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Review article

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The status of extracellular vesicles as drug carriers and therapeutics

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Abstract

Owing to their natural origin and biocompatibility, extracellular vesicles (EVs) are being recognized as next-generation vehicles for targeted drug delivery. Despite their potential as therapeutic carriers, EVs suffer from heterogeneity, low yields, limited cargo loading efficiency and rapid clearance by the mononuclear phagocyte system. Since the first EV-based clinical trial in 2005, more than 100 clinical trials have investigated the use of EVs as therapeutics and drug carriers. Despite this, no EV-based therapies have received regulatory approval to date. This gap between preclinical research activity and clinical translation underscores persistent scientific challenges and regulatory hurdles that continue to impede the advancement of EV-based therapeutics. In this Review, we examine the research articles published in the field between 2012 and 2024 (38,177 articles), highlighting key developments, persistent challenges and evolving assumptions. We review the current EV landscape and clinical trials, focusing on their organotropism and use as carriers for therapeutics. We compare their advantages and limitations in relation to other nanoparticles, such as lipid nanoparticles and liposomes, and examine how labelling strategies and cell sources influence EV biodistribution. Finally, we outline translational considerations for EV-based therapeutics and propose additional reporting standards, complementing the MISEV 2023 guidelines.

Sections

Introduction

The preclinical research landscape of EVs

The clinical research landscape of EVs

Drug loading, efficiency and dose–response correlations

EVs and tissue tropism

EVs as drug delivery carriers

Outlook

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Key points

- Extracellular vesicles (EVs) have emerged as promising drug carriers owing to their biocompatibility, stability and ability to transport a wide range of molecular cargo.
- Most current clinical studies focus on the intrinsic therapeutic potential of EVs, with only about 5% incorporating exogenous drugs.
- Of the articles reporting EV-mediated small interfering RNA delivery, 87% do not report dose–response curves, which are essential for evaluating therapeutic efficacy, ensuring pharmacological specificity and enabling comparisons across studies.
- The cell source of intravenously administered EVs minimally influences organ targeting, with most accumulating primarily in organs of the mononuclear phagocyte system.
- Tumour-derived and non-tumour-derived EVs show similar tumour accumulation in biodistribution studies, suggesting that passive mechanisms, such as the enhanced permeability and retention effect, might be responsible.

Introduction

The promise of leveraging a biologically evolved vesicle for targeted drug delivery has focused the efforts of biologists, engineers and drug delivery scientists on nature's very own nanocarriers, extracellular vesicles (EVs). EVs are membrane-bound structures composed of a lipid bilayer, secreted from eukaryotic cells via the endosomal pathway or plasma membrane budding[1]. Originally thought of as a cellular waste management system, EVs mediate cell-to-cell communication to maintain homeostasis via packaging and delivering several active molecules[2,3]. Under pathological conditions, EVs can become potent and damaging signalling vectors, modulating the phenotype of recipient cells[4]. Disruption of parental cell homeostasis can alter the protein, lipid and RNA content of EVs and, consequently, the messages that they deliver[5]. Thus, efforts to understand how normal and diseased cells package and deliver EVs are important not only for elucidating fundamental biology but also for harnessing EVs as therapeutics and drug delivery carriers[6–8].

Unlike conventional synthetic nanocarriers, EVs typically exhibit intrinsic biological and therapeutic effects, and their cargos are genetically encodable, facilitating the incorporation of various biomacromolecules such as nucleic acid therapies and protein-based drugs[9–11]. Furthermore, EVs exhibit desirable traits for drug delivery such as their favourable biocompatibility, stability and capacity to transport diverse molecular cargoes[6,7,12]. A recent meta-analysis of 21 clinical trials showed that EV-based treatments display a low incidence (0.7%) of serious adverse events (SAEs), which is comparable or better than the incidence displayed by commercially available lipid nanoparticle (LNP) formulations (0.675–1.36%)[13,14]. However, EVs have a limited delivery efficiency and poor organ targeting capabilities[15,16].

With over 117 clinical trials currently investigating EVs as therapeutic agents, the field is experiencing rapid growth (Figs. 1a and 2c). Despite this momentum, no EV-based therapy has yet received regulatory approval. This gap between preclinical research activity and clinical translation underscores persistent scientific challenges and regulatory hurdles that continue to impede the advancement of EV-based therapeutics. A thorough evaluation of the current landscape is urgently needed to identify these barriers and guide the development of safe, effective and clinically translatable EV platforms. As interest in EVs continues to rise, now is a critical time to coordinate efforts that will enable promising technologies to move successfully from bench to bedside. Based on research published between 2012 and 2024 (Supplementary Fig. 1), we examine the different therapeutic applications of EVs and discuss their intrinsic advantages and the key parameters that influence their clinical translation. We further assess the drug-loading capacity of EVs and analyse how labelling strategies, cell sources and surface modifications influence targeting. We conclude by addressing the barriers to clinical translation and propose updated reporting standards to improve the reproducibility and translatability of EV-based therapies.

The preclinical research landscape of EVs

Preclinical research on EVs has predominantly focused on their therapeutic applications, followed by diagnostic, mechanistic and method development studies (Fig. 1a).

Stem cell-derived EVs (particularly from mesenchymal, umbilical cord and embryonic sources) were the most widely used, appearing in ~16% of studies. The strong focus on therapeutic applications (70%) reflects the potential of EVs for regenerative purposes[17,18] (Fig. 1b); for example, stem cell-derived EVs possess RNA and protein profiles reflective of their parent cells, enabling them to stimulate tissue-repair processes[17–19]. Unlike stem cells, EVs are non-replicative and less likely to cause tumour formation, addressing key safety concerns[20].

Similarly, about 36% of blood-derived and plasma-derived EVs (~16% of total studies) were used for therapeutic applications, in particular from red blood cells. These EVs can deliver antisense oligonucleotides, Cas9 mRNA and guide RNAs for indications such as cancer and acute liver disease[21–24]. However, a key limitation is that red blood cells do not divide, which restricts their scalability and production as repeated donor sourcing can introduce variability in EV isolations[21]. Similarly, platelet-derived EVs contain several pro-reparative molecules, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGFβ)[25,26], but they are also limited by low scalability and donor dependence.

Tumour-derived EVs (~14% of total studies) have also been explored extensively for therapeutic purposes (~46%), often used as cancer vaccines owing to their high tumour antigen content but also as drug carriers if tumour-tropic[27–30]. Although tumour-derived EVs might also promote tumour growth and pre-metastatic niche formation[31,32], loading them with chemotherapeutics shifted their function towards counteracting tumorigenic processes in vitro, halting the growth of endothelial cells[29].

Immune cells are another popular source of EVs (~7.4% of total studies), with 53% of them used for therapeutic purposes. For example, when administered via the jugular vein, brain-derived neurotrophic factor (BDNF)-loaded macrophage-derived EVs can achieve a brain accumulation of ~0.1% injected dose (%ID)/g in healthy mice. In both in vitro and in vivo models, dendritic and T regulatory cell-derived EVs modulated immune responses through microRNA (miRNA) transfer and T cell suppression[33–36], and hold potential for autoimmunity, cancer and infectious disease applications[34,37–39].

Human embryonic kidney (HEK)-derived EVs, referenced in 0.32% of studies, have been primarily used as drug carriers (64%). This preference is probably because of their broad accessibility, cost-effectiveness,

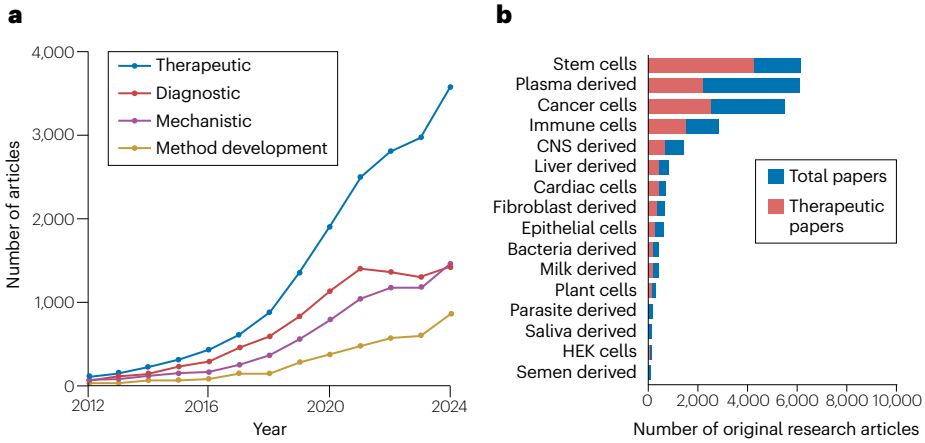


Fig. 1 | Preclinical research on extracellular vesicles. **a**, Research articles from 2012 to 2024 were divided into four key categories: (1) diagnostics, (2) therapeutics (and drug carriers), (3) mechanistic roles, and (4) method or protocol development. **b**, Research articles containing the terms 'exosome*' or 'extracellular vesicle*' were categorized by donor cell type. CNS, central nervous system; HEK, human embryonic kidney.

ease of genetic modification and high-yielding EV production[40]. HEK EV modifications are highly diverse, ranging from decorating EVs with targeting peptides[41,42] to conjugating tracking probes onto EV surface proteins[43]. HEK EVs are frequently regarded as 'model carriers' due to their relative biological inertness[44–46]. For example, when HEK EVs were administered to HepG2 cells, only 0.6% of genes exhibited altered expression, with all changes remaining below a twofold difference[47]. Similarly, minimal immunological or physiological effects following intravenous administration of native HEK EVs have been observed in healthy C57BL/6 and BALB/c mice[46,47]. However, contrasting findings have also been reported. For example, HEK EVs enhance the migratory capacity of MCF-7 breast cancer cells via modulation of cadherin signalling pathways[48]. These observations underscore the importance of considering both the EV donor cell type and the recipient cell context, as their interactions might yield divergent biological outcomes.

For example, EVs from different sources induce distinct gene expression profiles in human fibroblasts in vitro, especially at low doses (20 particles per cell); uterine EVs promoted DNA synthesis, HEK EVs influenced metabolism, mesenchymal stem cell (MSC) EVs supported wound healing and angiogenesis, whereas immune EVs activated immune-related genes[49]. Thus, choosing the right cell source is critical when developing EV-based therapies[6,49].

The clinical research landscape of EVs

The first EV-based clinical trial, launched at the Institut Gustave Roussy, aimed at treating metastatic melanoma using autologous dendritic cell-derived EVs loaded with MAGE3 peptides, with results first published in 2005 (ref. 50). Despite failing to elicit substantial proliferation of MAGE3-specific cytotoxic T cells, the study demonstrated that large-scale EV production was feasible and well tolerated[50]. This study marked the beginning of clinical research into EV therapeutics and has since spurred 117 additional trials investigating their safety and efficacy (Fig. 2a).

This rapid adoption can be attributed to several advantages of EVs over traditional cell therapies. Because EVs inherit many functional traits from their parent cells, they offer therapeutic benefits without the complexities of live cell administration such as dosing variability, tumorigenic risk and uncontrolled differentiation[20]. Unlike cells, EVs are non-replicative, thereby reducing biocontainment concerns[20,51]. Furthermore, they are more amenable to clinical workflows as they can be sterilized by filtration and stored long-term through freezing or lyophilization[52–56]. These practical and biological advantages have positioned EVs as a promising and increasingly viable platform for therapeutic development. However, EVs also face several limitations; for example, their isolations are typically low-yielding and heterogeneous, complicating large-scale production and consistency[57]. Unlike cells, EVs lack chemotactic ability and are rapidly cleared by mononuclear phagocyte system (MPS) organs such as the liver and spleen, reducing circulation time and target tissue accumulation[16].

Interestingly, published clinical trials reveal considerable variability in the dose of administered EVs, possibly because of the variability in composition and efficacy as a product of their diverse cellular sources (Fig. 2). For example, the highest median single dose (~1 trillion EV particles; NCT04493242)[58] was administered intravenously, whereas the lowest dose (~5 billion EV particles; NCT04491240)[59] was delivered via inhalation. This wide range might be caused by how the route of administration affects the dose of EVs needed to achieve therapeutic effects (Fig. 2b). However, such discrepancies might also be a byproduct of the different indications each study sought to treat (COVID-19-related acute respiratory distress syndrome for NCT04493242 and COVID-19-related pneumonia for NCT04491240). There is also variation in how doses are reported, with some studies reporting them in terms of EV protein weight, EV particle number or EV phospholipid weight (Fig. 2b). This lack of standardization can introduce additional variability and complicate direct comparisons across studies.

A large share of clinical studies (72.8%) used stem cell-derived EVs (mostly MSC EVs with ~62%) owing to regulatory precedents and ease of isolation[60] (Fig. 2c,d). Most trials (~80%) focus on regenerative and anti-inflammatory applications of MSC EVs, including several targeting COVID-19-related inflammation and acute respiratory distress syndrome[13,61,62] (Fig. 2c). These regenerative properties are appealing for skin-related applications, with 19.5% of total studies focusing on dermal conditions such as diabetic ulcers or psoriasis. For example, a recent phase I trial investigated the tolerability of topical MSC EVs for psoriasis, reporting no adverse drug reactions (NCT05523011)[63,64]. MSC EVs are also being explored in oncology, such as a trial using anti-*KRAS*^{G12D} small interfering RNA (siRNA)-loaded EVs for pancreatic cancer (NCT03608631)[65–68]. Despite the allogeneic source of EVs, no adverse effects were observed even at the highest dose (six doses of 4.8 mg siRNA EVs over 12 weeks)[69]. Intratumoural CD8$^+$ T cell infiltration was observed in human biopsies, suggesting enhanced anti-tumour efficacy[69]. Furthermore, lower levels of *KRAS*^{G12D} circulating DNA in

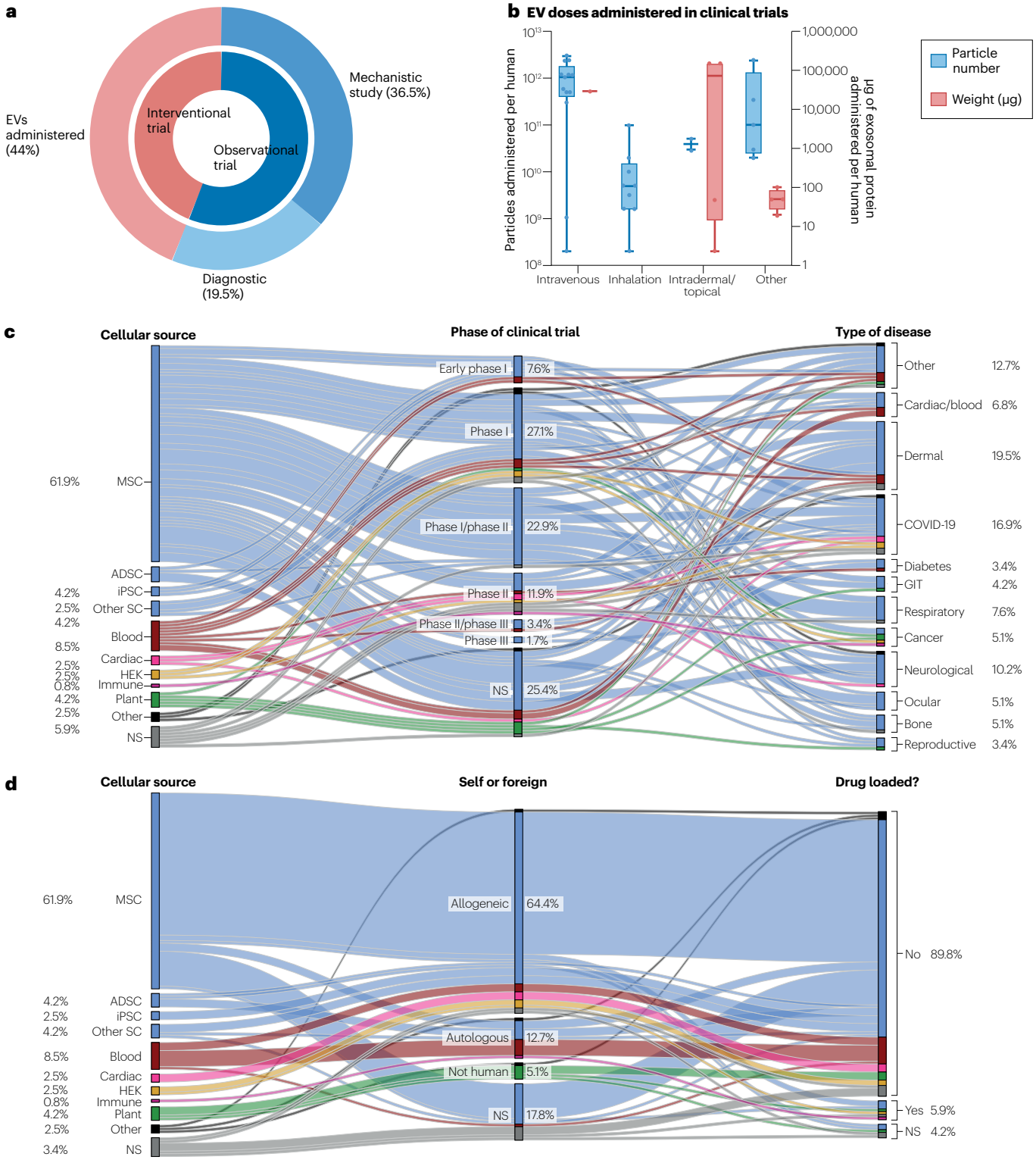


Fig. 2 | Clinical research on therapeutic EVs. a, Overview of 338 clinical studies categorized by study aim. **b**, Dose ranges administered to patients reported either by particle number or weight. **c**, Alluvial plot displaying extracellular vesicle (EV)-based therapies in clinical studies, showcasing the relationship between cell sources, their use in clinical trials from early phase I to phase III and their applications across different diseases. **d**, Alluvial plot illustrating the relationship between cell sources used for EV production, indicating whether the EVs are allogeneic, autologous or of non-human origin, and whether they have been loaded with therapeutic cargo. ADSC, adipose-derived stem cell; GIT, gastrointestinal tract; HEK, human embryonic kidney; iPSC, induced pluripotent stem cell; MSC, mesenchymal stem cell; NS, not specified; SC, stem cell. See Supplementary Information and Supplementary Data for detailed information on studies included.

the blood and lower ERK (a downstream effector of KRAS) detected in biopsied tissues revealed suppression of oncogenic *KRAS*^{G12D} (ref. 69). Although promising for this indication, MSC EVs could also promote M2-like macrophage polarization, angiogenesis and tissue repair in preclinical studies — traits that might inadvertently support tumour progression[70–74]. However, incorporation of anti-*KRAS*^{G12D} siRNA could potentially counteract these effects, shifting the role of MSC EVs from pro-tumorigenic to anti-tumorigenic.

Autologous EVs account for just 12.7% of those used in clinical trials compared to 64.4% for allogeneic EVs (Fig. 2d). Allogeneic EVs offer advantages such as pre-manufacturing, longer shelf-life and on-demand availability, improving scalability and cost-effectiveness[75]. However, donor variability can lead to batch-to-batch heterogeneity, hindering therapeutic consistency[57]. Additionally, they might carry unmatched human leukocyte antigen (HLA) complexes and alloantigens, which can trigger adaptive immune responses[76]. For instance, in a humanized mouse model, EVs from human skin grafts activated alloreactive B cells[77].

To reduce the heterogeneity of MSC-derived EV products, clonally expanded, human telomerase reverse transcriptase (hTERT)-immortalized MSCs have been explored in vitro and in preclinical animal models[78]. However, their safety and immunogenicity must be fully assessed to ensure enhanced proliferation without malignancy or altered traits[79]. Caution is needed, as some immortalization methods, such as SV40 T antigen introduction, can cause genomic instability, potentially altering EV composition and influencing therapeutic consistency[80,81].

Most EV clinical trials are phase I and II (70.0%, 82 trials), with only a minority of trials reported as phase III (~5.1%, 6 trials), highlighting the early stage of their clinical adoption (Fig. 2c). Despite promising clinical findings, the shortage of large, multicentre phase III trials makes it difficult to fully evaluate their clinical efficacy.

Drug loading, efficiency and dose–response correlations

Only ~6% of the clinical trials used drug-loaded EVs (Fig. 2d), suggesting a preference for unmodified EVs[2,17,82–84]. Although this simplifies production and characterization, intrinsic activity alone might not always achieve therapeutic efficacy, highlighting the need for engineered strategies to improve potency[12,53,85].

EV drug loading generally falls into two categories: exogenous loading, in which isolated EVs are loaded with drug cargo, and cellular (or endogenous) loading, in which cells package the cargo before EV release[86] (Fig. 3a). Exogenous methods leverage physical approaches, such as coincubation, electroporation and sonication, or chemical strategies such as surfactant permeabilization[86]. Coincubation is the simplest, involving passive drug incorporation by incubating EVs with the desired compound[86]. Drug loading can be further improved through electroporation or sonication, which transiently disrupt EV membranes to facilitate drug uptake[87–90]. However, physical methods can influence size and membrane integrity and damage EVs and biologically active transmembrane proteins[91–93]. Chemical permeabilization with surfactants is gentler but can introduce contaminants, alter EV composition and increase the risk of haemolysis[94]. Furthermore, mixtures of surfactants, lipids and water can form aggregates that might be misinterpreted as EVs.

To accurately assess the therapeutic contribution of drug-loaded EVs, it is critical to remove any unincorporated or free drug following exogenous loading. Ultracentrifugation is the most common purification method (Fig. 3b). However, it can lead to substantial loss of EVs, potential EV aggregation, and co-isolation of protein or drug contaminants[95]. Size exclusion chromatography and tangential flow filtration can improve EV recovery as they may reduce structural harm to EVs compared to ultracentrifugation[96].

Unlike exogenous methods, cellular loading enables genetic expression of drug cargo within the EV-producing cell, making it ideal for incorporating biologics and gene therapies into EVs[86]. Furthermore, cellular approaches can harness active loading strategies such as using RNA-binding proteins or surface protein display systems to selectively load desired drugs into EVs[97,98].

Definitions of drug loading

Loading capacity (LC) has mainly been reported in two ways in the literature: in terms of EV proteins (weight-to-weight ratio of drug to EV proteins) or EV particles (weight-to-particle ratio of drug to EV particles). Although multiple EV databases exist (which report metrics such as EV composition, particle number and relative abundance of proteins), there is currently no reliable way to correlate EV protein content to the number of EV particles[99,100]. Thus, it is challenging to compare LCs across articles. Either strategy has limitations; for example, typical EV isolation methods, such as ultracentrifugation or precipitation, can retain non-EV proteins if conducted incorrectly, which might skew the LC when reported as the weight-to-weight ratio of drug to EV proteins[95]. Similarly, reporting LC as the weight-to-particle ratio of drug to EV particles could lead to underestimation of the true LC value because of particulate contaminants such as lipoprotein aggregates[101,102]. Detergent controls help eliminate non-vesicular particles from EV counts but the detergent choice is important, as EV subpopulations vary in sensitivity. For example, apoptotic bodies and microvesicles are more susceptible, whereas exosomes require higher concentrations of agents such as SDS and Triton-X[103].

An additional parameter to consider is the loading efficiency, which is defined as the ratio of the final weight of the drug in the carrier to the initial weight of the drug added. Both LC and loading efficiency parameters should be reported. On the one hand, LC describes the dose of carrier required to achieve drug therapeutic concentration; if the LC is too low, then using EVs as carriers might not be viable as excessive amounts of EVs would have to be administered. On the other hand, the loading efficiency reflects process and economic efficiency as it describes the potential waste of unloaded drug.

Loading of small molecules

Among the articles reporting LC, small-molecule drug loading was the most frequently studied (65%). The method mostly used for incorporating small-molecule drugs into EVs was passive diffusion or coincubation (47%), probably owing to the simplicity of the technique (Fig. 3b,c).

Across published studies, sonication and chemical permeabilization demonstrated slightly higher median LC, reaching 9.5% and 9.2% wt/wt, respectively, while passive diffusion yielded a comparable LC of 8.6% wt/wt. Electroporation, by contrast, showed a lower median LC at 3.1% wt/wt (Fig. 3c). These findings align with previous reports demonstrating the superior efficiency of sonication. One study showed that sonication was five times more effective than electroporation for loading paclitaxel into RAW264.7 EVs[90].

The LCs of small molecules loaded via passive diffusion exhibited a wide range (0.73–93.45%), which probably stems from the varied physicochemical properties of the drugs, including their molecular weight and ionization state at the buffer pH (Fig. 3c). These characteristics influence drug incorporation into lipid membranes, potentially

explaining the high variability[104,105]. Interestingly, no correlation was observed between log*P* values and LC percentages, suggesting that hydrophobicity alone does not predict LC for this drug class. However, differences in molecular characteristics between drugs might influence LC values; therefore, caution should be exercised when making direct comparisons between them.

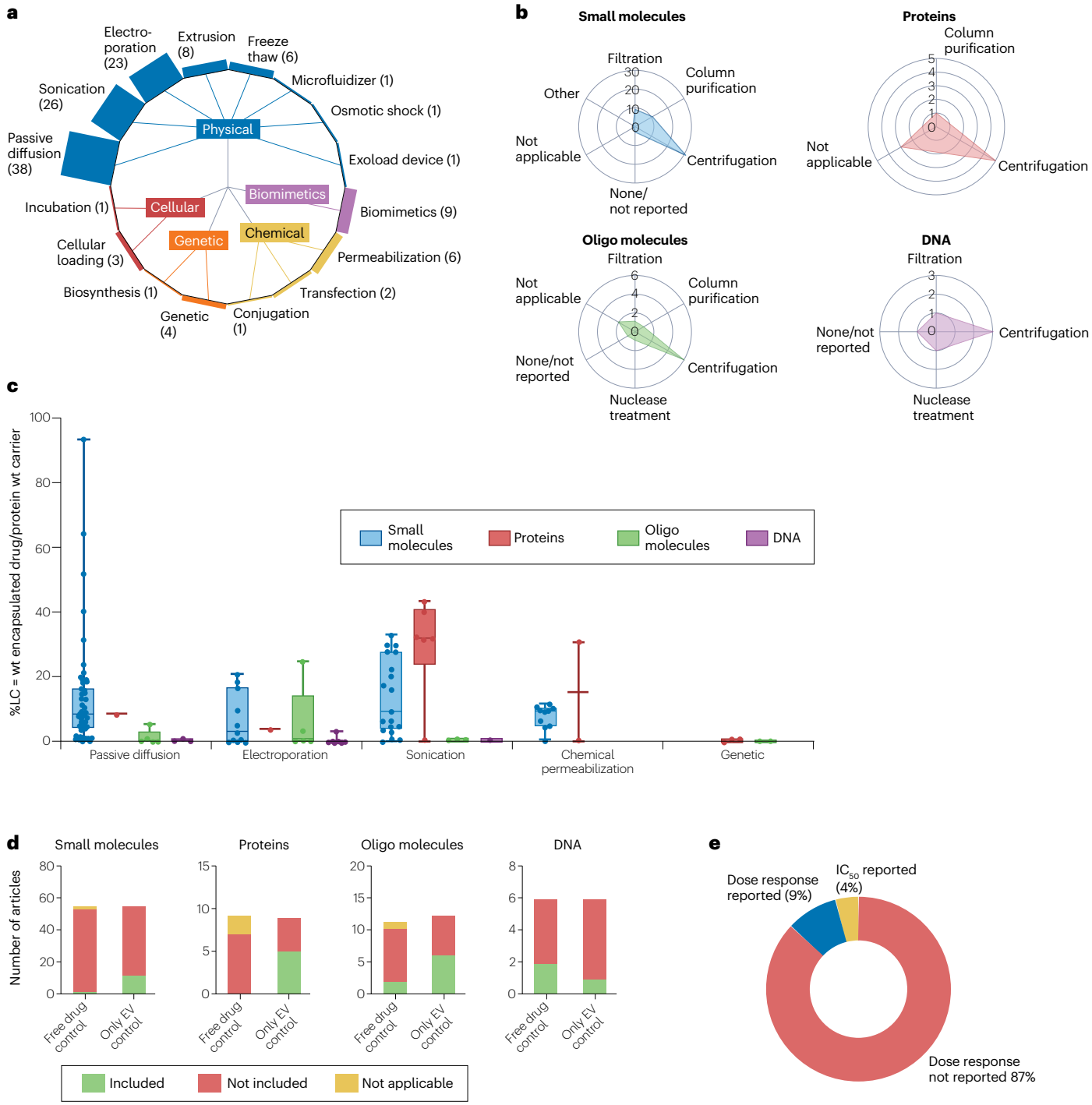


Fig. 3 | EVs as drug carriers. a, Distribution of studies using various drug-loading methods. **b**, Methods used for post-loading removal of unloaded drugs. **c**, Loading capacity (%LC) of various cargoes achieved using each method from 131 articles. **d**, Percentage of articles performing necessary controls to evaluate loading capacities. **e**, Percentage of articles reporting a dose response with a half-maximal inhibitory concentration (IC_{50}; 4%), without an IC_{50} (9%) and not reporting a dose response (87%) from 98 articles that delivered small interfering RNA gene knockdown with extracellular vesicles (EVs). wt, weight. See Supplementary Fig. 1 and Supplementary Data for the list of publications included.

Loading of proteins

Among the articles reporting LC, protein drugs were the most frequently loaded using sonication (38%) (Fig. 3a,c). Sonication also led to the highest LC for proteins, with a median LC of 32.8% wt/wt as opposed to genetic expression (0.5% wt/wt, the lowest median). This low LC for genetic expression is possibly caused by the inefficient trafficking of components from the cytoplasm into compartments that are part of the EV biogenesis pathway[106].

Nonetheless, protein loading can be boosted using protein domains that are highly enriched on EVs[10]. For example, Codiak Biosciences harnessed a proprietary scaffold protein, PTGRFN, to enrich IL-12 into EVs, with expression levels reaching ~1,000 copies per EV[107]. This genetic approach is less damaging than electroporation or sonication and better preserves EV function while simplifying scalable manufacturing by eliminating post-isolation steps.

An advantage of EVs is that they are well adapted to carrying transmembrane proteins while maintaining a small size, unlike liposomes and LNPs[108–110]. For example, EVs engineered to bear ACE2 receptors function as decoys to sequester viruses, protecting mice from infection[111]. This approach is difficult to achieve with traditional carriers as transmembrane proteins are hard to express and often lose their biological activity outside of their native membrane environment[1,112].

Loading of nucleic acids

Analysis of the LC for nucleic acids revealed that no methods achieved a median LC greater than 1% wt/wt (Fig. 3a,c). One possible explanation is that nucleic acids are hydrophilic and require encapsulation into the aqueous core of EVs. Furthermore, both nucleic acids and EV membranes are negatively charged, thereby leading to electrostatic repulsion[113]. Nonetheless, LCs of 35% have also been reported when electroporating EVs derived from C2C12 cells with anti-BACE1 siRNA[114]. However, during electroporation, siRNA molecules tend to aggregate, forming precipitates that are subsequently spun down with EVs, possibly inflating the measured LC[115].

To overcome this challenge, ethylenediaminetetraacetic acid (EDTA) can be used to prevent aggregation; however, this further reduced the measured LC (less than 0.05% wt/wt)[115]. These findings underscore the complexity of loading therapeutic cargo into EVs and emphasize the importance of rigorously evaluating loading techniques to ensure accurate assessments of their LC and loading efficiency.

Nucleic acids have also been loaded into EVs by chemical transfection, wherein the cargo is delivered to cells (cellular loading) or EVs (exogenous loading) using positively charged complexation agents such as lipofectamine or polyethyleneimine[116]. However, this method carries the risk of contamination caused by the fusion of the positively charged transfection reagents with the negatively charged EV membrane, resulting in a product that is a mixture rather than a fully native EV[116,117].

Loading of plasmid DNA into EVs presents an even greater challenge than siRNAs due to the substantially larger molecular weight of DNA, resulting in a median LC of less than 0.4 wt/wt. One study demonstrated a strong correlation between the size of DNA and the measured LC; a 250-bp linear DNA had a 45-fold higher LC than 4,000-bp linear DNA when electroporated into EVs[118]. mRNA exhibited a similar trend, with only 8.71×10^{-13} pmol/EV particle[23]. The loading efficiencies of plasmid DNA and mRNA are generally lower compared to those of siRNA and miRNA (in the range of 0.11–25% wt/wt) owing to the larger size of the former.

The broad range of LCs possibly reflects the absence of critical experimental controls in a substantial number of studies. For example, 88% of articles did not report any free drug control and 71% did not include a native EV control (Fig. 3d). These controls are essential for assessing the efficiency of post-loading isolation methods in removing excess, unencapsulated or aggregated drug. Thus, it is challenging to recommend one loading method over another without robust experimental controls (Box 1).

Loading uniformity

Loading of cargo into EVs might not occur uniformly, with certain EV subpopulations being more enriched than others[119,120]. This heterogeneity in payload distribution can influence the therapeutic efficacy and consistency of EV-based treatments. For example, lower density EVs are enriched with specific proteins (such as ACTN4 and CCNY) and contain 18S and 28S ribosomal RNA, whereas higher density EVs predominantly carry EPHA2 and a broader spectrum of RNA species. These compositional differences translate into distinct effects on recipient cells, as variations in EV cargo lead to differential gene expression outcomes in endothelial cells[121]. Similar trends can be observed with endogenously loaded cargoes. For example, electroporation of a fluorogenic RNA Mango/EXO-Code aptamer into MDA-MB-231 cells revealed that the EVs released could be classified into three distinct populations — 'low', 'medium' and 'high' incorporation of the RNA aptamer[119]. Similar loading heterogeneity has also been observed in LNPs when loaded with mRNA encoding green fluorescent protein (GFP)[122]. This variability poses challenges for standardization and highlights the need to control payload distribution. Methods capable of isolating EV subpopulations and assessing their efficacy in relation to cargo loading would help clarify underlying mechanisms. Moreover, uncovering why certain EV subpopulations carry more cargo than others could inform strategies to improve their therapeutic performance.

Reporting of dose–response relationships

In addition to drug loading, it is essential to report dose–response relationships and the corresponding inhibitory (IC_{50}) or effective (EC_{50}) concentrations — these parameters represent the concentration of a compound required to elicit 50% of its maximal inhibitory or stimulatory effect, respectively, in a biological system. Reporting IC_{50} and EC_{50} values provides a standardized metric for comparing the efficacy of different substances but also establishes whether an observed effect is specific and dose-dependent, facilitating benchmarking and guiding therapeutic development. Our analysis revealed that 87% of the studies on siRNA delivery using EVs did not report a dose–response relationship, and only 4% of studies reported an IC_{50} (Fig. 3e). This absence limits quantitative insight into pharmacological potency and specificity, hinders understanding of underlying biological mechanisms and poses challenges for meeting safety and regulatory requirements[123]. Furthermore, dose–response curves should be reported based on the quantity of active molecules in the EVs in addition to EV count or EV weight, when possible. Quantifying the active drug enables more accurate benchmarking of therapeutic efficacy across different EV formulations, especially when LCs vary substantially.

EVs and tissue tropism

EVs are also being investigated as mediators of targeted intercellular communication owing to the diverse biomolecules decorating their surface. This molecular diversity, which reflects their cell of origin, has prompted exploration into whether EVs exhibit organotropism, that is, preferential targeting of specific organs (Fig. 4). Although some studies suggest that EVs derived from specific donor cells display cell-specific

Box 1 | EV heterogeneity and mechanisms of action

Despite their therapeutic potential, identifying the active components in extracellular vesicles (EVs) is challenging because they contain subpopulations with varying lipid, protein and nucleic acid composition, and because most purification methods isolate them by size and density and not based on biomarkers, which is not sufficient for quality control purposes[6].

EVs are complex mixtures of macromolecules, influenced by culture conditions, cellular passage number and cell density[214]. Despite efforts to elucidate specific mechanisms of action, it is important to identify if a single molecule, a subpopulation of EVs or a synergy of both is responsible for the effects observed. Moreover, the use of fetal bovine serum introduces batch-to-batch variability[215], hindering reproducibility and therapeutic translation[146,216].

To improve rigour and reproducibility, appropriate controls must be selected; negative controls should include fresh media and detergent-treated EV samples, whereas positive controls should consist of non-EV molecules that produce a positive response in functional assays. The field would also benefit from EV standards, enabling comparisons across laboratories (Box 2).

Nanoparticle flow cytometry enables high-throughput, single-particle analysis of analytes[217], and can be used to identify different EV subpopulations based on surface markers (such as CD63, CD81 and CD9) and distinguish them from non-vesicular particles of similar size[218,219]. For example, EVs can be labelled with carboxyfluorescein diacetate succinimidyl ester, which is activated by cytosolic esterases within the EV lumen when present. This strategy can be combined with surface marker stains to differentiate them from similar-sized nanoparticles such as lipoproteins, protein aggregates, dye aggregates or dust[220]. Fluorescence-activated cell sorting or immunomagnetic separation can also separate nano-sized particles with high purity[220–222]. When drugs are loaded into EVs, nano flow cytometry can further delineate which subset of EVs contains the most drug and evaluate payload distribution across different particles. Combining nano flow cytometry with functional assays will enable the assessment of major and minor contributors to therapeutic activity.

Super-resolution microscopy, such as direct stochastic optical reconstruction microscopy (dSTORM), enables sensitive, single-particle analysis of EVs[223]. dSTORM uses photo-switchable fluorophores to achieve super-resolution (that is, below the diffraction limit of light), resolving nanostructures and providing detailed insights not possible with conventional microscopy[224]. Despite its lower throughput compared to nano flow cytometry, dSTORM can characterize EVs based on surface protein expression and visualize protein microdomains on the EV surface[217,225].

To identify the active components of EVs, each fraction should be tested using a functional assay. Follow-up cargo profiling with mass spectroscopy and RNA sequencing can clarify mechanisms and support clinical translation.

uptake in vitro[124–127] or exhibit tissue tropism based on their origin[128], others report that EVs predominantly accumulate in MPS organs[16]. In this section, we discuss patterns of EV biodistribution accounting for differences in labelling methods, engineering strategies and cell sources (Figs. 4 and 5).

The role of labelling in biodistribution

Three main labelling strategies have been used to assess the biodistribution of EVs: fluorescence, radiolabelling and luciferase labelling. About 51% of the studies used fluorescent lipid dyes to study in vivo biodistribution of EVs (Fig. 4a). These dyes intercalate into the EV membrane similarly to how liposomes are labelled[86]. However, dyes such as PKH can increase vesicle size and non-specifically stain lipoprotein aggregates (which are similar in size to EVs)[129–132], thereby skewing biodistribution results.

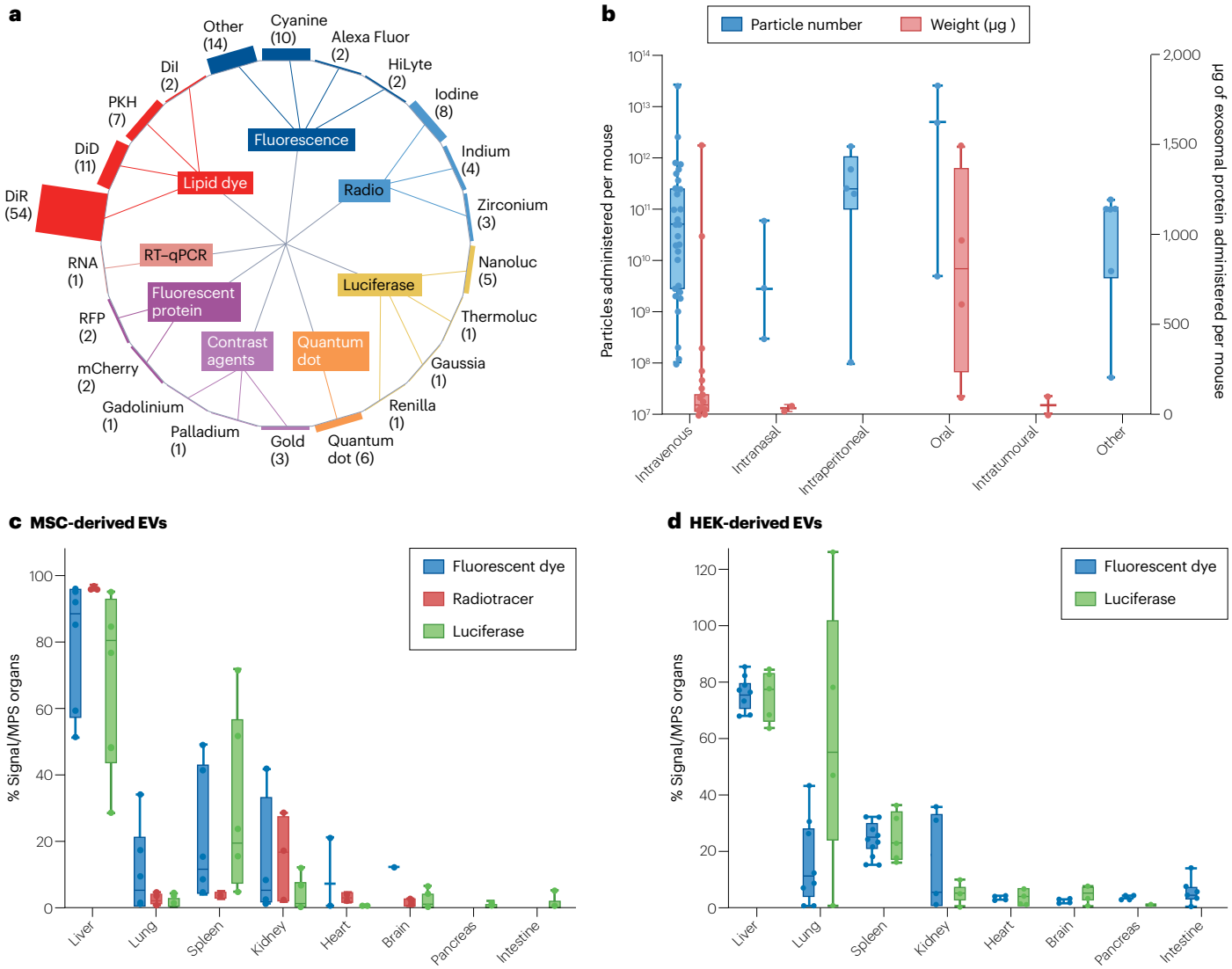
A smaller percentage of studies used fluorescent dyes chemically conjugated to EV surface proteins (19%), offering more stable labelling than lipophilic, lipid-inserting dyes by avoiding dye transfer or non-specific staining of lipoproteins[133]. Other strategies label EV tetraspanins (such as CD63 or CD9, which are enriched on the EV bilayer) with luciferase (~6%) or fluorescent proteins (~3%)[134,135]. However, tetraspanin expression is heterogeneous and varies by cell type, and their knockdown does not fully impair EV biogenesis, suggesting that tetraspanins are not universally present on all EVs[136,137]. Thus, EV subpopulations lacking surface tetraspanins might be overlooked.

About 11% of reports used radiotracers to study the in vivo biodistribution of EVs (Fig. 4a). This method involves chelating a radioisotope and integrating the complex onto the EV surface[138]. Radioisotopes can even be directly attached to EVs without the need for chelation[139]. Radiotracer labelling offers excellent sensitivity and signal-to-noise ratio, making it an attractive strategy[140]. However, surface modification of EVs might influence their targeting or internalization, potentially altering biodistribution patterns[140,141].

Notably, despite the implication of miRNAs and other non-coding RNAs in disease progression, only 0.5% of articles tracked the in vivo biodistribution of RNA cargo[142–144] (Fig. 4a). This under-exploration might stem from the lack of reliable and sensitive RNA tracking tools. However, recent advances, such as the use of fluorogenic RNA aptamers, offer promising solutions. These aptamers can be selectively sorted into EVs and produce a fluorescent signal upon binding to thiazole orange dye, enabling real-time visualization of RNA cargo[119]. These aptamers were actively loaded into EVs via a network of EV-associated RNA sorting proteins and used to determine the extent of EV RNA uptake within cells under in vitro conditions.

Labelling strategies used to track EV biodistribution vary widely across the field, each offering distinct advantages and limitations (Fig. 4e). Notably, although liver accumulation remains consistent across most studies regardless of the labelling method (Fig. 4c,d), certain strategies (such as fluorescence and luciferase labelling) appear to more strongly show EV distribution in other organs, particularly the kidneys and spleen (Fig. 4c). Interestingly, one study demonstrated that higher doses of nanoluciferase-labelled HEK EVs result in unusually high lung accumulation (52.7% of distribution of detected EVs) in healthy NMRI mice when injected intravenously[135]. Further analysis revealed that a high EV dose (5E11 particles/mouse) was used to compensate for the weak luciferase signal, which might have led to EV aggregation and MPS saturation (liver and spleen), enhancing lung distribution[145]. Although fluorescence and luciferase-labelled EVs showed a comparable organ

distribution, radio-labelled EVs tended to show lower signals in non-MPS organs. Given the limited number of studies available and included in our analysis, caution should be taken in drawing conclusions from these biodistribution trends. Moreover, comparative studies are needed to confirm how labelling method and dosing influence biodistribution outcomes. Reproducibility remains a challenge due to experimental



e

	Lipophilic dye	Radiotracer	Luciferase reporter
Pros	• Easy and fast labelling and imaging • No ionizing radiation exposure • FACS for cellular uptake	• High resolution images • No tissue penetration limit • Wide dynamic range	• No label exchange from EV to tissue membranes • Indicates successful payload delivery • Tracks endogenous secretion of EVs
Cons	• Low tissue penetration and lower spatial resolution • Longer half-life than EVs • Can form aggregates • May partition into other organ membranes	• Requires ionizing radiation • No cellular level information • Radiolabels may be cleaved and cleared • Contrast agents may interfere • Residualization may affect biodistribution	• Relies on luciferin biodistribution • Difficult to load EVs uniformly

Fig. 4 | In vivo biodistribution of EVs in murine models. a, Distribution of labelling methods used for tracking the in vivo biodistribution of extracellular vesicles (EVs). **b**, Reported EV doses used in mice by route of administration. **c**,**d**, Biodistribution profiles of mesenchymal stem cell (MSC) (**c**) and human embryonic kidney (HEK) cell (**d**) EVs, labelled using different strategies, observed between 0 and 6 h post-intravenous administration. For ease of comparison, studies reporting units normalized by weight of tissue (such as total radiant efficiency/grams of organ) were multiplied by standard organ weights[213]. See Supplementary Fig. 1 and Supplementary Data for the list of publications included. **e**, Advantages and disadvantages of the three most common labelling strategies used to study EV biodistribution. FACS, fluorescence-activated cell sorting; MPS, mononuclear phagocyte system; RT–qPCR, reverse transcription–quantitative PCR.

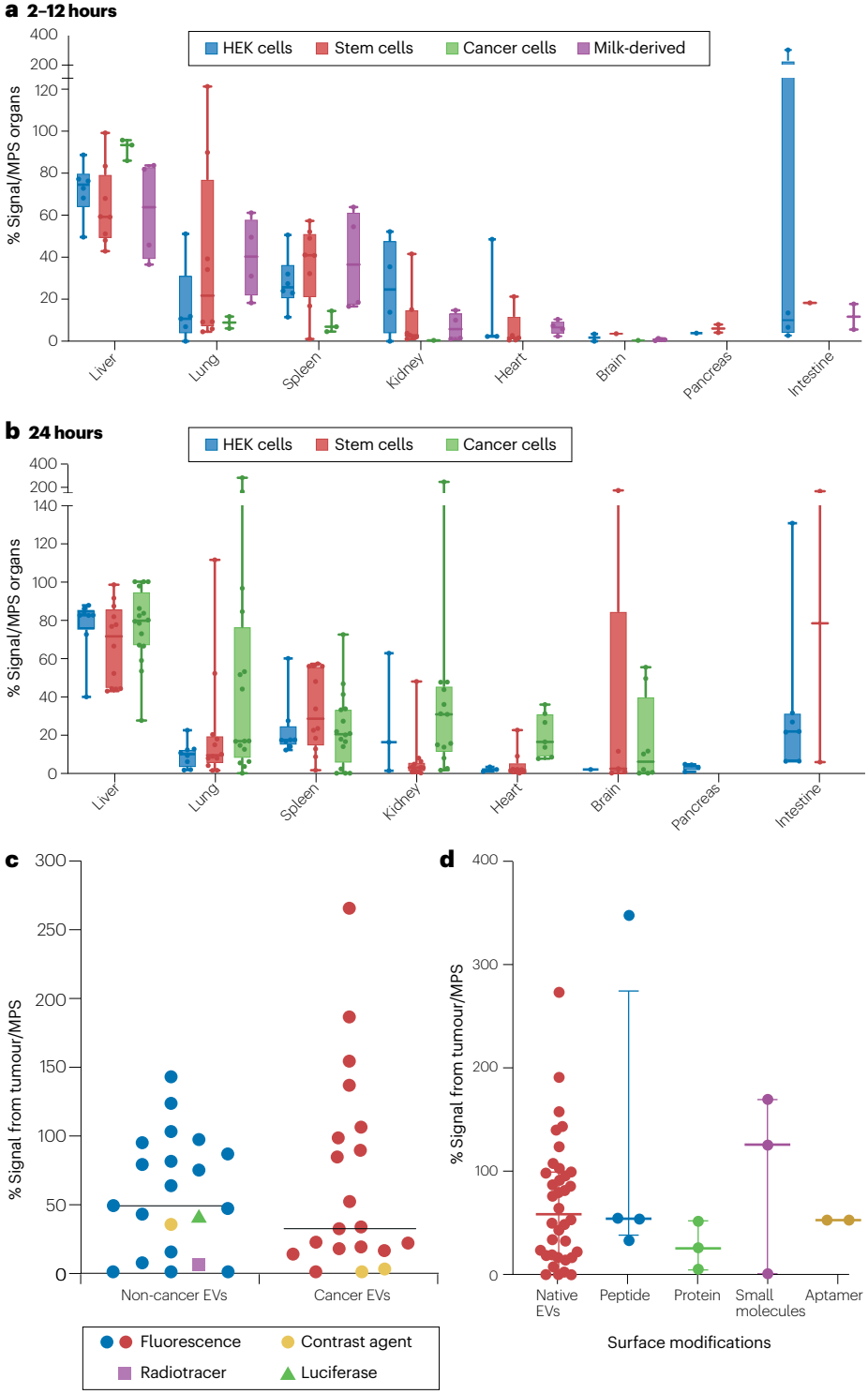


Fig. 5 | In vivo biodistribution of EVs derived from different cell sources. a,**b**, Biodistribution profiles of extracellular vesicles (EVs) labelled with fluorescent dyes from various cell sources observed at 2–12 h (**a**) and at 24 h (**b**) post-intravenous administration. **c**, Accumulation of native EVs derived from cancerous and non-cancerous cell sources in tumours post-intravenous administration. **d**, Accumulation of engineered and native EVs in tumour with respect to mononuclear phagocyte system (MPS) organs. For parts **a**–**d**, see Supplementary Fig. 1 and Supplementary Data for the list of publications included. HEK cells, human embryonic kidney cells. Keywords used for the search in addition to the initial EV screen: ('biodistribution') OR ('tissue AND distribution').

variability and differences in isolation methods, even from the same cell source. Thus, establishing robust standards and reporting practices is key. Although MISEV 2023 (ref. 146) provides broad recommendations for reporting in vivo data, standards for reporting biodistribution and pharmacokinetic data are still needed (Box 2).

The role of cell source in biodistribution

Biodistribution in healthy mice. Tumour-derived EVs express integrins that mediate organ-specific targeting via interactions with the extracellular matrix[128,147]. However, it remains unclear whether the distinct protein profiles of EVs from different cell sources lead to differences

in biodistribution. Thus, we qualitatively reviewed studies that used fluorescently labelled EVs, selected for their relative abundance and consistent use, and tracked their distribution at early (2–12 h) and later (24 h) time points in healthy animal models to minimize confounding factors from disease state or labelling artefacts. We included studies using murine models, such as atherosclerosis and obesity, which have limited impact on EV biodistribution in major organs while maintaining overall trends[148–150].

We focused on widely used EV sources such as HEK cells, stem cells, cancer cells and milk-derived EVs, selected for their prominence and available data, and included only intravenous administrations for consistency. EVs from various cell sources exhibited similar biodistribution trends, with the liver consistently being the primary site of accumulation at both early (2–12 h) and later (24 h) time points (Fig. 5a,b), which aligns with previous findings[16]. One study reported high MCF-7 EV accumulation in the kidney (~2.5-fold higher than in MPS tissues)[151], which might be an artefact caused by the fluorescence-based labelling method; because the size of EVs exceeds the glomerular filtration cut-off (30–50 kDa), renal clearance is unlikely. The signal might instead reflect accumulation of cleaved dye or tracer[152].

EV biodistribution has been reported to vary with source and labelling strategies; however, when key control variables are accounted for — such as methods of isolation, purity, injection route, time points and labelling — differences in organotropism are generally modest. For example, intravenous injection of EVs from C2C12, B16F10 and dendritic cells predominantly accumulate in the liver, followed by accumulation in the lung or spleen of mice[145]. By contrast, administration of lung-tropic EVs (MDA-MB-231) to healthy mice revealed threefold higher uptake in the lungs compared to liver-tropic EVs (BxPC-3, HPFA-II). On the other hand, liver-tropic EVs (BxPC-3, HPAF-II) displayed fourfold higher uptake in the liver compared to lung-tropic EVs (MDA-MB-231)[128]. Importantly, a full biodistribution expressed as a percentage of the %ID was not reported in these studies, limiting the ability to draw definitive conclusions and further highlighting the need for more rigorous, quantitative analysis.

Similarly, EVs from ten different cell sources, despite all crossing the blood–brain barrier[153] with varying rates, accumulate in the brain only minimally compared to the liver and spleen; for example, only 0.12% ID/g of EVs from macrophages accumulated in the brain of healthy mice and less than 0.5% ID/g accumulated in mice with neuroinflammation[34].

Biodistribution in tumour-bearing mice. Tumour tropism has been heavily explored in drug delivery applications owing to its promise of improving drug potency while limiting off-target toxicities[154]. Of all biodistribution studies included in our analysis, a substantial number were performed in tumour-bearing models (~24%). Some studies report that

Box 2 | Improving data reporting practices

In addition to the MISEV guidelines, which set reporting standards to enhance the rigour and reproducibility of extracellular vesicle (EV) studies[146,226], we recommend incorporating additional key metrics and reporting practices to further facilitate their translation into clinical applications.

- **In vitro and in vivo dose–response curves:** Studies investigating the therapeutic activity of EVs should report inhibitory and effective concentrations (IC_{50} and ED_{50}) as a function of particles, protein content and drug concentration (in the case of drug-loaded EVs). Without these values, the efficacy and specificity of different EV formulations cannot be compared.
- **Loading capacity (LC):** When loading drugs into EVs, LC should be reported as the weight of drug loaded normalized to the number of particles and weight of the carrier. For weight/particle count, detergent control should be used to determine the background count of non-vesicular contaminants. For weight/protein content, an unconditioned media control should be used to determine the background amount of media protein contaminants. The process parameters of loading must be described in detail. When using transfection agents, it must be verified whether the agents have been co-loaded within the EVs.
- **Biodistribution dosing:** The dose administered must be reported as total protein and particles dosed, including proper controls (such as dye only) because lipid dyes are known to form aggregates of similar size as EVs. Ideally, a minimum of three dose levels should be performed to assess the effect of saturation of the mononuclear phagocyte system (MPS) on organotropism. Details of the bioanalytical assays used to quantify EV concentrations in blood and in tissues should be reported. Ideally, two orthogonal assays should be used to confirm results (for example, tracing two EV components or using two labelling strategies). At a minimum, the method of sample preparation, assay dynamic range with a representative standard curve and R^2 values, the equation format used for interpolation of unknowns (for example, linear, four-parameter), and the sources of all reagents used in the bioassay should be reported.
- **Labelling strategy and efficiency:** Labels can alter the surface properties and size of EVs, ultimately influencing their biodistribution. Thus, a detailed labelling report should include, but is not limited to, dye concentrations, incubation times, particle recovery/purification methods and percentage of labelled particles.
- **Biodistribution reporting:** Biodistribution should be reported as a percentage of injected dose per gram of tissue in key organs (minimum of blood, lungs, heart, liver, spleen, kidneys) as is standard in pharmacokinetic analyses. The time point of sample collection and whether animals were perfused prior to organ harvesting should also be reported. This is in addition to the MISEV 2023 recommendation to report the dose administered, route of administration, and details of labelling strategy and detection methods.
- **Standards:** The EV field would greatly benefit from establishing EV reference standards and materials defined by function, composition and efficacy, similar to those issued by the National Institute of Standards and Technology (NIST) in other fields (for example, NIST human plasma standards (SRM 1950), NIST gold nanoparticles standards (SRM 8013), or NIST single-wall carbon nanotubes standards (SRM 2483)). These standards and reference materials should include different properties, including size, surface markers and cargo content.
- **Purity:** The polydispersity index (the sample variance in particle size distribution) can be used to capture the size distribution and to assess batch-to-batch variability.

tumour-derived EVs have tropism to their respective tumours (such as in 4T1, HT1080 and HeLa tumour-bearing mouse models), providing a promising strategy for targeted cancer therapies[124,155].

To explore how tumours influence EV biodistribution, we reviewed 24 studies focusing on intravenous administration of either tumour-derived or non-tumour-derived EVs in tumour-bearing mouse models, across all reported time points. To reduce variability associated with bioengineering and targeting strategies, studies involving engineered EVs were discussed separately (Fig. 5d).

Despite variation in methodology and EV source, the overall trend suggests that EV origin (cancer or non-cancer derived) does not substantially influence tumour accumulation as accumulation in the MPS tissues remains the dominant trend (Fig. 5c). These findings challenge the view that tumour-derived EVs preferentially target tumours[124]. For example, administration of EVs from K7M2 osteosarcoma cells (cancerous) and MC3T3-E1 preosteoblasts (non-cancerous) to tumour-bearing BALB/c mice revealed no difference in tumour accumulation[156]. These findings suggest that tumour accumulation might not be due to EV-specific targeting but rather driven by the enhanced permeability and retention effect, a phenomenon by which nanoparticles accumulate into tumours due to leaky vasculature within the tumour microenvironment[154].

We also investigated how engineering EVs with various targeting ligands (such as aptamers, small molecules or proteins) influences their tumour-targeting ability (Fig. 5d). Despite the slight difference, the amount of tumour accumulation of engineered EVs normalized to MPS organs is comparable (0.82–348%) to those of naive EVs (0.2–273.4%), with no substantial improvements. Various improvements have been reported in tumour biodistribution; for example, ligating anti-EGFR nanobodies to EVs improves in vitro uptake by $EGFR^+$ A431 cancer cells by up to fivefold, but the in vivo A431 tumour biodistribution in Crl:NU-$Foxn1^{nu}$ mice was below the limit of detection and comparable to unmodified EVs when injected intravenously[157]. Other studies show more modest improvements; for example, EVs over-expressing CD47 (a tetraspanin that attenuates uptake by MPS organs)[158] had higher tumour distribution (approximately twofold higher) than unmodified EVs[158]. In a similar vein, conjugating epherin-B2 targeting peptides onto HEK293T cell-derived EVs more than doubled tumour accumulation compared to non-modified EVs (~2.5-fold increase in fluorescence signal) in a murine model of ovarian cancer (ID8-ip1 tumour-bearing mice)[159]. Finally, more robust enhancements of tumour biodistribution were achieved by conjugating cell-penetrating peptides and magnetic nanoparticles onto EVs, resulting in an approximately eightfold increase in tumour signal when a magnetic force was applied[160]. However, such approaches might be restricted to more accessible tumours, as lesions buried deep within the body are more challenging to target with magnets. Given these discrepancies, more studies are needed to further elucidate the mechanisms driving EV biodistribution. For example, EVs bind serum proteins, forming a 'protein corona'. Surface modification of EVs can alter protein corona composition, thus altering their biodistribution and physiological effects[161]. Further elucidating how protein corona composition can be modulated to influence EV biodistribution will enable the field to refine engineering strategies for the targeted delivery of EVs.

EVs as drug delivery carriers

EVs have been hailed as 'nature's nanocarriers' owing to their structural similarities to laboratory-formulated nanoparticles and their ability to deliver a diversity of functional biomolecules to recipient cells. Although EVs share compositional and structural similarities with synthetic nanoparticles such as liposomes and LNPs, notable differences exist in terms of structure, delivery efficiency and cargo.

EVs typically contain a broader and more diverse lipid composition than liposomes and LNPs, making them more complex, variable and less defined[6,162,163]. Liposomes are structurally similar to EVs in that they contain an aqueous core and a lipid bilayer but are compositionally simpler[164]. By contrast, LNPs incorporate cationic or ionizable lipids, forming an aqueous compartment with inverse micelles containing the nucleic acid cargo, thereby creating structural differences from EVs and liposomes[6,165,166].

The compositional and structural differences between EVs and synthetic nanoparticles give rise to nuanced differences in their function, which must be considered when choosing an appropriate carrier. To compare EVs with FDA-approved liposomes and LNPs, key metrics should be evaluated, including (1) the capacity to load drugs and therapeutics, (2) the intracellular uptake and endosomal escape, (3) the homing capabilities, and (4) the biocompatibility and immunogenicity of the carrier.

Drug loading

LC directly influences therapeutic efficacy, dosing requirements and manufacturing efficiency. The LCs of EVs vary from 7.4×10^{-7}% to 93.45% wt/wt for small molecules, 0.14–25% wt/wt for RNA interference therapeutics and 0.1–43.5% wt/wt for proteins. In comparison, FDA-approved liposomes and LNPs have LCs of 12.53% wt/wt for Doxil (doxorubicin/liposomes), 8.3% wt/wt for Onpratto (siRNA/LNPs) and 3.96% wt/wt for BioNTech/Pfizer COVID-19 vaccine (mRNA/LNPs). However, differences in reporting make it difficult to directly compare loading across particles; the LC of EVs is determined by protein weight excluding lipid mass, whereas the LC of LNPs or liposomes is calculated using the entire lipid mass. Thus, standardized reporting (such as reporting LC as weight of drug per number of particles) is essential to compare LCs across carriers.

Drug loading varies by cargo type and carrier; for example, porphyrin (a hydrophobic compound) can be loaded twofold more efficiently into EVs than into liposomes composed of phosphocholine and cholesterol (lipid membrane components)[167]. By contrast, LNPs typically exhibit higher nucleic acid loading than EVs owing to their cationic or ionizable lipids that facilitate complexation with negatively charged nucleic acids[168]. For instance, ~4,000-bp plasmid DNA can be loaded with ~95% loading efficiency into LNPs[169] as opposed to less than 1% efficiency for ~6,000–8,000-bp plasmid DNA in EVs[118,168]. Despite the difference in plasmid size making it difficult to draw conclusions, the stark contrast in loading efficiency suggests that the carrier itself influences the loading efficiency.

CRISPR–Cas9 has emerged as a transformative therapeutic modality in recent years[170]. To enhance its clinical applicability, various delivery platforms have been explored, among which EVs and LNPs have shown promise in facilitating the efficient and targeted delivery of CRISPR–Cas9 components[171,172]. For EVs, electroporation is a technique commonly used for loading whole CRISPR–Cas9 ribonucleoproteins (RNPs), plasmids and mRNA[172,173]. By contrast, cellular loading strategies often leverage genetic engineering techniques to load expressed RNPs or mRNA into EVs, avoiding disruption of EV membranes that may otherwise be damaged by exogenous loading approaches[97,98]. The cargo can be loaded passively, relying solely on the overexpression of the desired cargo[174,175]. Alternatively, the mRNA or RNP can be

actively loaded into EVs by harnessing protein dimerization systems or RNA-binding proteins to immobilize the cargo onto EV-associated membranes[97,98]. However, these immobilization strategies might not be suitable for CRISPR–Cas9 as these constructs must dissociate from the EV membrane and translocate into the nucleus to become active. To address this, some have expressed CRISPR–Cas9 constructs fused to a small intein protein engineered with self-cleavage activity to release RNPs from the inner membrane of EVs after loading[9].

mRNA-LNPs are a more conventional platform used to deliver CRISPR–Cas9 for therapeutic applications[176]. A drawback of this approach is that LNP formulations contain ionizable lipids that are known to be immunogenic[177,178] whereas EVs may be less immunogenic depending on the cell source used[46,47,117,179]. Limiting the immunogenicity of the carrier is crucial to CRISPR delivery as Cas9 and its guide RNAs already exert inflammatory effects, which could be compounded by the carrier itself[180–182]. However, a downside of using EVs is that they exhibit very short circulating half-lives in plasma (70–180 min), potentially limiting their therapeutic applications for CRISPR delivery[135,183,184]. Typically, LNP formulations of CRISPR–Cas9 result in in vivo editing efficiencies ranging from ~15% to ~30%, or even higher (70%)[185–187]. By contrast, EVs exhibit lower editing efficiencies ranging anywhere from ~0.2% to ~26%[98,188–190].

Uptake and endosomal escape

There are conflicting views on how EVs bind to and are taken up by cells. For example, compared to similarly sized phosphatidylcholine:cholesterol liposomes (molar ratio of 2:1), tumour-derived EVs (MDA-MB-231, PC3 and MCF-7 cells) showed at least a tenfold higher association with the cancer cells in vitro[191].

In addition to cellular uptake, endosomal escape is a key aspect influencing the delivery efficiency of therapeutic compounds. For example, HeLa cells dosed with HEK EVs released 30% of the internalized EVs into the cytosol[192]. However, delivery efficiencies vary depending on the type of cargo and EV composition. For instance, EVs loaded with Cre recombinase only achieved functional delivery (that is, measurable Cre activity) when engineered to express vesicular stomatitis virus G glycoprotein (VSV-G), a protein that promotes endosomal escape[9]. For instance, when reporter cells (HeLa-TL) were treated with VSV-G-expressing Cre-loaded EVs, ~66% of cells achieved GFP expression, whereas ~0% achieved GFP expression when treated with Cre-loaded EVs lacking VSV-G[9]. In vitro, LNPs exhibit suboptimal endosomal escape, with only a limited release of payload observed in the cytoplasm[193–195]. For example, only 1–2% of the internalized siRNA in HeLa cells treated with siRNA–gold-loaded LNPs were detected in the cytosol using transmission electron microscopy[194]. Of note, both endosomal escape and delivery efficiency are relative metrics influenced by the initial dose and experimental conditions, limiting cross-study comparisons. Additionally, delivery efficiency can vary by recipient cell type, as some cells are inherently more receptive to uptake or transfection[196].

It is unclear whether LNPs or EVs offer better overall delivery efficiency. For example, treating reporter cells (HEK293T CROSS-FIRE) with either single guide RNA (sgRNA) loaded into Onpattro-like LNPs or sgRNA loaded into MDA-MB-231 EVs, revealed that the lowest dose of EVs needed to elicit GFP expression was 2.3 fM sgRNA, more than two orders of magnitude lower than with LNPs (which required 1 pM of sgRNA)[15]. These findings suggest that EVs might be substantially more efficient at delivering RNA species to the cytosol. Similarly, EVs from NSC-34 cells require up to 300-fold less siRNA to achieve equivalent GFP knockdown in the liver of healthy mice compared with LNPs[197]. By contrast, MC3 LNPs delivering erythropoietin mRNA elicited approximately 6–8-fold higher erythropoietin expression in the plasma of healthy mice compared to mRNA-loaded EVs (derived from HTB-177 cells)[117]. Given that the studies use different RNA species, EV cell sources, LNP formulations and mice strains, it is difficult to decipher the cause of such discrepancies. These conflicting findings highlight the need for more comprehensive, side-by-side comparisons to identify the most effective drug carrier for specific drug types.

Homing

A major obstacle for targeted drug delivery is the inability to drive nanoparticles to extrahepatic organs[198]. PEGylation is commonly used to reduce liver uptake and extend nanoparticle circulation[199]. Compared to FDA-approved PEGylated liposomes and LNPs, which have circulating half-lives of over 10 h, EVs have much shorter half-lives (70–180 min), similar to non-PEGylated liposomes[184,200]. Although PEGylation has been used to extend circulating half-life[157], there is growing concern about its immunogenicity caused by anti-PEG antibodies[201]. These have been associated with a range of adverse effects such as hypersensitivity reactions, accelerated blood clearance of PEGylated drugs, reduced therapeutic efficacy, and various toxicities (for example, injection site reactions, hepatotoxicity and haematological complications)[201]. Thus, there is growing interest in alternative polymeric strategies. For example, modifying mouse MSC-derived EVs with polyoxazolines (pseudo-polypeptides that impart 'stealth behaviour' similar to PEGylation) stabilized EVs in the blood and increased PANC-1 tumour accumulation in Swiss nude mice while preserving their native properties[202]. However, when functionalizing the surface of EVs with PEG or other polymers, critical surface proteins need to remain accessible to maintain functionality.

It is important to distinguish between the organotropism of drug carriers and the selective expression or functional delivery of nucleic acid cargo within a specific organ. Many studies report selective expression or downregulation of targeted genes or proteins following LNP administration[203]; however, this effect does not necessarily imply true organotropism. LNPs might accumulate extensively in organs such as the liver and spleen, yet this accumulation may not result in measurable protein expression or knockdown in these tissues[204]. Importantly, even in the absence of expression, such deposition can cause toxicity and organ damage.

In genetic medicine, selective expression or downregulation is extremely valuable. However, equating functional expression of the nucleic acid cargo with absolute deposition of the carrier can be misleading. Careful interpretation is therefore necessary, with emphasis on distinguishing biodistribution of the carrier from downstream functional outcomes. For an accurate assessment of organotropism, samples must be labelled in the same way. Comparing the biodistribution of EVs with similarly labelled LNPs provides a more appropriate basis for comparison than contrasting EVs with functional readouts of LNP distribution such as luciferase expression from delivered mRNA. This approach ensures a clear understanding of carrier targeting specificity, avoiding erroneous conclusions.

Biocompatibility and immunogenicity

EVs, LNPs and liposomes have different immunogenicity and overall biocompatibility profiles. For example, particles composed of negatively charged or neutral lipids are less immunogenic than those prepared from cationic and ionizable lipids[205–208]. Positively charged liposomes

and LNPs are known for their immunogenicity; several of their components can bind pattern recognition receptors leading to the production of various inflammatory cytokines (such as IL-6 and IFNγ)[208].

In terms of safety, EVs seem to be very well tolerated. A meta-analysis of 21 interventional clinical studies revealed that EV treatments are safe in humans with a low incidence of SAEs[13]. Of the 335 patients treated across the analysed studies, only 6 SAEs were reported (0.7%), which corresponded to liver dysfunction ($n = 2$), pyrexia ($n = 1$), vomiting ($n = 1$) and an acute asthmatic exacerbation ($n = 1$). Only one study reported a drug-related SAE where one patient suffered from grade 3 hepatic dysfunction[209]. Moreover, there was no difference in the occurrence of SAEs between groups treated with autologous versus allogeneic, or engineered versus native EVs.

Despite general tolerance, the immune privilege of EVs has yet to be rigorously proven. For example, repeated intravenous administration of Expi293F cell-derived EVs in *Macaca nemestrina* resulted in accelerated clearance and production of EV-specific antibodies, highlighting their immunogenic potential[183,210]. These findings highlight the need for comprehensive analyses on the immunogenicity of EVs and how it varies by cell source.

In conclusion, each delivery platform offers distinct advantages based on the nature of the therapeutic cargo. LNPs are well suited for delivering negatively charged molecules (such as nucleic acids) owing to their ability to complex with and protect these cargoes while facilitating cellular uptake[211]. Liposomes, with their amphiphilic bilayer structure, are ideal for encapsulating both small hydrophilic drugs in their aqueous core and hydrophobic compounds within the lipid bilayer, making them versatile carriers for a broad range of small-molecule therapeutics[212]. By contrast, EVs are uniquely suited for delivering complex biomolecular cargo that is either naturally incorporated during biogenesis or actively introduced through genetic engineering. Furthermore, EVs offer distinct advantages as cell-free alternatives and in applications in which synergistic delivery of proteins, lipids and nucleic acids is desirable, such as the case of MSC EVs, which exert their wound healing effects through a diversity of growth factors, signalling lipids and miRNAs[11]. Their composition makes them attractive for therapies requiring coordinated biological activity across multiple molecular components.

Outlook

Despite the abundance of articles that have explored the therapeutic potential of EVs, a substantial fraction of them lack critical information on dose–response relationships (such as the ratio of EVs to recipient cells and LC, and the resulting IC_{50} and ED_{50}), without which establishing cutoffs for therapeutic and toxic doses remains difficult (Box 2).

Furthermore, EV biodistribution patterns point to dominant uptake by the MPS, and engineering EVs to target specific disease sites by modifying them with ligands such as peptides and antibodies has only marginally increased targeting efficacy (Fig. 5d). Thus, new strategies need to be developed to improve cell, organ and tissue targeting.

EV heterogeneity remains a major barrier to clinical translation. Their therapeutic effects arise from complex interactions among lipid, RNA and protein components, which can act synergistically, additively or antagonistically. Clarifying these roles is key to designing well-defined, effective and safe EV-based therapies. Moreover, engineering complexity must be balanced with clinical practicality; over-engineering EVs can raise costs, regulatory barriers and safety concerns. Still, EVs are biocompatible and well tolerated in clinical trials, and excel in applications requiring transmembrane proteins, such as antigen display for vaccinations, which they naturally incorporate while maintaining small vesicle size, a feature that liposomes and LNPs struggle to replicate.

In conclusion, understanding the progress and challenges in the EV field is essential for guiding the development of next-generation therapeutics. Although proof-of-concept studies highlight the potential of EVs, the lack of robust, systematic and consistent data collection and reporting limits advancements in the field.

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Author contributions

A.P.C., E.E.B. and J.N. conceptualized the work. A.P.C., O.M.B., E.E.B., C.C.K., M.S.B. and K.J.L. extracted data from articles to compile the source dataset. A.P.C., Y.L., M.L.B., P.M.G. and J.N. analysed the data. A.P.C., O.M.B. and J.N. wrote the manuscript. A.P.C., O.M.B., L.H. and J.N. edited and revised the manuscript. J.N. provided resources and supervision.

Competing interests

J.N. is an inventor on the patent applications of the EXO-Code technology that is cited in this Review. The technology has been licensed to Exopharm. These relationships have been disclosed to and are under management by UNC-Chapel Hill.

Additional information

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