DANISH MEDICAL JOURNAL

Invited State-Of-The-Art Review

Extracellular vesicles – small messengers with a wide range of applications?

Antonia Hufnagel[1] & Romain Barrès[1, 2]

1) The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark, 2) Institut de Pharmacologie Moléculaire et Cellulaire, Université Côte d'Azur & Centre National pour la Recherche Scientifique (CNRS), Valbonne, France

Dan Med J 2024;71(8):A04240239. doi: 10.61409/A04240239

SUMMARY

ABSTRACT

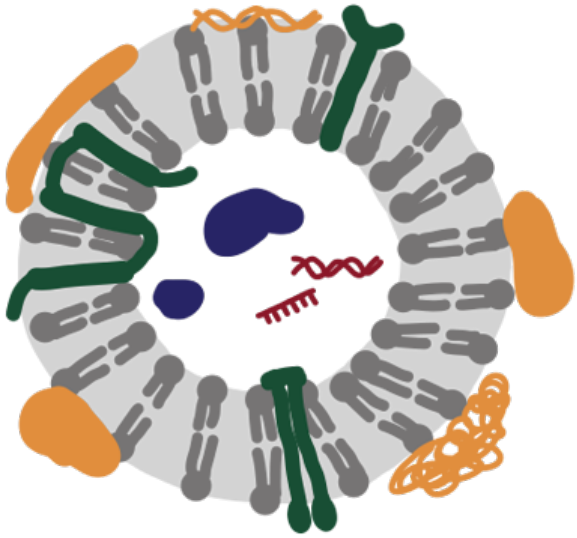
Extracellular vesicles (EVs) are small membranous vesicles secreted by many different cell types that have emerged as potentially important mediators of organ crosstalk. EVs are of research interest in health and disease and as biomarkers and therapeutic agents in various fields, including metabolism, reproduction, cancer, and others. Despite promising data and a growing understanding of their role, challenges and limitations of EV research remain, leaving room for optimisation regarding methods for pure isolations of EVs and translation into clinical practice.

KEY POINTS

- Extracellular vesicles (EVs) are membranous particles that carry RNA, DNA, proteins, and lipids, and are secreted by many cell types.
- As EVs provide a snapshot of the current state of the secreting cell, they can be mediators of organ crosstalk.
- In the clinic, EV-based biomarkers are used for prostate cancer diagnosis, and EVs are being tested as therapeutics via immunotherapy or drug delivery vehicles. Current clinical studies are in early stages.

Communication between different tissues and organs in the body orchestrates virtually all fundamental biological processes within an organism. While classically, metabolites, hormones and neural circuits have been described as the key enablers of cross-talk among organs, small membranous vesicles, termed extracellular vesicles (EVs), have emerged as messenger particles that execute communication within an organism [1]. EVs are defined as particles without a functional nucleus that are released from cells and surrounded by a lipid bilayer [2]. EVs carry ribonucleic acid (RNA), DNA and proteins (**Figure 1**) and, recently, the biomolecular corona attached to the EV membrane has also gained attention [3]. This corona consists of glycans, proteins, lipids and nucleic acids that are not directly integrated into the EV but attached to the outside of the EV's lipid bilayer. EV research has grown dramatically and is now expanding from basic understandings of the physicochemical properties of EVs to potential roles and applications in health and disease. This review aims to 1) introduce the history and concepts of the EV field, 2) point out important considerations within EV research, 3) highlight the role of EVs in different research fields and 4) discuss the clinical use of EVs now and in the future.

FIGURE 1 Schematic view of an extracellular vesicle with its associated and integrated cargo. Drawn in Adobe Fresco.



extracellular vesicles' journey from waste product to cross-talk mediator within the body

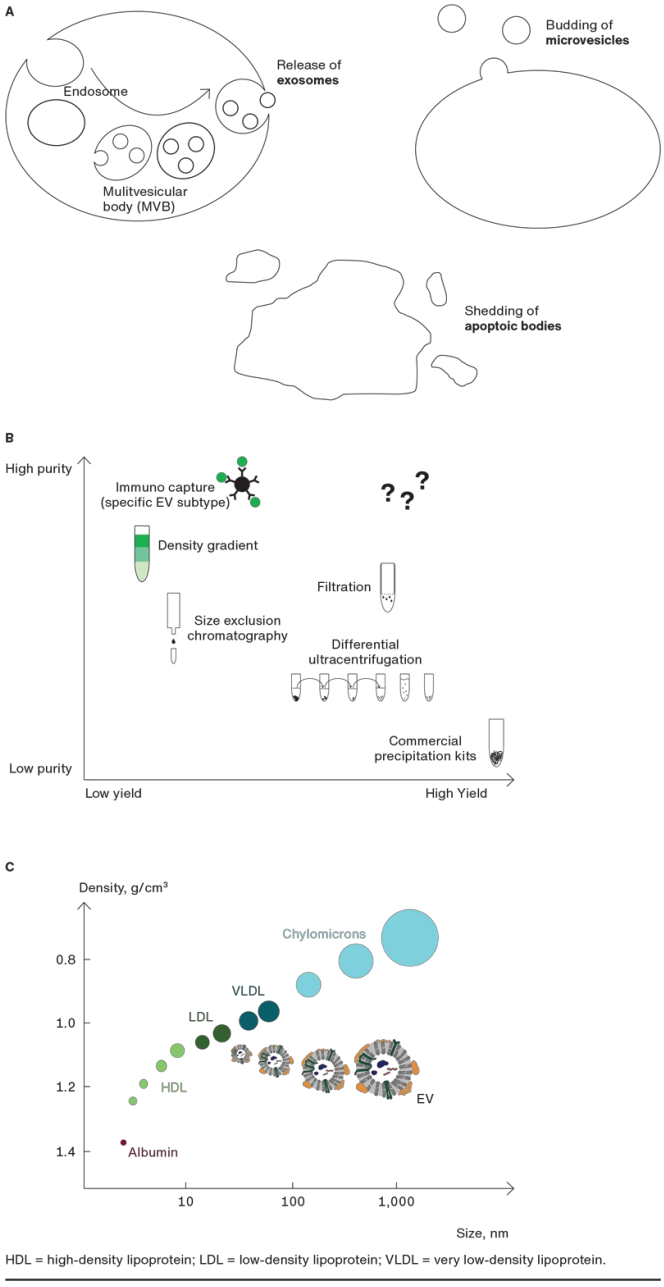
EV research has increased rapidly in the past decade, but the discovery of structures similar to EVs dates back to 1946 [4]. Researchers working on blood clotting discovered so-called *particulate fractions* that pelleted at 31,000 g, which they attributed to tiny breakdown products of the blood cells [4]. Twenty years later, these particulate fractions were called *platelet dust* [5]. In 1987, the term *exosome* was used for vesicles which formed via reticulocytes shedding off unwanted material during their maturation into erythrocytes [6]. A decade later, additional functions were attributed to exosomes, such as their ability to induce a T-cell response. This function was observed in exosomes secreted from B lymphocytes, harbouring the major histocompatibility complex (MHC) [7]. The phenomenon was harnessed just two years later as an approach to suppress tumour growth in mice, termed an exosome-based cell-free vaccine [8]. In this study, antigen-presenting cells (dendritic cells) were exposed to tumour peptides in vitro; exosomes were then isolated from the supernatant of these cells and injected back into tumour-bearing mice. The MHC molecules on the exosomes helped to prime the immune system against the tumour, thereby diminishing tumour growth. This discovery marked the beginning of a growing interest in vesicles, like exosomes. This interest was further accelerated with the discovery in 2007 that exosomes can deliver miRNA and intact mRNA to target cells [9].

Challenges and considerations within extracellular vesicle research

The exponential growth of EV research and its broad spectrum of applications and findings triggered the formation of the International Society for Extracellular Vesicles (ISEV) in 2011. In their first published framework in 2014, the ISEV aimed to clarify the various subtypes of EVs [10]. Therein, exosomes were defined as a subtype of EVs. Previously, the term exosome had been used for any vesicle. However, this revised term now specifically refers to vesicles generated via the endosomal route (**Figure 2**A). Exosomes have a 30-150 nm diameter and are formed via inward budding from an endosome that intracellularly forms a multivesicular body.

When the multivesicular body fuses with the cell membrane, the vesicles are released (Figure 2A). Another subtype of EVs is microvesicles, which are usually larger (100-1,000 nm diameter) than exosomes and are generated by budding off the plasma membrane (Figure 2A). An additional EV subtype includes apoptotic bodies, which are shed off by a cell during apoptosis and are 50-5,000 nm in size. While differentiating small and large EVs may be done through their proteomic signatures [14], other biogenesis pathways may be challenging to identify. Thus, the overarching term "EV" is commonly used to name all EVs, regardless of their origin.

FIGURE 2 The basics of extracellular vesicle (EV) research – EV classification and isolation. **A**. EV is an overarching term for the different subtypes: exosomes, microvesicles and apoptotic bodies (from [11]). **B**. Properties of the different methods used for isolation of EVs from biofluids, showing different yields and purity levels. A method with great yield and great purity has yet to be found (from [11]). **C**. In a complex fluid like blood, EVs overlap in size and density with other components such as lipoproteins (adapted from [12, 13]).



HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

Since 2014, the Minimal Information for Studies of Extracellular Vesicles *(MISEV)* guidelines have been regularly published to standardise isolation and characterisation methods within the field [2, 10]. Most approaches to

isolating EVs from their respective fluid separate EVs from other components via size and/or density. Initially, EVs were often isolated via differential centrifugation, where the centrifugation speed is sequentially increased to first pellet cells, then cell debris and, eventually, at around 20,000 g, large EVs and, usually at 100,000 g, small EVs [15]. In recent years, other methods, such as size-exclusion chromatography, have gained popularity [16]. Density centrifugation and flow- and charge-based methods are also used. In contrast, caution is advised for the use of commercial kits claiming to precipitate EVs but likely also many other contaminants [2]. As one can imagine, the different methods vary greatly in their performance regarding yield and purity (Figure 2B). A method with high purity and yield has yet to be found, with companies and research groups still aiming to develop new methods, e.g., using ultrasonic waves [17]. The characterisation of an obtained EV isolation is often performed via transmission electron microscopy, nanoparticle tracking analysis and protein analysis (mass spectrometry or western blotting) to assess typical protein markers commonly found to be associated with EVs.

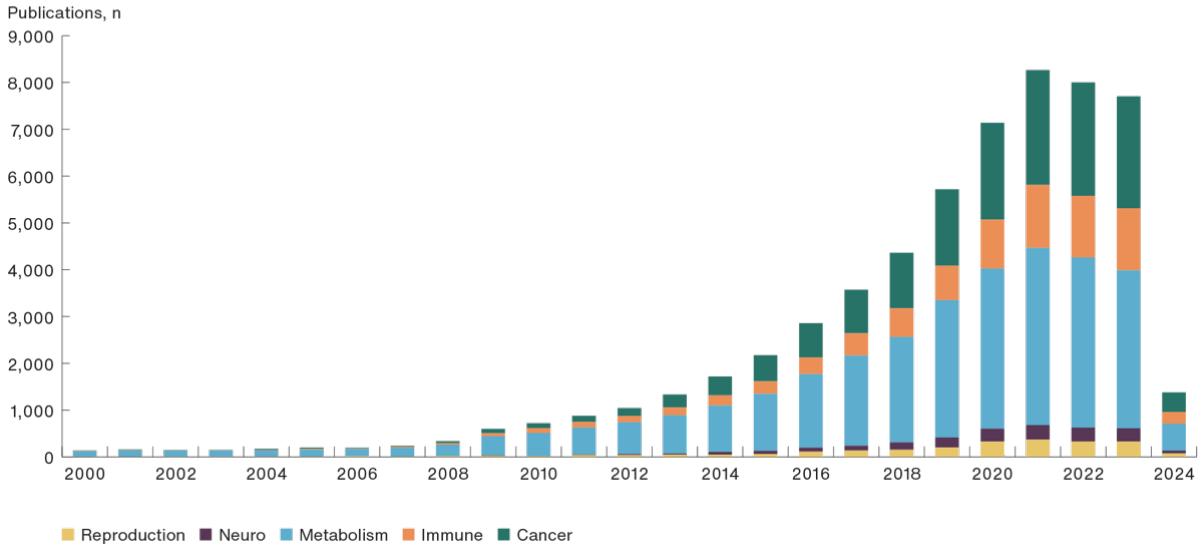
In a complex fluid - like blood - that is often used in EV research, obtaining a pure EV sample is almost impossible due to the high overlap of lipoproteins with the size and density of EVs (Figure 2C). Recent studies have also investigated the platelet dust found in 1967, showing that many EVs in blood originate from platelets, especially depending on how the sample is handled [18]. Ideally, several prerequisites should be met for the preparation of blood samples for EV research, which is not always feasible and within the scope of the research question posed in this paper. Ideally, platelet activation should be avoided to reduce the amount of EVs secreted by platelets, leading to a high background signal. This can be achieved by avoiding cold exposure and by collecting blood with an anticoagulant – such as ethylenediamine tetraacetic acid (EDTA) - that inhibits platelet activation. The blood task force of the ISEV Committee recommends centrifuging the blood twice at 2,500 g, carefully leaving the buffy coat behind to obtain pure blood plasma [19]. Additionally, considerations like overnight fasting of the blood donor can reduce the lipoprotein content, thereby increasing the purity of an EV preparation. As the EV field keeps expanding across different research topics, various starting materials are used for EV studies, each with its own limitations and challenges. In addition to the blood task force within the ISEV, additional task forces are in place to focus on EV research in urine, cerebrospinal fluid, reproductive fluids or milk. These task forces aim to standardise the EV field [20]. Despite the increasing research on EVs, many questions remain unanswered. One central question is whether the primary function of EVs is to act as messengers targeting specific recipient cells or to simply signal to whatever cell type they may passively reach. In other words, are EVs acting as a letter in an envelope with a specific address or more like a message in a bottle? Several engineering strategies suggest that EVs have the potential to target specific tissues. For instance, the addition of albumin to the EV corona improves selective uptake by hepatocytes [21], fusion of neuron-specific peptides to an EV membrane protein leads to uptake of EVs to the brain [22] and engineering of glycans on the EV corona achieved specific targeting to endothelial or dendritic cells [23]. In favour of a specific targeting in physiology are data from the cancer field where EVs originating from tumour cells have distinct patterns of membrane proteins (integrins) that lead to organ-specific uptake, and thereby metastases, in mouse models [24]. But how universal this is for all released EVs remains to be uncovered [25]. Equally, it is discussed how organised the sorting of material, especially RNAs, occurs into EVs. As reviewed by Fabbiano et al. [26], accumulating evidence suggests that EV packaging is controlled by RNA-binding proteins that are associated with the vesicle formation machinery. However, several questions remain unanswered, including the exact mechanism of cargo sorting, why studies report contradicting results, and whether sorting is altered depending on variables such as health status [27].

Role of extracellular vesicles in a variety of different research areas

Because of the large amount of EV research in the past 20 years in a range of fields (**Figure 3**), an exhaustive

overview is not possible. This section therefore aims to give a current snapshot of some fields.

FIGURE 3 Surge of extracellular vesicle papers from 2000-2024 in different research fields. Visualisation of the number of publications resulting from pubmed searches using "Extracellular vesicles" AND "cancer"/"immune"/"metabolism"/"neuro"/"reproduction". Data from February 2024.



Research in the field of metabolism and metabolic dysfunction showed that EV concentrations in plasma are increased in individuals with obesity (reviewed in [28]). Additionally, EVs secreted from adipose tissue macrophages of obese mice caused insulin resistance when injected into lean mice and vice versa [29], highlighting the potential of EVs for the field of metabolism. A recent study claimed that circulating EVs may present a link between metabolic and vascular health, with results showing that EVs from patients with metabolic syndrome blunted insulin-induced vasodilation in mouse arteries [30].

In reproductive biology research, EVs are studied in various fluids and tissues such as follicular fluid, blood-borne placenta-derived EVs from pregnant females, uterine fluid, embryo culture and seminal fluid. It is now well-known that blood EV concentration is increased in pregnancy and that the placenta secretes EVs needed for a successful pregnancy because of their role in modulating the maternal immune response (reviewed in [31]). In pathological states, placental EVs are heavily studied for their role in preeclampsia, a severe pregnancy condition characterised by hypertension and proteinuria [32]. EVs from females with preeclampsia have reduced levels of endothelial nitric oxide synthase, which could therefore participate in vascular dysfunction inherent to the disease [32]. Additionally, neprilysin levels are increased on placental EVs from women with preeclampsia and are likely involved in the disease as the peptidase neprilysin breaks down vasoactive molecules [33]. In males, EVs have been reported as highly abundant in seminal fluid [34], with effects on sperm function and the priming of the female reproductive tract for a potential pregnancy [35]. Important interest has been expressed in multiple reviews, hypothesising that EV RNA could be involved in soma-germline communication [36] and that EVs might have an application in assisted reproductive technologies [37].

As highlighted in the previous sections, significant EV research has progressed in the field of cancer, especially regarding clinical applications, as discussed in the next section.

Clinical use of extracellular vesicles

EVs have been widely studied for their use as biomarkers owing to their availability in various easily obtainable

biofluids. EVs are considered a snapshot of the state of an organ or cell at the sampling time point. Additionally, EV isolation can enable the detection of a change that is otherwise masked in a complex fluid by other highly abundant constituents. However, despite active research to identify EVs as biomarkers, very few EVs are currently used as biomarkers in clinical practice [38]. Contributing to this low success rate is likely the lack of a robust and approved pipeline for EV biomarker discovery and clinical testing that would be essential for enabling the translation of research into the clinic [38].

Nonetheless, in the diagnosis of prostate cancer, four urine-based diagnostic tests and three serum-based diagnostic tests based on EVs have been approved for use by the Food and Drug Administration (FDA) [39]. One of these tests, called ExoDx, measures mRNA in urine EVs and has been incorporated into the Prostate Cancer Early Detection guidelines. This test was proven superior to the previous standard of care to determine biopsy need [40].

No therapeutic EV applications are currently approved by drug administration authorities. Yet, non-approved EV therapies are available from various merchants without thorough testing, particularly for skin care products sold in the US [41]. Whereas EVs hold great potential as therapeutic agents, the first preclinical results are only now emerging, and validating tests are only in the early stages of clinical trials. A recent review reported that, in 2023, 60 studies using EVs as a therapy were registered as clinical trials, with 60% being in phase I or II [42]. Applications under clinical investigation include diabetes, myocardial infarction, wound healing, polycystic ovary syndrome and host-vs-graft rejection, to name a few. The most advanced applications, with published data from phase I trials, concern cancer immunotherapy as originally reported in 1998. Three phase I studies highlighted the feasibility and safety of EV-based immunotherapy in lung, melanoma and colorectal cancer patients 15 years ago [43-45]. Another highly researched therapeutic potential comes from EVs derived from mesenchymal stem cells, where preclinical models have shown beneficial effects in models for stroke, newborn brain injury, multiple sclerosis and Alzheimer's disease [46]. A human study has also demonstrated beneficial effects of treatment with mesenchymal stem cell EVs for graft-vs-host disease [47]. An additional type of EV therapeutics harnesses the use of EVs as a delivery vehicle, loading them with RNAs or drugs. EVs secreted from tumour cells and packaged with the chemotherapeutic agent methotrexate have recently been shown to successfully treat malignant pleural effusion in advanced non-squamous non-small cell lung cancer in a randomised controlled trial [48].

Conclusions

This review highlights the wide range of functions of EVs, with roles in immunomodulation, crosstalk, reproduction, cancer, and metabolism, with growing research interest over the past decades in several fields. Despite promising results in EV research, critical knowledge gaps must be addressed before advancing to use EVs in disease treatment. Translation into the clinic requires more standardisation with a subgroup of the ISEV committee dealing with regulatory affairs aiming to streamline manufacturing processes and quality control assessments to enable the safe translation of EVs into the clinical setting [46].

Indeed, it will be exciting to see if the booming EV interest will translate into a wide range of applications in the near future, including potential novel innovative diagnostics and therapies.

Correspondence *Romain Barrès/Antonia Hufnagel*. E-mail: barres@sund.ku.dk/antonia.hufnagel@sund.ku.dk

Accepted 3 June 2024

Conflicts of interest Potential conflicts of interest have been declared. Disclosure forms provided by the authors are available with

the article at ugeskriftet.dk/dmj

Acknowledgements We want to thank *Jessica Preston* for proofreading the manuscript

References can be found with the article at ugeskriftet.dk/dmj

Cite this as Dan Med J 2024;71(8):A04240239

doi 10.61409/A04240239

REFERENCES

1. Abbott A. FedEx for your cells: this biological delivery service could treat disease. 2023. www.nature.com/articles/d41586-023-02906-w
2. Welsh JA, Goberdhan DCI, O'Driscol L et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. J Extracell Vesicles. 2024;13(12):e12404. https://doi.org/10.1002/jev2.12404
3. Buzas EI. Opportunities and challenges in studying the extracellular vesicle corona. Nat Cell Biol. 2022;24(9):1322-1325. https://doi.org/10.1038/s41556-022-00983-z
4. Chargaff E, West R. The biological significance of the thromboplastic protein of blood. J Biol Chem. 1946;166(1):189-97. https://doi.org/10.1016/s0021-9258(17)34997-9
5. Wolf P. The nature and significance of platelet products in human plasma. Br J Haematol. 1967;13(3):269-88. https://doi.org/10.1111/j.1365-2141.1967.tb08741.x
6. Johnstone RM, Adam M, Hammond JR et al. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem. 1987;262(19):9412-20. https://doi.org/10.1016/s0021-9258(18)48095-7
7. Raposo G, Nijman HW, Stoorvogel W et al. B Lymphocytes secrete antigen-presenting vesicles. J Exp Med. 1996;183:1161-72 https://doi.org/10.1084/jem.183.3.1161
8. Zitvogel L, Regnault A, Lozier A et al. Eradication of established murine tumours using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med. 1998;4(5):594-600. https://doi.org/10.1038/nm0598-594
9. Valadi H, Ekström K, Bossios A et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654-9. https://doi.org/10.1038/ncb1596
10. Lötvall J, Hill AF, Hochberg F et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. J Extracell Vesicles. 2014;3:26913. https://doi.org/10.3402/jev.v3.26913
11. Hufnagel A. The effect of maternal obesity/GDM and metformin intervention on maternal, placental and fetal health. 2021. https://doi.org/10.17863/CAM.85661
12. Karimi N, Cvjetkovic A, Jang SC et al. Detailed analysis of the plasma extracellular vesicle proteome after separation from lipoproteins. Cell Mol Life Sci. 2018;75(15):2873-86. https://doi.org/10.1007/s00018-018-2773-4
13. Busatto S, Zendrini A, Radeghieri A et al. The nanostructured secretome. Biomater Sci. 2019;8(1):39-63. https://doi.org/10.1039/c9bm01007f
14. Lischnig A, Bergqvist M, Ochiya T, Lässer C. Quantitative proteomics identifies proteins enriched in large and small extracellular vesicles. Mol Cell Proteomics. 2022;21(9):100273. https://doi.org/10.1016/j.mcpro.2022.100273
15. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol. 2006;chapter 3:3.22.1-3.22.29. https://doi.org/10.1002/0471143030.cb0322s30
16. Royo F, Théry C, Falcón-Pérez JM et al. Methods for separation and characterization of extracellular vesicles: results of a worldwide survey performed by the ISEV Rigor and Standardization Subcommittee. Cells. 2020;9(9):1955. https://doi.org/10.3390/cells9091955
17. Ku A, Lim HC, Evander M et al. Acoustic enrichment of extracellular vesicles from biological fluids. Anal Chem. 2018;90(13):8011-9. https://doi.org/10.1021/acs.analchem.8b00914

18. Bettin B, Gasecka A, Li B et al. Removal of platelets from blood plasma to improve the quality of extracellular vesicle research. 2022;20(11):2679-85. https://doi.org/10.1111/jth.15867
19. Nieuwland R, Siljander PRM. A beginner's guide to study extracellular vesicles in human blood plasma and serum. J Extracell Vesicles. 2024;13(1):e12400. https://doi.org/10.1002/jev2.12400
20. About the International Society for Extracellular Vesicles. International Society for Extracellular Vesicles, 2021. www.isev.org/taskforces
21. Liam-Or R, Faruqu FN, Walters A et al. Cellular uptake and in vivo distribution of mesenchymal-stem-cell-derived extracellular vesicles are protein corona dependent. Nat Nanotechnol. 2024;19(6):846-55. https://doi.org/10.1038/s41565-023-01585-y
22. Alvarez-Erviti L, Seow Y, Yin H et al. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011;29(4):341-5. https://doi.org/10.1038/nbt.1807
23. Zheng W, HE R, Liang X et al. Cell-specific targeting of extracellular vesicles through engineering the glycocalyx. J Extracell Vesicles. 2022;11(12):12290. https://doi.org/10.1002/jev2.12290
24. Hoshino A, Costa-Silva B, Shen TL et al. Tumour exosome integrins determine organotropic metastasis HHS Public Access. Nature. 2015;527(7578):329-35. https://doi.org/10.1038/nature15756
25. van Niel G, Carter DRF, Clayton A et al. Challenges and directions in studying cell–cell communication by extracellular vesicles. Nat Rev Mol Cell Biol. 2022;23(5):369-82. https://doi.org/10.1038/s41580-022-00460-3
26. Fabbiano F, Corsi J, Gurrieri E et al. RNA packaging into extracellular vesicles: An orchestra of RNA-binding proteins? J Extracell Vesicles. 2020;10(2):e12043. https://doi.org/10.1002/jev2.12043
27. Mateescu B, Kowal EJK, van Balkom BWM et al. Obstacles and opportunities in the functional analysis of extracellular vesicle RNA - an ISEV position paper. J Extracell Vesicles. 2017;6(1):1286095. https://doi.org/10.1080/20013078.2017.1286095
28. Samuelson I, Vidal-Puig AJ. Fed-EXosome: extracellular vesicles and cell-cell communication in metabolic regulation. Essays Biochem. 2018;62(2):165-75. https://doi.org/10.1042/EBC20170087
29. Ying W, Riopel M, Bandyopadhyay G et al. Adipose tissue macrophage-derived exosomal miRNAs can modulate in vivo and in vitro insulin sensitivity. Cell. 2017;171(2):372-84.e12. https://doi.org/10.1016/J.CELL.2017.08.035
30. Ragland TJ, Heiston EM, Ballantyne A et al. Extracellular vesicles and insulin-mediated vascular function in metabolic syndrome. Physiol Rep. 2023;11(1):e15530. https://doi.org/10.14814/phy2.15530
31. Nair S, Salomon C. Extracellular vesicles and their immunomodulatory functions in pregnancy. Semin Immunopathol. 2018;40(5):425-37. https://doi.org/10.1007/s00281-018-0680-2
32. Motta-Mejia C, Kandzija N, Zhang W et al. Placental vesicles carry active endothelial nitric oxide synthase and their activity is reduced in preeclampsia. Hypertension. 2017;70(2):372-81. https://doi.org/10.1161/HYPERTENSIONAHA.117.09321
33. Gill M, Motta-Mejia C, Kandzija N et al. Placental syncytiotrophoblast-derived extracellular vesicles carry active NEP (Neprilysin) and are increased in preeclampsia. Hypertension. 2019;73(5):1112-9. https://doi.org/10.1161/HYPERTENSIONAHA.119.12707
34. Höög JL, Lötvall J. Diversity of extracellular vesicles in human ejaculates revealed by cryo-electron microscopy. J Extracell Vesicles. 2015;4(1):1-11. https://doi.org/10.3402/jev.v4.28680
35. Zhang X, Liang M, Song D et al. Both protein and non-protein components in extracellular vesicles of human seminal plasma improve human sperm function via CatSper-mediated calcium signaling. Hum Reprod. 2024;39(4):658-73. https://doi.org/10.1093/humrep/deae018
36. Phillips D, Noble D. Bubbling beyond the barrier: exosomal RNA as a vehicle for soma–germline communication. J Physiol. 2024;602(11):2547-63. https://doi.org/10.1113/JP284420
37. Aleksejeva E, Zarovni N, Dissanayake K et al. Extracellular vesicle research in reproductive science: paving the way for clinical achievements. Biol Reprod. 2022;106(3):408-24. https://doi.org/10.1093/biolre/ioab245
38. Yekula A, Muralidharan K, Kang K et al. From laboratory to clinic: translation of extracellular vesicle based cancer biomarkers. Methods. 2020:177:58-66. https://doi.org/10.1016/j.ymeth.2020.02.003
39. Borbiev T, Kohaar I, Petrovics G. Clinical biofluid assays for prostate cancer. Cancers (Basel). 2023;16(1):165. https://doi.org/10.3390/cancers16010165
40. Tutrone R, Lowentritt B, Neuman B et al. ExoDx prostate test as a predictor of outcomes of high-grade prostate cancer - an

interim analysis. Prostate Cancer Prostatic Dis. 2023;26(3):596-601. https://doi.org/10.1038/s41391-023-00675-1

41. Asadpour A, Yahaya BH, Bicknell K et al. Uncovering the gray zone&;: mapping the global landscape of direct-to&;consumer businesses offering interventions based on secretomes, extracellular vesicles, and exosomes. Stem Cell Res Ther. 2023;14(1):111. https://doi.org/10.1186/s13287-023-03335-2
42. Sanz-Ros J, Mas-Bargues C, Romero-García N et al. Extracellular vesicles as therapeutic resources in the clinical environment. Int J Mol Sci. 2023;24(3):2344. https://doi.org/10.3390/ijms24032344
43. Andre F, Schartz NEC, Movassagh M et al. Malignant effusions and immunogenic tumour-derived exosomes. Lancet. 2002;360(9329):295-305. https://doi.org/10.1016/S0140-6736(02)09552-1
44. Dai S, Wei D, Wu Z et al. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. Mol Ther. 2008;16(4):782-90. https://doi.org/10.1038/mt.2008.1
45. Morse MA, Garst J, Osada T et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med. 2005;3(1):9. https://doi.org/10.1186/1479-5876-3-9
46. Lener T, Gimona M, Aigner L et al. Applying extracellular vesicles based therapeutics in clinical trials? an ISEV position paper. J Extracell Vesicles. 2015;4:30087. https://doi.org/10.3402/jev.v4.30087
47. Kordelas L, Rebmann V, Ludwig AK et al. a-derived exosomes: A novel tool to treat therapy-refractory graft-versus-host disease. Leukemia. 2014;28(4):970-3. https://doi.org/10.1038/leu.2014.41
48. Dong X, Huang Y, Yi T et al. Intrapleural infusion of tumor cell-derived microparticles packaging methotrexate or saline combined with pemetrexed-cisplatin chemotherapy for the treatment of malignant pleural effusion in advanced non-squamous non-small cell lung cancer: a double-blind randomized, placebo-controlled study. Front Immunol. 2022;13:1002938. https://doi.org/10.3389/fimmu.2022.1002938

