumors with PD-1/PD-L1 blockade. *Front. Immunol.* **2020**, *11*, 339. DOI PubMed PMC
71. Wang, B.; Bai, W.; Ma, H.; Li, F. Regulatory effect of PD1/PD-Ligand 1 (PD-L1) on treg cells in patients with idiopathic pulmonary fibrosis. *Med. Sci. Monit.* **2020**, *26*, 927577. DOI PubMed PMC
72. Shergold, A. L.; Millar, R.; Nibbs, R. J. Understanding and overcoming the resistance of cancer to PD-1/PD-L1 blockade. *Pharmacol. Res.* **2019**, *145*, 104258. DOI PubMed
73. Cloughesy, T. F.; Mochizuki, A. Y.; Orpilla, J. R.; et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nat. Med.* **2019**, *25*, 477-86. DOI
74. Chen, G.; Huang, A. C.; Zhang, W.; et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature* **2018**, *560*, 382-6. DOI
75. Ricklefs, F. L.; Alayo, Q.; Krenzlin, H.; et al. Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles. *Sci. Adv.* **2018**, *4*, eaar2766. DOI PubMed PMC
76. Theodoraki, M.; Yerneni, S. S.; Hoffmann, T. K.; Gooding, W. E.; Whiteside, T. L. Clinical significance of PD-L1+ exosomes in plasma of head and neck cancer patients. *Clin. Cancer. Res.* **2018**, *24*, 896-905. DOI PubMed PMC
77. Yang, Y.; Li, C.; Chan, L.; et al. Exosomal PD-L1 harbors active defense function to suppress T cell killing of breast cancer cells and promote tumor growth. *Cell. Res.* **2018**, *28*, 862-4. DOI PubMed PMC
78. Fleming, V.; Hu, X.; Weller, C.; et al. Melanoma extracellular vesicles generate immunosuppressive myeloid cells by upregulating PD-L1 via TLR4 signaling. *Cancer. Res.* **2019**, *79*, 4715-28. DOI
79. Du, Z.; Feng, Y.; Zhang, H.; Liu, J.; Wang, J. Melanoma-derived small extracellular vesicles remodel the systemic onco-immunity via disrupting hematopoietic stem cell proliferation and differentiation. *Cancer. Lett.* **2022**, *545*, 215841. DOI
80. Maus, R. L. G.; Jakub, J. W.; Nevala, W. K.; et al. Human melanoma-derived extracellular vesicles regulate dendritic cell maturation. *Front. Immunol.* **2017**, *8*, 358. DOI PubMed PMC
81. Valenti, R.; Huber, V.; Filipazzi, P.; et al. Human tumor-released microvesicles promote the differentiation of myeloid cells with transforming growth factor-β-mediated suppressive activity on t lymphocytes. *Cancer. Res.* **2006**, *66*, 9290-8. DOI
82. Hosseini, R.; Asef-kabiri, L.; Yousefi, H.; et al. The roles of tumor-derived exosomes in altered differentiation, maturation and function of dendritic cells. *Mol. Cancer.* **2021**, *20*, 83. DOI PubMed PMC
83. Marton, A.; Vizler, C.; Kusz, E.; et al. Melanoma cell-derived exosomes alter macrophage and dendritic cell functions in vitro. *Immunol. Lett.* **2012**, *148*, 34-8. DOI
84. Düchler, M.; Czernek, L.; Peczek, L.; Cypryk, W.; Sztiller-Sikorska, M.; Czyz, M. Melanoma-derived extracellular vesicles bear the potential for the induction of antigen-specific tolerance. *Cells* **2019**, *8*, 665. DOI PubMed PMC
85. Sharma, P.; Diergaarde, B.; Ferrone, S.; Kirkwood, J. M.; Whiteside, T. L. Melanoma cell-derived exosomes in plasma of melanoma patients suppress functions of immune effector cells. *Sci. Rep.* **2020**, *10*, 92. DOI PubMed PMC
86. Whiteside, T. L. Immunosuppressive functions of melanoma cell-derived exosomes in plasma of melanoma patients. *Front. Cell. Dev. Biol.* **2023**, *10*, 1080925. DOI PubMed PMC
87. Crucitta, S.; Cucchiara, F.; Marconcini, R.; et al. TGF-β mRNA levels in circulating extracellular vesicles are associated with response to anti-PD1 treatment in metastatic melanoma. *Front. Mol. Biosci.* **2024**, *11*, 1288677. DOI PubMed PMC
88. Tatischeff, I. Extracellular vesicle-DNA: the next liquid biopsy biomarker for early cancer diagnosis? *Cancers* **2023**, *15*, 1456. DOI PubMed PMC
89. Belviso, I.; Pastore, R.; Mileo, A.; et al. The role of fibroblasts in melanoma development: from tumor microenvironment remodeling to pre-metastatic niche formation. *Int. J. Mol. Sci.* **2025**, *26*, 6132. DOI PubMed PMC

90. Lu, M.; Yang, Y. Exosomal PD-L1 in cancer and other fields: recent advances and perspectives. *Front. Immunol.* **2024**, *15*, 1395332. DOI PubMed PMC
91. Chen, J.; Song, Y.; Miao, F.; et al. PDL1-positive exosomes suppress antitumor immunity by inducing tumor-specific $CD8^+$ T cell exhaustion during metastasis. *Cancer. Sci.* **2021**, *112*, 3437-54. DOI PubMed PMC
92. Liu, N.; Zhang, J.; Yin, M.; et al. Inhibition of xCT suppresses the efficacy of anti-PD-1/L1 melanoma treatment through exosomal PD-L1-induced macrophage M2 polarization. *Mol. Ther.* **2021**, *29*, 2321-34. DOI PubMed PMC
93. Christofides, A.; Strauss, L.; Yeo, A.; Cao, C.; Charest, A.; Boussiotis, V. A. The complex role of tumor-infiltrating macrophages. *Nat. Immunol.* **2022**, *23*, 1148-56. DOI PubMed PMC
94. Gerloff, D.; Lützkendorf, J.; Moritz, R. K.; et al. Melanoma-derived exosomal miR-125b-5p educates tumor associated macrophages (TAMs) by targeting lysosomal acid lipase A (LIPA). *Cancers* **2020**, *12*, 464. DOI PubMed PMC
95. Wang, R.; Zhu, T.; Hou, B.; Huang, X. An iPSC-derived exosome-pulsed dendritic cell vaccine boosts antitumor immunity in melanoma. *Mol. Ther.* **2023**, *31*, 2376-90. DOI PubMed PMC
96. Tucci, M.; Mannavola, F.; Passarelli, A.; Stucci, L. S.; Cives, M.; Silvestris, F. Exosomes in melanoma: a role in tumor progression, metastasis and impaired immune system activity. *Oncotarget* **2018**, *9*, 20826-37. DOI PubMed PMC
97. Zhou, J.; Yang, Y.; Wang, W.; et al. Melanoma-released exosomes directly activate the mitochondrial apoptotic pathway of CD4+ T cells through their microRNA cargo. *Exp. Cell. Res.* **2018**, *371*, 364-71. DOI
98. Li, X.; Liu, Y.; Yang, L.; Jiang, Y.; Qian, Q. TIM-3 shuttled by MV3 cells-secreted exosomes inhibits CD4+ T cell immune function and induces macrophage M2 polarization to promote the growth and metastasis of melanoma cells. *Transl. Oncol.* **2022**, *18*, 101334. DOI PubMed PMC
99. De Cicco, P.; Ercolano, G.; Ianaro, A. The new era of cancer immunotherapy: targeting myeloid-derived suppressor cells to overcome immune evasion. *Front. Immunol.* **2020**, *11*, 1680. DOI PubMed PMC
100. Peinado, H.; Alečković, M.; Lavotshkin, S.; et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* **2012**, *18*, 883-91. DOI
101. Peinado, H.; Zhang, H.; Matei, I. R.; et al. Pre-metastatic niches: organ-specific homes for metastases. *Nat. Rev. Cancer.* **2017**, *17*, 302-17. DOI
102. Kim, J.; Afshari, A.; Sengupta, R.; et al. Replication study: melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *eLife* **2018**, *7*, e39944. DOI
103. Rinderknecht, M.; Detmar, M. Tumor lymphangiogenesis and melanoma metastasis. *J. Cell. Physiol.* **2008**, *216*, 347-54. DOI PubMed
104. Hood, J. L.; San, R. S.; Wickline, S. A. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. *Cancer. Res.* **2011**, *71*, 3792-801. DOI PubMed
105. García-Silva, S.; Benito-Martín, A.; Nogués, L.; et al. Melanoma-derived small extracellular vesicles induce lymphangiogenesis and metastasis through an NGFR-dependent mechanism. *Nat. Cancer.* **2021**, *2*, 1387-405. DOI PubMed PMC
106. Hu, T.; Hu, J. Melanoma-derived exosomes induce reprogramming fibroblasts into cancer-associated fibroblasts via Gm26809 delivery. *Cell. Cycle.* **2019**, *18*, 3085-94. DOI PubMed PMC
107. Shelton, M.; Anene, C.; Nsengimana, J.; Roberts, W.; Newton-Bishop, J.; Boyne, J. The role of CAF derived exosomal microRNAs in the tumour microenvironment of melanoma. *Biochim. Biophys. Acta.* **2021**, *1875*, 188456. DOI PubMed
108. Benito-Martín, A.; Jasiulionis, M. G.; García-Silva, S. Extracellular vesicles and melanoma: new perspectives on tumor microenvironment and metastasis. *Front. Cell. Dev. Biol.* **2023**, *10*, 1061982. DOI PubMed PMC
109. Wang, C.; Wang, Y.; Chang, X.; et al. Melanoma-derived exosomes endow fibroblasts with an invasive potential via miR-21 target signaling pathway. *Cancer. Manag. Res.* **2020**, *12*, 12965-74. DOI
110. Tagliatela, A. C.; Hempstead, S. C.; Hibshman, P. S.; et al. Coronin 1C inhibits melanoma metastasis through regulation of MT1-MMP-containing extracellular vesicle secretion. *Sci. Rep.* **2020**, *10*, 11958. DOI PubMed PMC
111. Pavlides, S.; Whitaker-Menezes, D.; Castello-Cros, R.; et al. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell. Cycle.* **2014**, *8*, 3984-4001. DOI
112. Lazar, I.; Clement, E.; Dauvillier, S.; et al. Adipocyte exosomes promote melanoma aggressiveness through fatty acid oxidation: a novel mechanism linking obesity and cancer. *Cancer. Res.* **2016**, *76*, 4051-7. DOI
113. Clement, E.; Lazar, I.; Attané, C.; et al. Adipocyte extracellular vesicles carry enzymes and fatty acids that stimulate mitochondrial metabolism and remodeling in tumor cells. *EMBO. J.* **2020**, *39*, EMBJ2019102525. DOI PubMed PMC
114. Medhin, L. B.; Beasley, A. B.; Warburton, L.; Amanuel, B.; Gray, E. S. Extracellular vesicles as a liquid biopsy for melanoma: are we there yet? *Semin. Cancer. Biol.* **2023**, *89*, 92-8. DOI PubMed
115. Wu, N.; Sun, H.; Sun, Q.; et al. Circulating microRNAs as diagnostic biomarkers for melanoma: a systematic review and meta-analysis. *BMC. Cancer.* **2023**, *23*, 414. DOI PubMed PMC

116. Hu, C.; Jiang, W.; Lv, M.; et al. Potentiality of exosomal proteins as novel cancer biomarkers for liquid biopsy. *Front. Immunol.* **2022**, *13*, 792046. DOI PubMed PMC
117. Wang, M.; Cai, Y.; Peng, Y.; Xu, B.; Hui, W.; Jiang, Y. Exosomal LGALS9 in the cerebrospinal fluid of glioblastoma patients suppressed dendritic cell antigen presentation and cytotoxic T-cell immunity. *Cell. Death. Dis.* **2020**, *11*, 896. DOI PubMed PMC
118. Shimada, Y.; Matsubayashi, J.; Kudo, Y.; et al. Serum-derived exosomal PD-L1 expression to predict anti-PD-1 response and in patients with non-small cell lung cancer. *Sci. Rep.* **2021**, *11*, 7830. DOI PubMed PMC
119. Kluszczynska, K.; Czyz, M. Extracellular vesicles-based cell-cell communication in melanoma: new perspectives in diagnostics and therapy. *Int. J. Mol. Sci.* **2023**, *24*, 965. DOI PubMed PMC
120. Van Niel, G.; D'angelo, G.; Raposo, G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell. Biol.* **2018**, *19*, 213-28. DOI PubMed
121. Théry, C.; Witwer, K. W.; Aikawa, E.; et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesicle.* **2018**, *7*, 1535750. DOI PubMed PMC
122. Ullah, M. The future of bioorthogonal-chemistry for targeting of exosomes in precision medicine. *Oncotarget* **2022**, *13*, 1303-4. DOI PubMed PMC
123. Rayamajhi, S.; Sipes, J.; Tetlow, A. L.; Saha, S.; Bansal, A.; Godwin, A. K. Extracellular vesicles as liquid biopsy biomarkers across the cancer journey: from early detection to recurrence. *Clin. Chem.* **2024**, *70*, 206-19. DOI PubMed PMC
124. Xia, C.; Yin, S.; To, K. K. W.; Fu, L. CD39/CD73/A2AR pathway and cancer immunotherapy. *Mol. Cancer.* **2023**, *22*, 44. DOI PubMed PMC
125. Kurago, Z.; Guo, G.; Shi, H.; et al. Inhibitors of the CD73-adenosinergic checkpoint as promising combinatory agents for conventional and advanced cancer immunotherapy. *Front. Immunol.* **2023**, *14*, 1212209. DOI PubMed PMC
126. Saigí, M.; Mesía-Carbonell, O.; Barbie, D. A.; Guillamat-Prats, R. Unraveling the intricacies of CD73/adenosine signaling: the pulmonary immune and stromal microenvironment in lung cancer. *Cancers* **2023**, *15*, 5706. DOI PubMed PMC
127. Turiello, R.; Capone, M.; Morretta, E.; et al. Exosomal CD73 from serum of patients with melanoma suppresses lymphocyte functions and is associated with therapy resistance to anti-PD-1 agents. *J. Immunother. Cancer.* **2022**, *10*, e004043. DOI PubMed PMC
128. Yang, Y.; Zhu, L.; Xu, Y.; et al. The progress and prospects of targeting the adenosine pathway in cancer immunotherapy. *Biomark. Res.* **2025**, *13*, 75. DOI PubMed PMC
129. Rodrigues-junior, D. M.; Tsirigoti, C.; Lim, S. K.; Heldin, C.; Moustakas, A. Extracellular vesicles and transforming growth factor β signaling in cancer. *Front. Cell. Dev. Biol.* **2022**, *10*, 849938. DOI PubMed PMC
130. De Miguel-Perez, D.; Russo, A.; Gunasekaran, M.; et al. Baseline extracellular vesicle TGF-β is a predictive biomarker for response to immune checkpoint inhibitors and survival in non-small cell lung cancer. *Cancer* **2022**, *129*, 521-30. DOI
131. Biagioni, A.; Laurenzana, A.; Menicacci, B.; et al. uPAR-expressing melanoma exosomes promote angiogenesis by VE-Cadherin, EGFR and uPAR overexpression and rise of ERK1,2 signaling in endothelial cells. *Cell. Mol. Life. Sci.* **2020**, *78*, 3057-72. DOI PubMed PMC
132. Porcelli, L.; Guida, M.; De, Summa. S.; et al. uPAR^{+} extracellular vesicles: a robust biomarker of resistance to checkpoint inhibitor immunotherapy in metastatic melanoma patients. *J. Immunother. Cancer.* **2021**, *9*, e002372. DOI PubMed PMC
133. Serratì, S.; Di Fonte, R.; Porcelli, L.; et al. Circulating extracellular vesicles are monitoring biomarkers of anti-PD1 response and enhancer of tumor progression and immunosuppression in metastatic melanoma. *J. Exp. Clin. Cancer. Res.* **2023**, *42*, 251. DOI PubMed PMC
134. Cordonnier, M.; Nardin, C.; Chanteloup, G.; et al. Tracking the evolution of circulating exosomal-PD-L1 to monitor melanoma patients. *J. Extracell. Vesicle.* **2020**, *9*, 1710899. DOI PubMed PMC
135. Hur, J. Y.; Lee, K. Y. Characteristics and clinical application of extracellular vesicle-derived DNA. *Cancers* **2021**, *13*, 3827. DOI PubMed PMC
136. Waldenmaier, M.; Schulte, L.; Schönfelder, J.; et al. Comparative panel sequencing of DNA variants in cf-, ev- and tumorDNA for pancreatic ductal adenocarcinoma patients. *Cancers* **2022**, *14*, 1074. DOI PubMed PMC
137. Miceli, R. T.; Chen, T. Y.; Nose, Y.; et al. Extracellular vesicles, RNA sequencing, and bioinformatic analyses: challenges, solutions, and recommendations. *J. Extracell. Vesicle.* **2024**, *13*, e70005. DOI PubMed PMC
138. Li, M.; Yu, Y.; Liu, G.; et al. A promising frontier of circulating messenger RNA in liquid biopsy: from mechanisms to clinical applications. *Int. J. Cancer.* **2025**, *157*, 1519-37. DOI PubMed PMC
139. Bamodu, O. A.; Chung, C.; Pisanic, T. R. Harnessing liquid biopsies: exosomes and ctDNA as minimally invasive biomarkers for precision cancer medicine. *J. Liquid. Biopsy.* **2023**, *2*, 100126. DOI PubMed PMC
140. Sánchez-Herrero, E.; Serna-Blasco, R.; Robado De Lope, L.; González-Rumayor, V.; Romero, A.; Provencio, M. Circulating tumor DNA as a cancer biomarker: an overview of biological features and factors that may impact on ctDNA analysis. *Front. Oncol.* **2022**, *12*, 943253. DOI PubMed PMC

141. Tsering, T.; Nadeau, A.; Wu, T.; Dickinson, K.; Burnier, J. V. Extracellular vesicle-associated DNA: ten years since its discovery in human blood. *Cell. Death. Dis.* **2024**, *15*, 668. DOI PubMed PMC
142. Shen, Y.; Li, M.; Liao, L.; Gao, S.; Wang, Y. Plasma exosome-derived connexin43 as a promising biomarker for melanoma patients. *BMC. Cancer.* **2023**, *23*, 242. DOI PubMed PMC
143. Lim, S. Y.; Boyd, S. C.; Diefenbach, R. J.; Rizos, H. Circulating MicroRNAs: functional biomarkers for melanoma prognosis and treatment. *Mol. Cancer.* **2025**, *24*, 99. DOI PubMed PMC
144. De Martino, E.; Gandin, I.; Azzalini, E.; et al. A group of three miRNAs can act as candidate circulating biomarkers in liquid biopsies from melanoma patients. *Front. Med.* **2023**, *10*, 1180799. DOI PubMed PMC
145. Trilla-Fuertes, L.; Gámez-Pozo, A.; Laso-García, F.; et al. Protein content of extracellular vesicles from patients with advanced melanoma changes upon progression to anti-PD1 therapy. *Sci. Rep.* **2026**, *16*, 5891. DOI PubMed PMC
146. Bollard, S.; Howard, J.; Casalou, C.; et al. Proteomic and metabolomic profiles of plasma-derived Extracellular Vesicles differentiate melanoma patients from healthy controls. *Transl. Oncol.* **2024**, *50*, 102152. DOI PubMed PMC
147. Sirivolu, S.; Peng, C.; Neviani, P.; Xu, B. Y.; Berry, J. L.; Xu, L. Comparative single vesicle analysis of aqueous humor extracellular vesicles before and after radiation in uveal melanoma eyes. *Int. J. Mol. Sci.* **2024**, *25*, 6035. DOI PubMed PMC
148. Luz, Pessuti. C.; Fialho, Costa. D.; Ribeiro, K. S.; et al. Characterization of Extracellular vesicles isolated from different liquid biopsies of uveal melanoma patients. *J. Circ. Biomark.* **2022**, *11*, 36-47. DOI PubMed PMC
149. Beasley, A. B.; Chen, F. K.; Isaacs, T. W.; Gray, E. S. Future perspectives of uveal melanoma blood based biomarkers. *Br. J. Cancer.* **2022**, *126*, 1511-28. DOI PubMed PMC
150. Marsavela, G.; Mcevoy, A. C.; Pereira, M. R.; et al. Detection of clinical progression through plasma ctDNA in metastatic melanoma patients: a comparison to radiological progression. *Br. J. Cancer.* **2021**, *126*, 401-8. DOI PubMed PMC
151. De, Simoni. E.; Spagnolo, F.; Gandini, S.; et al. Circulating tumor DNA-based assessment of molecular residual disease in non-metastatic melanoma. *Cancer. Treat. Rev.* **2024**, *129*, 102788. DOI
152. Fathi, M.; Zarei, A.; Moghimi, A.; et al. Combined cancer immunotherapy based on targeting adenosine pathway and PD-1/PDL-1 axis. *Expert. Opin. Ther. Targets.* **2024**, *28*, 757-77. DOI
153. Shan, Z.; Liu, F. Targeting ferroptosis, pyroptosis and necroptosis for cancer immunotherapy in melanoma: mechanistic insights and clinical perspectives. *Front. Immunol.* **2025**, *16*, 1629620. DOI PubMed PMC
154. Sato, Y. T.; Umezaki, K.; Sawada, S.; et al. Engineering hybrid exosomes by membrane fusion with liposomes. *Sci. Rep.* **2016**, *6*, 21933. DOI PubMed PMC
155. Lee, J.; Kim, J.; Na, K.; Ahn, H. GMP manufacturing of umbilical cord blood mesenchymal stem cell-released exosomes and verification of wound healing efficacy. *Nanotheranostics* **2025**, *9*, 95-109. DOI PubMed PMC
156. Yang, F.; Wang, M.; Guan, X. Exosomes and mimics as novel delivery platform for cancer therapy. *Front. Pharmacol.* **2022**, *13*, 1001417. DOI PubMed PMC
157. Thakur, A.; Sidu, R. K.; Zou, H.; Alam, M. K.; Yang, M.; Lee, Y. Inhibition of glioma cells' proliferation by doxorubicin-loaded exosomes via microfluidics. *Int. J. Nanomed.* **2020**, *15*, 8331-43. DOI PubMed PMC
158. Zhao, Y.; Hang, R.; Li, H.; et al. Biomaterial surface-mediated macrophages exert immunomodulatory roles by exosomal CCL2-induced membrane integrin β1 trafficking in recipient cells. *Adv. Sci.* **2025**, *12*, 2409809. DOI PubMed PMC
159. Chen, J.; Zhang, G.; Wan, Y.; et al. Immune cell-derived exosomes as promising tools for cancer therapy. *J. Controlled. Release.* **2023**, *364*, 508-28. DOI
160. Wang, W.; Jiang, Y.; Zhang, D.; et al. USP5 inhibition via bone marrow-targeted engineered exosomes for myeloproliferative neoplasms therapy. *J. Nanobiotechnol.* **2025**, *23*, 501. DOI PubMed PMC
161. Yin, Y.; Han, X.; Li, C.; et al. The status of industrialization and development of exosomes as a drug delivery system: a review. *Front. Pharmacol.* **2022**, *13*, 961127. DOI PubMed PMC
162. Nguyen, V. D.; Kim, H. Y.; Choi, Y. H.; Park, J.; Choi, E. Tumor-derived extracellular vesicles for the active targeting and effective treatment of colorectal tumors *in vivo*. *Drug. Deliv.* **2022**, *29*, 2621-31. DOI PubMed PMC
163. Zhan, Q.; Yi, K.; Qi, H.; et al. Engineering blood exosomes for tumor-targeting efficient gene/chemo combination therapy. *Theranostics* **2020**, *10*, 7889-905. DOI PubMed PMC
164. Jiang, L.; Gu, Y.; Du, Y.; Tang, X.; Wu, X.; Liu, J. Engineering exosomes endowed with targeted delivery of triptolide for malignant melanoma therapy. *ACS. Appl. Mater. Interfaces.* **2021**, *13*, 42411-28. DOI
165. Liu, J.; Srivastava, S.; Li, T.; et al. On the feasibility of SERS-based monitoring of drug loading efficiency in exosomes for targeted delivery. *Biosensors* **2025**, *15*, 141. DOI PubMed PMC
166. Wu, J.; Jin, Z.; Fu, T.; et al. Extracellular vesicle-based drug delivery systems in cancer therapy. *Int. J. Mol. Sci.* **2025**, *26*, 4835. DOI PubMed PMC

167. Li, Z.; Ma, Y.; Ren, Y.; Lin, X.; Su, Z.; Zhang, S. Thermal-triggered loading and GSH-responsive releasing property of HBc particles for drug delivery. *J. Controlled. Release.* **2023**, *362*, 784-96. DOI
168. Alvarez-erviti, L.; Seow, Y.; Yin, H.; Betts, C.; Lakhal, S.; Wood, M. J. A. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnol.* **2011**, *29*, 341-5. DOI
169. Wang, C.; Li, N.; Li, Y.; et al. Engineering a HEK-293T exosome-based delivery platform for efficient tumor-targeting chemotherapy/internal irradiation combination therapy. *J. Nanobiotechnol.* **2022**, *20*, 247. DOI PubMed PMC
170. Wu, K.; Chang, Y.; Chiang, R. Y.; Ding, D. CAP-LAMP2b-modified stem cells' extracellular vesicles hybrid with CRISPR-Cas9 targeting ADAMTS4 to reverse IL-1β-induced aggrecan loss in chondrocytes. *Int. J. Mol. Sci.* **2025**, *26*, 9812. DOI PubMed PMC
171. Gu, Y.; Du, Y.; Jiang, L.; et al. αvβ3 integrin-specific exosomes engineered with cyclopeptide for targeted delivery of triptolide against malignant melanoma. *J. Nanobiotechnol.* **2022**, *20*, 384. DOI PubMed PMC
172. Kang, C.; Han, P.; Lee, J. S.; Lee, D.; Kim, D. Anchor, spacer, and ligand-modified engineered exosomes for trackable targeted therapy. *Bioconjugate. Chem.* **2020**, *31*, 2541-52. DOI
173. Tian, Y.; Li, S.; Song, J.; et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. *Biomaterials* **2014**, *35*, 2383-90. DOI
174. Tian, J.; Han, Z.; Song, D.; et al. Engineered exosome for drug delivery: recent development and clinical applications. *Int. J. Nanomed.* **2023**, *18*, 7923-40. DOI PubMed PMC
175. Macdougall, L. J.; Wiley, K. L.; Kloxin, A. M.; Dove, A. P. Design of synthetic extracellular matrices for probing breast cancer cell growth using robust cyctocompatible nucleophilic thiol-yne addition chemistry. *Biomaterials* **2018**, *178*, 435-47. DOI PubMed PMC
176. Lim, G. T.; You, D. G.; Han, H. S.; et al. Bioorthogonally surface-edited extracellular vesicles based on metabolic glycoengineering for CD44-mediated targeting of inflammatory diseases. *J. . Extracell. Vesicle.* **2021**, *10*, e12077. DOI PubMed PMC
177. Aare, M.; Lazarte, J. M. S.; Muthu, M.; Rishi, A. K.; Singh, M. Genetically bio-engineered PD-L1 targeted exosomes for immunotherapy of resistant triple negative breast cancer. *Drug. Deliv. Transl. Res.* **2025**, *16*, 693-710. DOI PubMed PMC
178. Wang, J. S.; Yassin, S.; Lin, A. Y. Utilization of exosome-based therapies to augment anti-PD-1/PD-L1 therapies. *J. Immunother. Cancer.* **2025**, *13*, e010603. DOI PubMed PMC
179. Yin, Z.; Yu, M.; Ma, T.; et al. Mechanisms underlying low-clinical responses to PD-1/PD-L1 blocking antibodies in immunotherapy of cancer: a key role of exosomal PD-L1. *J. Immunother. Cancer.* **2021**, *9*, e001698. DOI PubMed PMC
180. 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. Controlled. Release.* **2020**, *326*, 599-614. DOI
181. Bolaños, K.; León, D.; Jara-Guajardo, P.; et al. In vivo targeted delivery of extracellular vesicle-gold nanorod hybrids to metastatic melanoma lung tumors. *Int. J. Nanomed.* **2025**, *20*, 6983-98. DOI PubMed PMC
182. Chen, H.; Jiang, Y.; Li, X. Adriamycin-loaded exosome with anti-CD20 aptamers selectively suppresses human CD20+ melanoma stem cells. *Skin. Res. Technol.* **2022**, *29*, e13259. DOI PubMed PMC
183. Li, L.; He, D.; Guo, Q.; et al. Exosome-liposome hybrid nanoparticle codelivery of TP and miR497 conspicuously overcomes chemoresistant ovarian cancer. *J. Nanobiotechnol.* **2022**, *20*, 50. DOI PubMed PMC
184. Xie, Q.; Gao, Y.; Chen, W.; et al. Membrane fusogenic liposomes facilitate the production of immunostimulatory extracellular vesicles for enhanced cancer therapy. *J. Mater. Chem. B.* **2025**, *13*, 7490-501. DOI
185. Lee, Y.; Huang, C. Engineered perfluorochemical cancer-derived exosomes loaded with indocyanine green and camptothecin provide targeted photochemotherapy for effective cancer treatment. *Int. J. Nanomed.* **2025**, *20*, 327-42. DOI PubMed PMC
186. Ho, M. Y.; Liu, S.; Xing, B. Bacteria extracellular vesicle as nanopharmaceuticals for versatile biomedical potential. *Nano. Converg.* **2024**, *11*, 28. DOI PubMed PMC
187. Gao, Y.; Liu, J.; Wu, M.; et al. Photosensitive hybrid γδ-T exosomes for targeted cancer photoimmunotherapy. *ACS. Nano.* **2025**, *19*, 4251-68. DOI PubMed PMC
188. Zhang, M.; Hu, S.; Liu, L.; et al. Engineered exosomes from different sources for cancer-targeted therapy. *Sig. Transduct. Target. Ther.* **2023**, *8*, 124. DOI PubMed PMC
189. Hazekawa, M.; Nishinakagawa, T.; Hosokawa, M.; Ishibashi, D. Development of an organ-directed exosome-based siRNA-carrier derived from autologous serum for lung metastases and testing in the B16/BL6 spontaneous lung metastasis model. *Pharmaceutics* **2022**, *14*, 815. DOI PubMed PMC
190. Santos, N. L.; Bustos, S. O.; Reis, P. P.; Chammas, R.; Andrade, L. N. S. Extracellular vesicle-packaged miR-195-5p sensitizes melanoma to targeted therapy with kinase inhibitors. *Cells* **2023**, *12*, 1317. DOI PubMed PMC
191. Park, J. H.; Lee, S.; Jeon, H.; et al. A novel convex acoustic lens-attached ultrasound drug delivery system and its testing in a murine melanoma subcutaneous model. *Int. J. Pharm.* **2023**, *642*, 123118. DOI
192. Duan, M.; Chen, B.; Yi, X.; et al. Doxorubicin delivery by pYEEIE peptide-functionalized rhodiola rosea-derived exosome-like nanovesicles for targeted melanoma therapy. *Front. Pharmacol.* **2025**, *16*, 1619998. DOI PubMed PMC

193. Lee, J. H.; Song, J.; Kim, I. G.; et al. Exosome-mediated delivery of transforming growth factor-β receptor 1 kinase inhibitors and toll-like receptor 7/8 agonists for combination therapy of tumors. *Acta. Biomater.* **2022**, *141*, 354-63. DOI
194. Huang, C.; Zhou, Y.; Feng, X.; Wang, J.; Li, Y.; Yao, X. Delivery of engineered primary tumor-derived exosomes effectively suppressed the colorectal cancer chemoresistance and liver metastasis. *ACS. Nano.* **2023**, *17*, 10313-26. DOI
195. Ehexige, E.; Bao, M.; Bazarjav, P.; et al. Silencing of STAT3 via peptidomimetic LNP-mediated systemic delivery of RNAi downregulates PD-L1 and inhibits melanoma growth. *Biomolecules* **2020**, *10*, 285. DOI PubMed PMC
196. Faruqu, F. N.; Wang, J. T.; Xu, L.; et al. Membrane radiolabelling of exosomes for comparative biodistribution analysis in immunocompetent and immunodeficient mice - a novel and universal approach. *Theranostics* **2019**, *9*, 1666-82. DOI PubMed PMC
197. Villa, A.; Crescenti, D.; De, Mitri. Z.; et al. Preclinical pharmacology of patient-derived extracellular vesicles for the intraoperative imaging of tumors. *Theranostics* **2024**, *14*, 6301-18. DOI PubMed PMC
198. Sadeghi, S.; Tehrani, F. R.; Tahmasebi, S.; Shafiee, A.; Hashemi, S. M. Exosome engineering in cell therapy and drug delivery. *Inflammopharmacology* **2023**, *31*, 145-69. DOI PubMed PMC
199. Antimisiaris, S. G.; Mourtas, S.; Marazioti, A. Exosomes and exosome-inspired vesicles for targeted drug delivery. *Pharmaceutics* **2018**, *10*, 218. DOI PubMed PMC
200. Kostyusheva, A.; Romano, E.; Yan, N.; Lopus, M.; Zamyatnin, A. A.; Parodi, A. Breaking barriers in targeted therapy: advancing exosome Isolation, Engineering, and imaging. *Adv. Drug. Delivery. Rev.* **2025**, *218*, 115522. DOI PubMed
201. Zhao, S.; Di, Y.; Fan, H.; et al. Targeted delivery of extracellular vesicles: the mechanisms, techniques and therapeutic applications. *Mol. Biomed.* **2024**, *5*, 60. DOI PubMed PMC
202. Hu, M.; Han, Y.; Zhang, X.; et al. Extracellular vesicles for targeted drug delivery: advances in surface modification strategies and therapeutic applications. *J. Transl. Med.* **2025**, *23*, 1028. DOI PubMed PMC
203. Leary, N.; Walser, S.; He, Y.; et al. Melanoma-derived extracellular vesicles mediate lymphatic remodelling and impair tumour immunity in draining lymph nodes. *J. Extracell. Vesicle.* **2022**, *11*, e12197. DOI PubMed PMC
204. Zhang, W.; Zhong, W.; Wang, B.; et al. ICAM-1-mediated adhesion is a prerequisite for exosome-induced T cell suppression. *Dev. Cell.* **2022**, *57*, 329-43.e7. DOI PubMed PMC
205. Jackson Cullison, S. R.; Flemming, J. P.; Karagoz, K.; Wermuth, P. J.; Mahoney, M. G. Mechanisms of extracellular vesicle uptake and implications for the design of cancer therapeutics. *J. Extracell. Bio.* **2024**, *3*, e70017. DOI PubMed PMC
206. Song, H.; Chen, X.; Hao, Y.; Wang, J.; Xie, Q.; Wang, X. Nanoengineering facilitating the target mission: targeted extracellular vesicles delivery systems design. *J. Nanobiotechnol.* **2022**, *20*, 431. DOI PubMed PMC
207. Bader, J.; Brigger, F.; Leroux, J. Extracellular vesicles versus lipid nanoparticles for the delivery of nucleic acids. *Adv. Drug. Delivery. Rev.* **2024**, *215*, 115461. DOI PubMed
208. Gandek, T. B.; Van Der Koog, L.; Nagelkerke, A. A comparison of cellular uptake mechanisms, delivery efficacy, and intracellular fate between liposomes and extracellular vesicles. *Adv. Healthc. Mater.* **2023**, *12*, 2300319. DOI PubMed PMC
209. Nieuwland, R.; Siljander, P. R. M. A beginner's guide to study extracellular vesicles in human blood plasma and serum. *J. Extracell. Vesicle.* **2024**, *13*, e12400. DOI PubMed PMC
210. György, B.; Pálóczi, K.; Balbisi, M.; et al. Effect of the 35 nm and 70 nm size exclusion chromatography (SEC) column and plasma storage time on separated extracellular vesicles. *Curr. Issues. Mol. Biol.* **2024**, *46*, 4337-57. DOI PubMed PMC
211. Alter, C. L.; Lotter, C.; Puligilla, R. D.; et al. Nano plasma membrane vesicle-lipid nanoparticle hybrids for enhanced gene delivery and expression. *Adv. Healthc. Mater.* **2024**, *14*, 2401888. DOI PubMed PMC
212. Li, Q.; Li, Y.; Shao, J.; et al. Exploring regulatory frameworks for exosome therapy: insights and perspectives. *Health. Care. Sci.* **2025**, *4*, 299-309. DOI PubMed PMC
213. Mizenko, R. R.; Feaver, M.; Bozkurt, B. T.; et al. A critical systematic review of extracellular vesicle clinical trials. *J. Extracell. Vesicle.* **2024**, *13*, e12510. DOI PubMed PMC
214. Sun, D.; Zhuang, X.; Xiang, X.; et al. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Mol. Ther.* **2010**, *18*, 1606-14. DOI PubMed PMC
215. Wahlgren, J.; Karlson, T. D. L.; Brisslert, M.; et al. Plasma exosomes can deliver exogenous short interfering RNA to monocytes and lymphocytes. *Nucleic. Acids. Res.* **2012**, *40*, e130. DOI PubMed PMC
216. Kim, M. S.; Haney, M. J.; Zhao, Y.; et al. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. *Nanomed. Nanotechnol. Biol. Med.* **2016**, *12*, 655-64. DOI PubMed PMC
217. Guo, P.; Busatto, S.; Huang, J.; Morad, G.; Moses, M. A. A facile magnetic extrusion method for preparing endosome-derived vesicles for cancer drug delivery. *Adv. Funct. Mater.* **2021**, *31*, 2008326. DOI PubMed PMC
218. Ohno, S.; Takanashi, M.; Sudo, K.; et al. Systemically injected exosomes targeted to EGFR deliver antitumor MicroRNA to breast cancer cells. *Mol. Ther.* **2013**, *21*, 185-91. DOI PubMed PMC

219. Pham, T. C.; Jayasinghe, M. K.; Pham, T. T.; et al. Covalent conjugation of extracellular vesicles with peptides and nanobodies for targeted therapeutic delivery. *J. Extracell. Vesicle.* **2021**, *10*, e12057. DOI PubMed PMC
220. Smyth, T.; Petrova, K.; Payton, N. M.; et al. Surface functionalization of exosomes using click chemistry. *Bioconjugate. Chem.* **2014**, *25*, 1777-84. DOI PubMed PMC
221. Ferraz, L. S.; Souza, A. S. O.; Rodrigues, T.; Cabral-Castro, M. J. Liquid biopsy as a potential tool for diagnosis and clinical monitoring of cutaneous and uveal melanoma. *J. Liquid. Biopsy.* **2025**, *9*, 100306. DOI PubMed PMC
222. Uman, S.; Worthington, K.; Dominic, J.; Atluri, P.; Burdick, J. A. Comparison of technologies for manufacturing extracellular vesicles for therapeutic applications. *Trends. Biotechnol.* **2026**, *44*, 1079-99. DOI PubMed PMC
223. Javdani-Mallak, A.; Mowla, S. J.; Alibolandi, M. Tumor-derived exosomes and their application in cancer treatment. *J. Transl. Med.* **2025**, *23*, 751. DOI PubMed PMC

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