Extracellular Vesicle 2 (2023) 100029

Contents lists available at ScienceDirect

Extracellular Vesicle

journal homepage: www.elsevier.com/locate/vesic

Guidelines for clinical translation and commercialization of extracellular vesicles and exosomes based therapeutics☆

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ARTICLE INFO

Keywords:
Extracellular vesicles and exosomes
Therapeutics
Diagnostics
Clinical translation
Commercialization

ABSTRACT

Extracellular vesicles (EVs) are lipid-bilayer delimited membrane structures released by cells, and play a role in intercell communication and disease development. The Global market for EVs in diagnostic and therapeutic applications is expanding. This review maps the current status of EV industry, summarizes the recent advances in EV manufacturing, and focuses on preclinical research involving EVs. The complexity and heterogeneity of EVs provide new opportunities for the development of clinical-grade EV products. The standardization of manufacturing and robust quality control must meet all the Food and Drug Administration requirements and expectations. We believe the evolution of EV research and their mass production with stringency will open a new era of EV-based products in the near future.

1. Introduction

Extracellular vesicles (EVs) are particles with a lipid bilayer that are naturally released from cells and cannot replicate. EVs generally fall into two major categories, ectosomes and exosomes.[1] Ectosomes are vesicles generated by the direct outward budding of the plasma membrane, which produces microvesicles, apoptosomes, migrasomes, and other large vesicles in the size range of 50 nm to 1 mm in diameter. By contrast, exosomes are of endosomal origin and in a size range of 40 to 160 nm diameter. However, a recent study challenged the dualism by tracking exosomal biomarkers, making the boundary between the membrane origin and endosome origin more ambiguous.[2] Of these EVs, exosomes have been extensively studied and generally characterized by classic tetraspanins like CD9, CD63, and CD81. But the heterogeneous exosome group cannot be defined by a single set of biomarkers and needs to be characterized by comprehensive approaches.[3]

The EV field is growing exponentially due to an increased understanding of the role of EVs in disease development and potential treatments. The global market for exosome diagnosis is projected to grow from $57.1 million in 2021 to $321.9 million by 2026, while the exosome therapeutics market is projected to grow from $33.1 million in 2021 to $169.2 million by 2026.[4] Despite the significant progress we have seen in the area of EV biology and its applications, the capacity of these particles has yet to be fully harnessed for any potential commercial use. The analysis and manufacturing of EVs need to be standardized for clinical applications. However, to date there is no consensus on the isolation methods and release criteria of EV-related products for clinical applications.

2. Mapping the EV industry status quo

EVs can serve as biomarkers and therapeutics in various diseases.[5] Additionally, EVs can be used as carriers for drugs and other therapeutics. Concomitant with the increasing need for scalable EV products and development of standard cell culture techniques, EV isolation and characterization-based companies are also thriving. Here, we focus on the current EV-based companies and clinical trials, highlighting their technology, products and applications. We also discuss the bottlenecks in large-scale production and outline difficulties in clinical translation.

☆ Given his role as Editor in Chief Professor Ke Cheng had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Professor Gangjian Qin.

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https://doi.org/10.1016/j.vesic.2023.100029
Received 2 November 2022; Received in revised form 24 July 2023; Accepted 25 July 2023
Available online 30 September 2023

Table 1
Naïve exosomes: Naturally produced by cells.

Company	Product	Clinical phase	Detail	Release Criteria	Concentration	Storage	Disease
Direct Biologics	Exoflo™	Phase I/II	BM-MSC-EVs	– proprietary cGMP processing – USP<71> sterility testing	10^10 particles/mL	−40 to − 80 °C, 5 years −20 to − 40 °C, 6 months	Crohn's Disease IBD - Irritable Bowel Disease Covid19 ARDS Pneumonia, Viral
Aegle Therapeutics	AGLE-102 AGLE-103	Phase I	BM-MSC-EVs	Not applicable	Not applicable	Not applicable	Second degree burn wounds Dystrophic epidermolysis bullosa (DEB)
RION	PEP™	Phase I	Platelets	A dry product with high purity and lot-to-lot consistency	Not applicable	Shelf-stable at room temperature and remains active at body temperature	Wound healing Myocardial infarction
Organicell Regenerative Medicine	Zofin™	Phase I/II	Human amniotic fluid	Sterility (14-day cultures), Endotoxin (<0.05 EU/mL) Particle composition Protein concentration Hyaluronic acid concentration	5.24 × 10^11 particles/mL	Not applicable	SARS COVID-19 ARDS
United Therapeutics	UNEX-42	Phase I	BM-MSC	Not applicable	20-200 pmol phospholipid/kg body weight	Not applicable	Infants born at <27 weeks of gestational age (GA) at high risk for bronchopulmonary dysplasia
ExoCoBio	ExoBRID-E™ Vexosome™	N/A	Human stem cell Hybrid exosome				Cosmeceutical products
The Cell Factory (Esperite)	CF-MEV-107 CF-MEV-132 CF-MEV-117	IP in Europe, China and Canada	MSC-derived EVs	Based on GMP guidelines			Crohn's Disease Bronchopulmonary dysplasia Epilepsy
Capricor Therapeutics	CDC-Exosomes	Phase I	Cardiosphere-derived cells and derived EVs		Intravenous infusion of 100-mL (total volume) infusion of 150M CDCs in 5% Human Serum Albumin (HSA)		Duchenne muscular dystrophy
ReNeuron	N/A	IP in Europe, Japan, China and Korea	Neural stem cells derived EVs	Not applicable			Brain cancer
Xsome Biotech	XO102	IP in United States	Lung spheroid cell-EVs	Not applicable	Inhalation Dose not applicable	Dry powders are stable at room temperature for at least one month	Idiopathic pulmonary fibrosis (IPF), Pulmonary hypertension –heart failure with preserved ejection fraction (PH-HFpEF), Chronic obstructive pulmonary disease (COPD)

2.1. EVs as therapeutics

EVs naturally inherit specific factors and nucleic acids from their parent cells. This feature has been largely used in regenerative medicine. Table 1 summarizes naïve EV based companies, the source of EVs and the application of their products. The widely used MSC-derived exosomes contain the bioactive molecules of stem cells and, as a result, inherit the multipotency and self-renewing properties from their parent cells. Besides, some tissue-specific exosomes have an advantage in targeting their local tissues and organs. For example, lung spheroid cells (LSCs)-derived exosomes are applied to treat idiopathic pulmonary fibrosis (IPF).[6–10]

2.2. EVs as drug delivery systems

The Food and Drug Administration (FDA) approved lipid nanoparticles (LNPs) developed by Alnylam Pharmaceuticals for the delivery of small interfering RNAs (siRNAs) to treat hereditary transthyretin amyloidosis in 2018 (NDA 210922), marking a big step forward for nanoparticle drug delivery systems. More recently, Moderna and Pfizer are also using LNPs in developing their COVID-19 vaccines. However, dose-dependent toxicity and ineffective delivery to target tissues are still the big limitations of current drug delivery carriers. More effective and safe carriers are still needed. EVs are a potential solution, with a natural lipid bilayer and with lack of immunogenicity and lower toxicity compared to lipid nanoparticles.

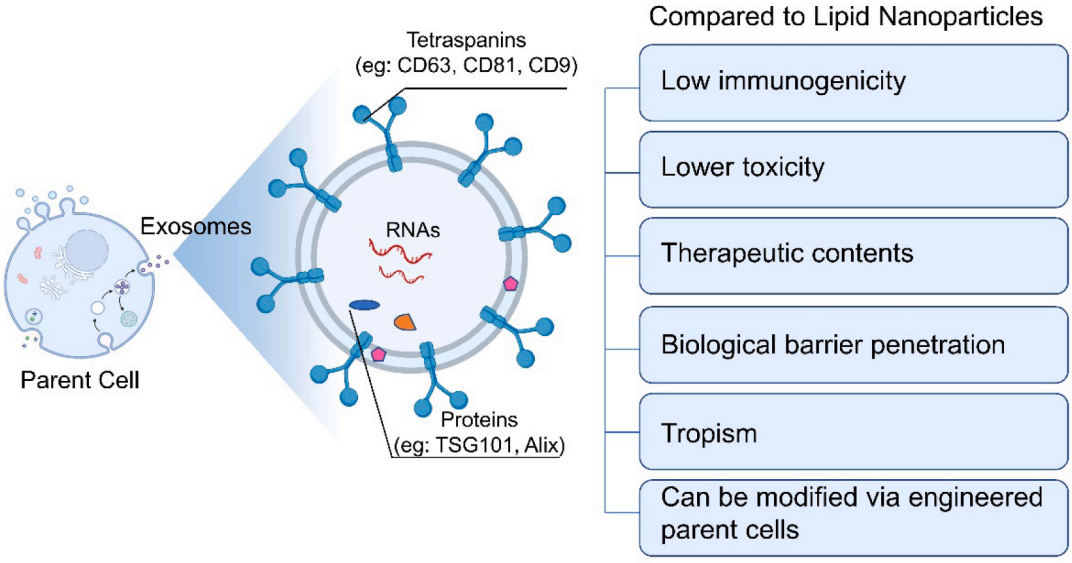

Fig. 1. Characterization and function of exosomes.

The complex structure is a double-edged sword for EVs (Fig. 1). On the one hand, EVs carry natural therapeutic contents and targeting proteins from parent cells, which will be further discussed in Section 3.1. EVs from specific cells can cross diverse biological barriers, such as the blood–brain barrier and the air-blood barrier.[11] Additionally, the modification of EVs can be achieved via engineered parent cells. On the other hand, the heterogeneity of EVs makes the purification process and release criteria difficult to standardize. In this regard, FDA needs to be convinced that such heterogeneity is an inherent biological feature of EVs with likely advantages, and there is no real need to overcome it in a therapeutic product.[12]

Looking at the engineered EV-based companies (Table 2), EVs have been used to deliver proteins, antisense oligonucleotides (ASO), micro RNAs (miRNA), small interfering RNAs (siRNA) and messenger RNAs (mRNA). In general, there are three ways to engineer EVs: (1) modifying host exosome producing cells to generate specific exosomes; (2) loading cargo into exosome producing cells, and (3) loading cargo after exosome purification. The first method is mainly used by companies, to modify host cells by gene editing to mass produce EVs containing specific functionalized miRNA or proteins.[13–15] The stable cell lines constructed can be patented. Companies like Codiak and Avalon are using this method to deliver RNA cargos. The second method is more transient — cargos were firstly loaded to cells, and the cells were stimulated to secrete EVs, to get the cargos covered by membrane.[16,17] The third method is also widely used due to its flexibility. Any modified EV delivery system and cargo can be combined by electroporation to achieve multiple functions. Carmine is using this method by isolating EVs from blood followed by loading therapeutic cargos.[18–20]

2.3. EVs as diagnostics

The enthusiasm for EVs as biomarkers in diagnostics and disease monitoring began a decade ago.[21] The role EVs play in disease development provided compelling evidence supporting the use of EVs as liquid biopsy in clinical applications.[5] The advantages of using EVs for diagnostics are evident. First, EVs exist in almost all biofluids, such as blood, urine, saliva, bronchoalveolar lavage fluid (BALF), and ascites fluid, which can be used in liquid biopsy.[22,23] Second, EVs are relatively stable due to their lipid bilayer membrane. The collected bio-samples can be stored at 4 °C for several days and at −20 °C or −80 °C for a substantially longer time.[24] Third, EVs are more representative of parent cells and show higher accuracy than other cell-free components as biomarkers.[25,26] DNA in EVs is representative of the entire genome and reflects the mutational status of parental pancreas tumor cells.[27] EVs isolated from cerebrospinal fluid contain a higher concentration of biomarkers (proteins, mRNA, and miRNA) than other components for diagnosing and prognosis of central nervous system diseases.[28,29] Fourth, EVs can be isolated and concentrated according to their specific protein markers or the markers of parent cells, which provides higher sensitivity.[30]

2.4. EV service companies

To cope with the increase in EV related research, companies have started to provide custom solutions to researchers to benefit and speed up the research and development process (Table 3). Companies focusing on exosome diagnosis develop EV isolation kits for multiple biofluids and combine downstream biomarker detection like RNA sequencing and immuno-analysis. To assist in EV based clinical trials, there are companies focusing on the manufacturing process, providing GMP-level products for scalable cures and clinical trials (Table 4). The whole process service includes cell culture, EV isolation and purification, EV characterization, and quantification. Kimera Exosomes® produces the first pharmaceutical-grade MSC exosome product meeting the FDA requirements for treating ARDS secondary to COVID-19.

3. Manufacturing of EVs

3.1. EV sources

Naive exosomes are unmodified exosomes naturally produced by cells (Table 5). Exosome surface properties and cargo reflect their cell of origin. For example, exosomes from mesenchymal stem cells (MSC) possess the inherent regenerative and anti-inflammatory activities of their parent cell. Exosomes derived from lung spheroid cells (LSCs) have been proven to improve respiratory diseases like pulmonary fibrosis.[31] It is promising for exosomes derived from neural stem cells (NSCs) to address the need for neuroprotective or regenerative therapy.[32] Tissue-specific cell-derived EVs are also used as naïve exosomes. Platelet-derived EVs provide pro-hemostatic support during uncontrolled bleeding.[33] EVs from dendritic cells injected into the tumor site can induce both primary and secondary immune responses, resulting in

Table 2
Engineered exosomes: Exosomes loaded with specific materials such as proteins, nucleic acids, or other biomolecules.

Company	Product	Therapeutics	Location	Feature	Administration	Disease
Codiak Biosciences	exoSTING™ (Phase I)	STING (stimulator of interferon genes) agonist	In the lumen of exosomes	– Potent, targeted for the treatment of multiple solid tumors enriched in the target APCs	Intratumorally	Solid tumors
	exoIL-12™ (Phase I)	IL-12 in a fully active form	On the surface of exosomes via PTGRFN	– Allowed for reduced IL-12 doses – No measurable systemic exposure of IL-12	Intratumorally	Solid tumors
	exoASO™-STAT6	Surface-loaded with an antisense oligonucleotide targeting STAT6	On the surface of exosomes	-Preferential uptake into M2 polarized macrophages	Intravenously	Myeloid rich cancers
Avalon Globocare Corp.	AVA-201 (Discovery)	miR-185	In the lumen of exosomes	– Engineered mesenchymal stem cells as the "bio-factory" to mass-produce miR-185	Externally applied to the oral leukoplakia lesions	Oral cancer
Aruna Bio, Inc.	AB126 (Discovery)	siRNA/ASO	In the lumen of exosomes	– Derived from proprietary non-transformed neural stem cells – Innate properties (effective at crossing the BBB; therapeutic properties; targeting neural cells)	Intravenously	Acute ischemic stroke
ILIAS Biologics	ILB-202	Anti-inflammatory protein srl κB	In the lumen of exosomes	– Lower the chance of off-target effects by directly targeting cytosolic core inflammation signals	Intraperitoneal	Acute/chronic inflammatory diseases
The Cell Factory (Esperite)	CF-MEV-126	miRNA in the lumen & bound surface molecule (i.e., Annexin V)		Penetrate the blood–brain barrier	Intravenously	Neurological disorders
Evox Therapeutics	Undisclosed (Discovery)	Intracellular protein/mRNA/gene editing/ transmembrane protein/siRNA		Undisclosed		Rare metabolic/ others
Exopharm	Undisclosed (Discovery)	Undisclosed		Provide tissue tropism Load active pharmaceutical ingredients		Neurology, cardiology and oncology
Carmine Therapeutics	Red Cell EV Gene Therapy (REGENT®) (Discovery)	Antisense oligonucleotides (ASO), Cas9 mRNA, and guide RNAs		RNA delivery	Subcutaneously	Cancer
Capricor Therapeutics	Exosome-mRNA vaccine (pre-clinical)	Undisclosed		Cardiosphere-derived cells derived EVs	Undisclosed	SARS-COV-2
VesiCURE Therapeutics	modEXO™ (Discovery)	Undisclosed				Myeloid; Tumor; COVID-19

suppression or eradication of the established tumor.[34]

Engineered exosomes from tissue-specific cells could be modified to handle local missions. EVs derived from brain endothelial cells could efficiently deliver drugs across the Blood Brain Barrier (BBB) into the brain, making it a promising treatment for brain cancers.[11] Macrophages-derived EVs loaded with paclitaxel were detected to preferentially accumulate in cancer cells.[35] *T* cell and CAR *T* cell-derived EVs can act as an alternative treatment since they can avoid cytokine-releasing syndrome and neurotoxicity compared to their parent cells.[36] Also, EVs derived from tumor cells, such as leukemia cells,[37] breast cancer cells,[38] lung cancer cells,[39] and so on,[40] are with the property to home to the tumor site. However, tumor EVs cannot be used in drug delivery due to their potential deleterious effects.[41,42] EVs secreted by the cancer cells (brain, bone, liver, lung, etc.) carry various molecular effectors like miRNA and play a role in reshaping the metastatic sites to help cancer cell colonization.[43] It has also been reported that EVs' specific metastasis may be influenced by a set of integrins on EV surface.[44]

The cells that produce EVs can be modified by varying culture conditions and exposure to stress, such as hypoxia or cytokine introduction, to produce more or different exosomes. The HEK293 cell line, commonly used for producing proteins and with mature, steady, and large-scale culturing conditions, has also been used to produce therapeutic exosomes.[45] HEK293 cells exhibit high efficiency in producing EVs and HEK293 derived EVs are considered to be safe and thus largely used in exosome engineering.[40]

The inherent function of EVs and uptake by target cells should be considered when thinking about what cell sources to choose. EVs from stem cells can be used for regeneration, immune cells are for attacking the tumor, and cells with safety and high yield like HEK293 are good choices for providing drug delivery platforms. It is also an outstanding property for tissue-specific-cell-derived EVs to home to specific sites.

3.2. 2D vs 3D culture vs scale-up

Exosome contents are altered during cell metabolism (Fig. 2). 3D culture will lead to a different protein/miRNA profile compared to 2D culture.[46,47] This needs to be taken into consideration when using exosomes for therapeutic purposes. Stirred tank bioreactors, rotating wall bioreactors, and bioreactors with a fixed bed system have been largely

Table 3
Companies focused on exosome diagnosis.

Company	Country	Year	Mission	Technology
Bio-Techne	United States	2010	Enrichment of exosomes from various biofluids/tissues to find clinical utility in diverse disease areas	Exosome Diagnostics Platform
Exosome Sciences (Aethlon Medical, Inc)	United States	2013	Exosome based diagnosis and monitoring	TauSome™ biomarkers
Exosomics	Italy	2011	Exosome based pre-analytical and analytical diagnostic assays	Purification kits and analytical assays
Echo Biotech	China	2017	Isolation and characterization of EVs	EV isolation kits Analysis of tissue EVs in combination with scRNA-seq

used for scaling up. Hollow fiber bioreactors also show promise in scalable production of EVs.[48]

3.3. Isolation methods

Pure exosomes are critically important when considering regulatory requirements for therapeutic applications of exosomes. FDA and other regulatory agencies throughout the world require purity, potency, safety, and efficacy to grant approval. A pure product without contaminants such as peptides, proteins, cell free DNA and other cell debris is essential for clinical use. This is important for exosome therapeutics, specifically. Although exosomes can be dosed based on protein or nucleic acid content, the current standard is to dose based on the number of exosomes (e.g., 1×10^{10}). If foreign nanoparticles are present as contaminants, the dose could potentially contain a mixture of exosomes and other nanoparticles that are not exosomes.[49]

The isolation efficiency of ultracentrifugation is rather low (10∼25%) and time-consuming. Tangential flow filtration (TFF) has the advantage of efficiency, flexibility, and scalability, and has been widely used in industry. However, size cannot be the only criterion for differentiating exosomes from other vesicles and exosome subtypes. Exosome subtypes are critical to research, diagnostic biomarker identification, and development of therapeutics. TFF allows similar-sized contaminants to flow through along with exosomes, such as other EV types and non-vesicle particles. TFF can be paired with other techniques, such as immunocapture, density gradient centrifugation, ion-exchange chromatography, and microfluidic system of various principles, which is the current trend in EV isolation.[50]

Immunocapture captures EVs with specific surface proteins; thus, only EV subtypes are enriched based on the antibodies used since there is no widely accepted EV marker. This method could produce exosomes with high purity and coherence but is hard to scale up. Microfluidics also hold great promise in producing purified exosomes. However, at this time the lack of method validation and lack of standardization has limited the application of microfluidics to exosomes (Table 6).

3.4. In process and final release criteria for EV products

Considering the lability of the bioactive components of EVs (proteins/RNAs), standardization of storage conditions is crucial.[51] The morphology changes before and after freeze-thaw are not enough to demonstrate the stability of EVs. The biological properties of EVs also need to be tested (Table 7). Buffer conditions and storage temperature are carefully discussed in several studies.[52–55] −80 °C is recommended for long-time storage.[53] Additions like HEPES, albumin, and trehalose into the PBS can significantly improve the maintenance of concentration, morphology, protein, and nucleic acid cargo of EVs after being frozen and will not change the in-vivo EV distribution.[52] Besides, lyophilized EV product is commercially valued due to easy storage and transportation and has been tried in COVID-19 vaccine studies.[56–58]

Currently, the concentration of EV product is generally measured using the number of EV particles, the consistency of EV product is determined by the size distribution, and the purity is decided by the number of particles per microgram of protein (particles/ug).[3,49] However, for the measurement of particle size and concentration, the results from different methods can vary significantly.

Nanoparticle tracking analysis (NTA) is commonly used to simultaneously measure size distribution and particle concentration. The main NTA manufacturers are Malvern® NanoSight and Particle Metrix® ZetaView. ZetaView was more accurate in EV concentration, whereas NanoSight performed better in size measurement, by comparing the accuracy and repeatability between the two machines.[59] The use of electron microscope photography (TEM/cryo-EM) and manual measurement are considered the gold standard for measuring particle diameter.[3] But it cannot provide absolute quantification of particle concentration and is subjective and cumbersome. NanoFCM, a flow

Table 4
Companies focused on exosome services.

Company	Country	Year	Misson	Technology
EVerZom	France	2017	Large scale GMP manufacturing platform	Turbulence stimulation, patented
RoosterBio®	MD, USA	2013	Commercialization of scalable regenerative cures	Stem cell culture medium
Clara Biotech	KS, USA	2018	Exosome purification	ExoRelease workflow Antibody conjugated beads
NanoView Biosciences	MA, USA	2015	Analytical testing of EVs	ExoView® ExoFlex®
Kimera Exosomes	FL, USA	2012	Provide pharmaceutical grade MSC exosomes for clinical research	XoGlo® (10^9/mL), XoGlo®Pro, and Equisome HC® First FDA IND using exosomes for treatment of ARDS secondary to COVID-19
System Biosciences	CA, USA	2004	EV research products	Deliver EV research related reagents
The Cell Factory	Canada	2018	EVs biologic drug products for multiple indications	Fully defined, serum-free, xeno-free defined media

Table 5
Exosome sources.

Application		Source	Details
Diagnostic	Biomarkers	Biofluids	Blood, urine, saliva, bronchoalveolar lavage fluid (BALF) and ascites fluids
Therapeutic	Native EVs	Mesenchymal stem cells (MSCs)	Regeneration properties
		Neural stem cells (NSCs)	Neuroprotective and regenerative therapy
		Lung spheroid cells (LSCs)	Lung regenerative and lung-specific diseases therapy
		Platelets	Wound healing properties
		Dendritic cells	Inducing primary and secondary immune responses
	Engineered EVs	HEK293 cells	Safe and high yield
		Brain endothelial cells	Ability to penetrate through the BBB
		Macrophages	Accumulation in cancer cells
		T lymphocytes/ CAR-T cells	Activating immune response and decreasing toxicity
		Cancer cells (Leukemia cells, Breast cancer cells, Lung cancer cells)	Homing to the site of the tumor
		Red blood cells	Safe and high yield
		Ascites-derived	Safe, nontoxic, and tolerable

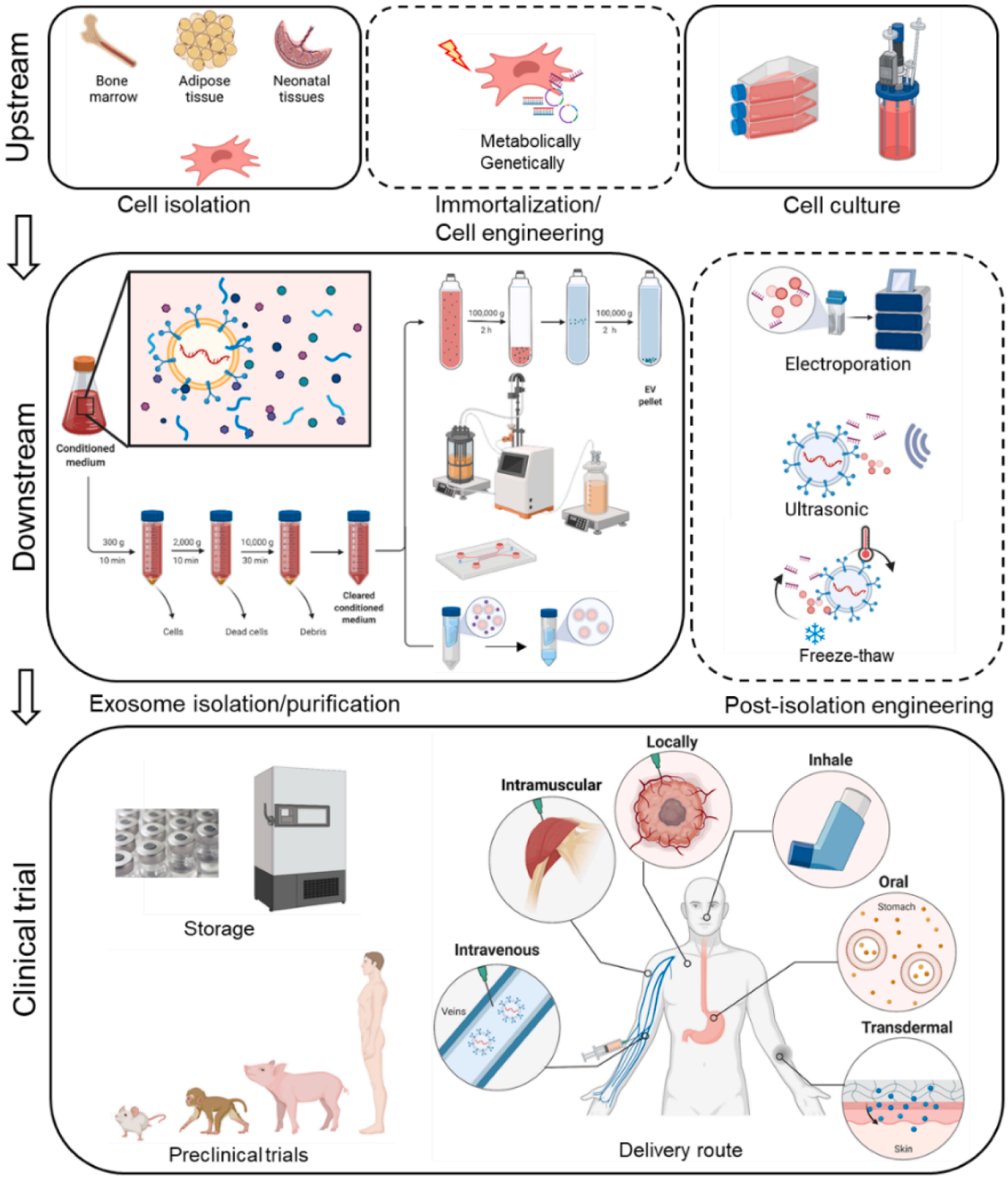

Fig. 2. Manufacturing, pre-clinical and clinical studies of exosomes.

Table 6
Isolation of EVs.

Method		Details	Advantage	Disadvantage
Conventional	Differential ultracentrifugation	100,000 g (up to 24 h)	High feasibility	Low yield, Time consuming
	Polymer precipitation	PEG	Easy to concentrate	Poor purity
Novel	Immunocapture	CD63, CD81, CD9	High purity	Hard to scale up
	Affinity-based	Tim-4	High affinity	Hard to scale up
	Size-based	TFF	Flexibility	Poor purity
	Size-based	SEC	High purity	Expansive, Hard to scale up
		Ion-exchange	High purity	Expansive, Hard to scale up
	Microfluidics	Flexible	Flexibility	Hard to scale up
	Combination	E.g. TFF+ chromatography	Scalability plus high purity	

Table 7
Characterization of EV products.

Parameter	Criteria	Method
Physiological properties	Particle number	Nanoparticle tracking analysis (NTA), NanoFCM
	Size	NTA, Dynamic light scattering (DLS), NanoFCM
	Morphology	Transmission electron microscopy (TEM), Cryo-electron microscopy, Scanning electron microscopy (SEM)
	Zeta potential	NTA
Biological properties	Surface marker	Western blotting; Flow cytometry, NanoFCM, ExoView
	Protein concentration	Quantitation assays
	RNA concentration	Quantitation assays
Microbial impurities	Endotoxin	Per standard of the country
	Sterility	
	Myocoplasma	

cytometer with a detection limit of 40–1000 nm, can detect EVs with specific fluorescent markers through multi-channel fluorescence detectors while measuring the size distribution and particle concentration.[60] This method combines quantitation and characterization. However, these several techniques for particle assay still need to be compared and standardized.

Different companies have their own standards for EV products. When considering the methods of purification summarized above, we should notice that different isolation methods have their preference for EV subgroups, thus leading to the variant composition of miRNA and surface proteins.[60,61] Much work is required because exosome products generally contain an unknown amount of exosome content, along with contaminants that may include proteins, cell-free DNA, viruses, and vesicles of other origins.[62] Purifying these samples is not trivial, and current methods require more rigorous controls to become approved therapies.

4. Considerations for EV preclinical research and clinical protocols

Pre-clinical studies include cell studies and animal studies. Currently, more researchers are using large animals or primates to further test the efficacy and safety of EV products. If the treatment is promising, researchers can file an investigational new drug (IND) application with the FDA. An IND application includes composition, stability, characterization of the drug, animal results, detailed outlines of clinical studies and the clinical trial team. Once the IND is approved, the drug can enter Phase I clinical trials. Most EV therapeutics are in Phase I trials, being tested in a few subjects with an accelerating dose to identify any side effects. The safety of EVs in human is being increasingly accepted. More detailed clinical protocols are required to launch Phase II clinical trials to test the efficacy of EVs in a larger group of people.

Dosing of EVs is usually based on EV protein content or particle concentration. For intravenous (IV) injection of MSC-EVs for example, 10^{12} EVs/kg of naïve EVs and 2×10^{10} EVs/kg of targeting EVs are needed according to our previous studies.[9] Local delivery doses may vary. We can extrapolate that approximately one trillion EVs might be needed for systemic EV treatments in humans.

Pharmacokinetic studies are important to elucidate the biological functions of exosomes, and for the development of exosome-based therapy. Exosomes are likely to be administrated parenterally, but it is possible they could be administered parenterally or orally.[63] Exosomes could also be used topically or incorporated into devices such as stents.[64] IV injected EVs mainly go to the liver and are taken up by macrophages.[65] However, the distribution of EVs largely depends on the cell source. For example, a remarkable accumulation of 4T1 cell derived EVs were found in the lung after IV injection. EV-based therapeutics and vaccines for lung diseases are usually administered to be inhaled to achieve a high lung accumulation.[66,67]

The FDA fast track process addresses a broad range of serious conditions and unmet medical needs. Drug companies can submit completed sections of their Biologic License Application (BLA) or New Drug Application (NDA) for review by FDA, rather than waiting until every section is completed. EV therapies to treat certain diseases hold the potential to be assigned for priority review and qualify for accelerated approval by the FDA via the fast-track process.

5. Conclusions

To date, no EV products have been approved by the FDA for therapeutic use. The FDA provides guidance to inform sponsors how to provide sufficient Chemistry, Manufacturing and Control (CMC) information required to assure product safety, identity, quality, purity, and strength including potency. In addition, the FDA and other regulatory authorities around the world have specific requirements for biologics used to mitigate, treat, cure, or prevent disease. Isolation and purification of exosomes from cell culture media require significant controls for quality, purity, potency, and reproducibility. Subsequent modifications to exosomes require additional controls. Specifications for

exosomes are likely to include specifications for their origin cells as well as their contents. Release criteria for EVs need more evaluation and standardization. With the evolution in our understanding of EVs and the technology of isolation, the era of EV-based products is poised to arrive in the near future.

CRediT authorship contribution statement

Ke Cheng: Drafting the article, Critical revision of the article. **Raghu Kalluri:** Drafting the article, Critical revision of the article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: K. C. is a co-founder and equity holder of Xsome Biotech Inc. Xsome Biotech Inc. is a licensee of the intellectual property covering exosome delivery technologies and antiviral therapies from North Carolina State University. UT MD Anderson Cancer Center and R.K. hold patents in exosome biology that are licensed to Codiak Biosciences, Inc. R. K is an equity holder and scientific advisory board member of Transcode Therapeutics.

Acknowledgments

K.C. and R.K. thank their lab members for their contributions to research in the area of extracellular vesicles. The graphical figures were created with BioRender.com. The work in the Cheng lab is supported by grants from the NIH (HL123920, HL137093, HL144002, HL146153, HL147357, and HL149940 to K.C.) and the American Heart Association (19EIA34660286 to K.C.). The EV work in the Kalluri lab is supported by MD Anderson Cancer Center, NIH R35CA263815, and NIH CA231465 and gift support from Fifth Generation (Love, Tito's), Lyda Hill Philanthropies and Bosarge Family Trust. R.K. is a Distinguished University Chair supported by Sid W. Richardson Foundation .

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