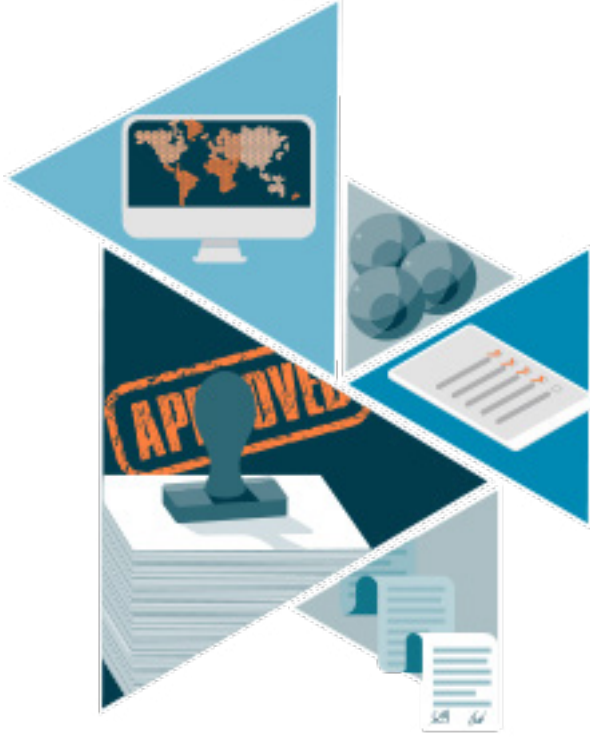
CELL & GENE THERAPY INSIGHTS



Regulatory Insights

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REGULATORY PERSPECTIVE

Exosomes as therapeutics and drug delivery vehicles: global regulatory perspectives

Shaun Stapleton

Exosomes are nano-sized extracellular vesicles secreted by all cells. Extensive research over recent years has now shown these vesicles to be important players in intercellular signalling and to have a role in tissue regeneration, immunomodulation and other biological functions. A lipid bilayer protects the exosome cargo of nucleic acids and proteins from degradation and permits signalling over long distances. The signalling characteristics of the cargo have led to exosomes being evaluated as therapeutics in their own right, while the protective lipid envelope and tissue tropism of exosomes suggests potential use as delivery vehicles for drugs that are usually rapidly degraded in the body. This range of potential uses raises interesting regulatory questions and challenges. Although derived from cells and containing nucleic acids, exosomes will not be considered as Advanced Therapy Medicinal Products (ATMPs) in Europe, unless they are used to deliver gene therapy. As biological medicinal products, whether exosomes would fall under the mandatory scope of the Centralised Procedure in Europe or fall under the jurisdiction of the Center for Biologics Evaluation and Research (CBER) or the Center for Drug Evaluation and Research (CDER) in the USA would depend on what they are being used to deliver. The legal classification and jurisdiction will go on to impact regulatory strategy and development plans. Characterisation of the exosome, reproducibility of production, potency assays, viral safety, toxicology study design and starting dose in man will be critical considerations as for other biological products.

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INTRODUCTION

Every so often new technologies emerge to change and augment the options available to treat human disease. These changes bring with them regulatory challenges, often as fundamental as how a new technology would be legally classified, requiring new legal definitions and whole sets of new guidelines to aid developers. Understanding the legal classification of a product is the first step in developing an appropriate regulatory strategy and development programme. This article discusses the regulatory control of exosomes, a new treatment paradigm that is emerging from the ATMP world and for which there are a wealth of regulatory questions - but because of the novelty of the technology, currently few firm answers.

WHAT ARE EXOSOMES?

Cell therapies have been under clinical investigation for many years and indeed the first products have now come to market. However, for many of these products, the regenerative therapeutic effect cannot be attributed to cell survival or differentiation and the possibility of trophic factors released from cells being involved has been investigated. The discovery that cells release extracellular vesicles (EVs) which elicit similar effects on surrounding tissue as the cells themselves led to great interest in isolating these extracellular vesicles from cell culture media and using them as possible "cell free" regenerative medicines [1]. One sub-type of extracellular vesicle is the exosome. These are nano-sized (50-120nm) lipid-bilayer bound vesicles released by fusion of multivesicular bodies (MVBs) with the cell surface plasma membrane. The exosomes contain a wide range of molecular components including microRNA (miRNA), pre-miRNA, messenger RNA (mRNA), cytokines, lipids and proteins [2]. A diagrammatic representation of an exosome is given in Figure 1.

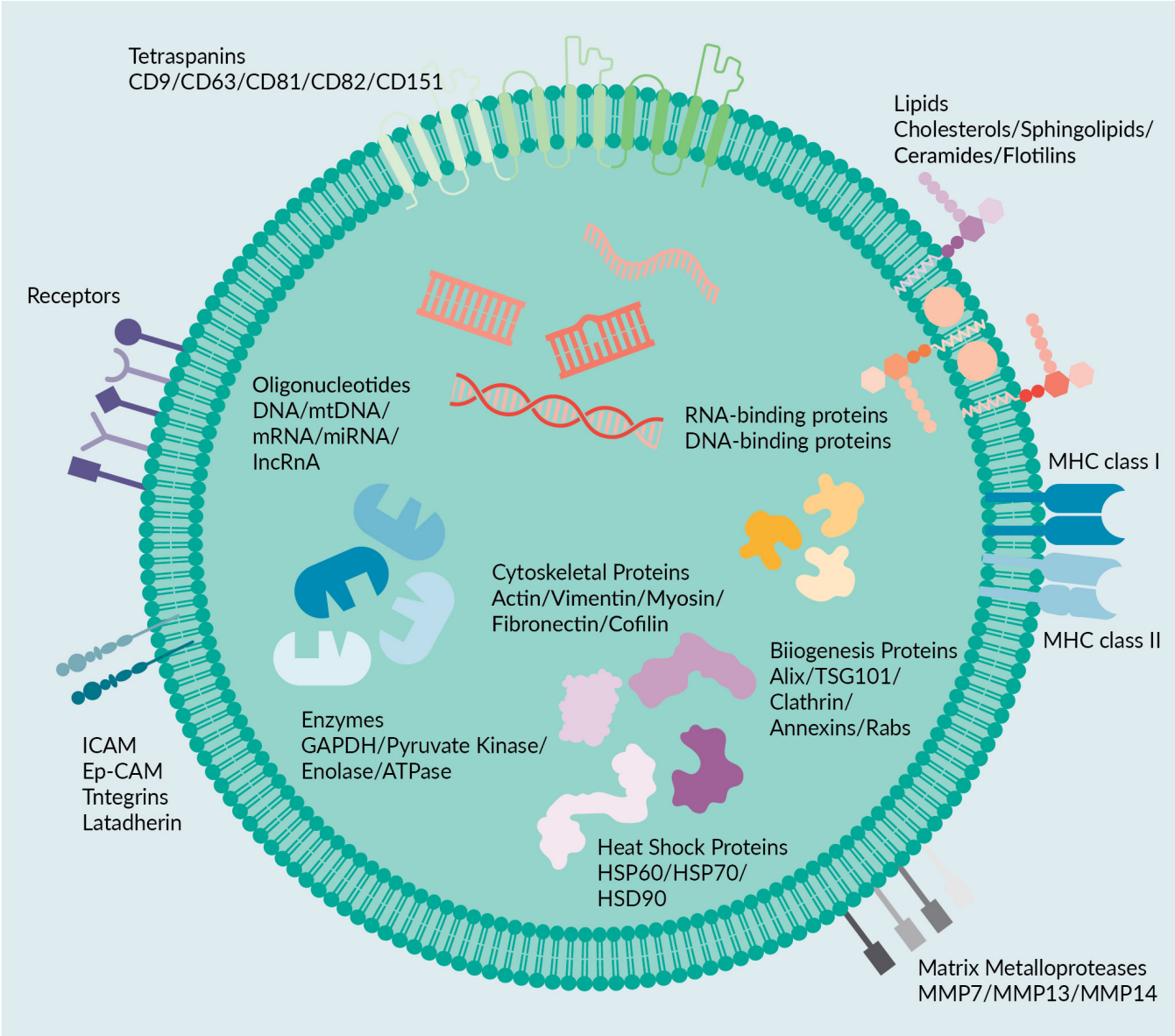
Research has shown that the molecular "cargo" is functional in sending signals from the source cell to the recipient cell and could be involved in a regenerative effect in the recipient. Other work has shown exosomes to be involved in immunoregulation including antigen presentation, immune activation and immune suppression [4] and they are potentially involved in many other biological functions such as viral pathogenicity, cardiovascular diseases, CNS diseases and cancer progression [2]. The exosome itself protects its contents from degradation and allows the signals to pass over long distances within tissues without degradation [1]. This suggests that native exosomes themselves could have therapeutic benefits and this is one area of ongoing research. A number of clinical trials with native, unmodified exosome-based therapies in conditions such as Duchenne muscular dystrophy, various cancers, epidermolysis bullosa and stroke have recently started or are planned [3].

In addition, considering the protective effect of the lipid bilayer of exosomes on the molecular cargo, exosomes also have potential as delivery vehicles. In this respect they could be considered like natural liposomes, but further research has shown that exosomes also display specific organotropic behaviour – the cellular origin of exosomes may influence the biodistribution profile, suggesting that EVs from different cell sources possess different targeting characteristics [5]. So exosomes could be used to deliver unstable molecules such as proteins and nucleic acids that are currently difficult to deliver effectively, more precisely to their target tissues. Several companies are evaluating such approaches to deliver replacement enzymes for metabolic diseases, siRNAs for genetic diseases and peptide therapeutics and small molecules for a range of conditions where traditional formulations are sub-optimal for reaching the target, for example crossing the blood-brain barrier.

This article aims to raise and discuss some of the unique regulatory issues and challenges that such a diverse range of potential uses brings, considering both native, unmodified exosomes and exosomes loaded with other active substances.

FIGURE 1

Diagrammatic representation of an exosome showing lipid bilayer, range of potential molecular cargos and surface molecules involved in tropism and signalling.



REGULATORY CLASSIFICATION AND AGENCY JURISDICTION OF EXOSOME BASED PRODUCTS

Exosomes are purified from spent cell culture media. So as they are derived from cells, does this make them ATMPs (somatic cell therapies, gene therapies or tissue-engineering products, as defined in EU legislation)? To answer this question, one has to consider what the active substance is in the product.

Native exosomes may contain hundreds or thousands of protein and nucleic acid components. If the exosome product has an intrinsic therapeutic or diagnostic effect that cannot be attributed to one or more specific molecules within the exosome then the exosome itself would be considered the active substance and the product would be a biological medicine. This is because the active substance would be extracted from a biological source and therefore meet the definition of a biological medicinal product in Section 3.2.1.1.b of Part I, Annex I of Directive 2001/83/EC. The product would not be an ATMP because the legal definitions of a somatic cell therapy

in Part IV of Annex I to Directive 2001/83/EC and of a tissue-engineered product in Article 2.1 of Regulation 1394/2007/EC require that these types of product "contain or consist of cells or tissues". Similarly, a native exosome is not a gene therapy, the other subtype of ATMPs. Even though exosomes may contain nucleic acids, the definition of a gene therapy requires that the gene therapy contain "recombinant" nucleic acids. It is true that they may regulate the expression of a genetic sequence within the target cell but these mRNAs and microRNAs are the "natural" product of the cell and are not modified or "recombinant". So native exosome products used as medicines would not be classified as ATMPs in Europe. Existing precedent supports this conclusion. In December 2009 the Committee for Advanced Therapies (CAT) concluded that mesenchymal stem cell-derived microvesicles (containing receptors, proteins, lipids, mRNA and microRNA) for treatment of renal disease are not considered to be an ATMP [6].

In the US the situation is not as clear. FDA defines gene therapy products [7]:

to include all products that mediate their effects by transcription or translation of transferred genetic material or by specifically altering host (human) genetic sequences.

Native, unmodified exosomes contain nucleic acid sequences (e.g. miRNA) that mediate their effect by binding target cell mRNAs and regulating translation to target cell proteins. This would not appear to meet the above definition of a gene therapy, as the transferred nucleic acids themselves are not transcribed or translated, nor do they alter host genetic sequences. There may also be a multi-modal mechanism of action that may include some of the transferred proteins within the exosomes. In the opinion of the author, it is unlikely that native, unmodified exosomes will be classified as gene therapies in the US and the situation will be similar to that in Europe, but this should be discussed with FDA on a case-by-case basis.

In the US, the concept of an advanced therapy analogous to the EU definition is restricted to the Regenerative Medicine Advanced Therapy (RMAT) expedited program introduced in 2017. The FDA and specifically its Center for Biologics Evaluation and Research (CBER) has determined that native, unmodified exosome products will be considered biologicals under the jurisdiction of the CBER Office of Tissues and Advanced Therapies (OTAT) because they are considered to be "parts of cells" (personal communication).

The situation is markedly different in the case that exosomes are loaded with a manufactured drug substance. As described above, there is growing interest in using exosomes as delivery vehicles for drug substances loaded into or onto the exosome. Various mechanisms exist for loading proteins or nucleic acids into or onto exosomes, either directly with techniques such as electroporation, chemical transfection and passive incubation or by modifying the producer cells to express a particular protein or nucleic acid, which is then expressed and trafficked into exosomes by the intracellular machinery.

The regulatory classification of exosomes used as delivery vehicles would depend on the nature of the therapeutic component being loaded and delivered. If the exosomes were used as a delivery system for a recombinant nucleic acid, then such a product would be considered to be an ATMP (gene therapy) in Europe if that recombinant nucleic acid or its expression was directly related to the therapeutic, prophylactic or diagnostic effect of the medicinal product. Recent precedent supports this conclusion – the European Medicines Agency (EMA) has classified exosomes carrying recombinant mRNA encoding for the cystic fibrosis transmembrane conductance regulator protein and microRNA-17 for the treatment of cystic fibrosis as a gene therapy product [8]. The same would apply in the US as such a product would meet the definition above.

On the other hand, if the exosome was delivering anything other than a recombinant nucleic acid, it would not be considered as a gene therapy product. This explains why the products that are being developed consisting

of siRNA molecules, which are produced synthetically, are not classified as ATMPs [9]. These may be regulated as biologics in Europe (if the exosome itself is considered part of the active substance), as would exosomes loaded with recombinant proteins for example (see Table 1). Whether loaded exosomes would necessarily be under the jurisdiction of CBER in the US is an open question. In 2003, jurisdiction for certain products, including proteins and monoclonal antibodies was transferred from CBER to the Center for Drug Evaluation and Research (CDER). CDER is also responsible for small molecules, including synthetic peptides and synthetic siRNA products (e.g. patisiran). So it is possible that for exosomes loaded with these types of active substances, oversight of the product at FDA could be under CDER rather than CBER if the exosome is contributing only to the delivery of the active substance. If the exosome has a specific tropism based on surface markers to a particular site in the body, then this could further confuse the situation and may result in jurisdiction under CBER.

In Europe, Article 3(1) of Regulation 726/2004/EC mandates certain types of product to obtain a marketing authorisation granted by the European Commission through the Centralised Procedure (CP). These products are described in Annex 1 to the Regulation and includes all ATMPs as well as orphan drugs and products for certain clinical conditions. It is however noteworthy that not all biological products fall within the mandatory scope of the CP – only those which are manufactured using recombinant DNA technology, controlled expression of genes coding for biologically active proteins in prokaryotes and eukaryotes including transformed mammalian cells, or hybridoma and monoclonal antibody methods. This would include exosome products derived from cells that have been modified with recombinant DNA to express a particular nucleic acid or protein in the exosome or immortalized to produce

TABLE 1

Classification and jurisdiction of various potential exosome based medicinal products.

Classification/ jurisdiction	Native, unmodified exosomes	Loaded exosomes					
		Synthetic siRNA, miRNA	Recombinant nucleic acid	Virus (for gene therapy)	Protein[a]		Small molecule
					Pre-loaded via producer cells	Post-loaded	
ATMP	x	x	✓	✓	x	x	x
Biological MP (not ATMP)	✓	x[b]	x	x	✓	✓	x[b]
Small molecule MP	x	✓[b]	x	x	x	x	✓[b]
EMA/CP mandatory scope	x	x	✓	✓	✓	✓/x[c]	x
EMA/CP optional scope	✓	✓	x	x	x	x/✓[c]	✓
CBER	✓	x[b]	✓	✓	x[b]	x[b]	x[b]
CDER	x	✓[b]	x	x	✓[b]	✓[b]	✓[b]

[a]Pre-loaded assumes producer cells express protein from recombinant DNA and package into exosomes. Post-loaded assumes protein loaded by electroporation or other loading techniques.
[b]Assuming that the exosome was shown not to play a part in the mechanism of action other than simple delivery of the active substance to the site of action (so not to be an "active substance"). However, it is unknown whether exosomes can be completely emptied of their cargo or if any specific tropisms can be eliminated. Therefore it is likely that exosomes will be considered part of a "combined active substance" rather than an "excipient" and will always be considered biologics. As far as the author is aware, this has yet to be tested with regulators.
[c]Would be optional scope only if the protein was not produced by recombinant DNA techniques (e.g. extraction from biological samples).
Note: The hypothetical products mentioned are all assumed to be new active substances. The table highlights only the considerations specific to the composition of the product itself and does not take into account specific clinical indications or orphan drug status which could influence the classifications/jurisdictions/CP scope.

native, unmodified exosomes but would not necessarily include native exosomes purified from spent culture media from non-genetically modified cells. In addition, Article 3(2) of Regulation 726/2004 gives optional access to the CP for all new active substances and any product that "constitutes a significant therapeutic, scientific or technical innovation".

Considering the relative novelty of the exosome field and the wide range of potential products, FDA and EMA will deal with these issues on a case-by-case basis (personal communication). Developers of exosome-based products will need to take the aforementioned points into consideration and consult the Agencies on the classification of their products at an early stage in order to clearly define expectations in relation to product development plans. The EMA offers a specific procedure for confirming the classification of a product as an ATMP.

The classification and jurisdiction of various types of future exosome based medicinal products (MP) are summarised in Table 1. The hypothetical products mentioned are all assumed to be new active substances. The table does not take into account specific clinical indications or orphan drug status which could influence the classifications/jurisdictions (specifically the EMA/CP mandatory/optional scope) and is meant to highlight only the considerations specific to the composition of the product itself. The classifications described in Table 1 correspond to the four scenarios described in a 2015 position paper published by the International Society for Extracellular Vesicles (ISEV) [10]. It should also be noted that the above discussion and the scenarios in Table 1 apply whether the exosome is derived from autologous or allogeneic cell sources.

THE IMPACT OF THE REGULATORY CLASSIFICATION ON DEVELOPMENT PLANS

Development plans will differ depending on whether native exosomes are being used as therapies themselves or whether they are being used as delivery vehicles for an added active component. Some key potential considerations are described below in general terms. The specifics will be largely dependent on the nature of the exosomes being developed (including whether the exosomes are derived from autologous or allogeneic cells), the target indication and the posology.

- Native, unmodified exosomes

1. Definition of starting materials in the exosome production process. Detailed information on the derivation of the cell line used to produce the exosomes (upstream process) and of the conditioned media from which the exosomes are purified will be required. The Master Cell Bank/Working Cell Bank are likely to be considered as starting materials, with the spent media from which the exosomes are purified as a key intermediate, although this should be discussed and agreed with regulators.

2. How can the product be characterised? Are there different populations of exosomes secreted by the cells that have different effects? How can these be separated to provide a purified population with the desired effect and reducing potential adverse effects. Or is a mixed population required for the therapeutic effect?

3. How can batch to batch reproducibility of the purified population be assured? What are the key identification markers for the desired exosome population, for example surface markers, specific miRNA content?

4. Thorough understanding of the upstream and downstream manufacturing process, including how different donations of cells and how even small process changes may have an impact on the quality of the exosomes produced will be critical [10].

5. Which components of the exosomes are responsible for the therapeutic effect? Is

it possible to identify these and if so can they be measured and a potency method developed? If not, how will potency of the exosome product be determined? Development of a potency method linked to mechanism of action and/or clinical effectiveness will be a key requirement as clinical development progresses to later stages, particularly in ensuring batch to batch consistency and comparability following any process changes.

6. Are there any impurities, either different populations of exosomes or residuals from the manufacturing process? How can these be measured and controlled?

7. What approaches to viral safety may be suitable? Viral clearance or inactivation is a key requirement for biological products but the available techniques such as chromatography or detergent inactivation are not suitable for exosomes due to their size and composition (lipids and proteins). So reassurance based on control and testing of the banks of the producer cell line and on the raw materials used in the process will be critical.

8. Are exosomes immunogenic and if so, how does this impact animal model selection for pharmacology/toxicology studies?

9. How would the proposed initial dose for a first in man clinical study be calculated? This would be based on the potency assay developed and doses used in nonclinical toxicology and pharmacology studies (with a justified safety margin), and could for example be expressed as potency/number of particles.

- Exosomes as delivery vehicles

Several of the items listed above for native, unmodified exosomes would also apply to exosomes used as delivery vehicles. However, in this case the development plan will be further complicated, or possibly simplified, based on exactly what is being delivered and the regulatory strategy will be central to designing an appropriate development plan. For exosomes used as delivery vehicles, the "drug substance" will be considered to be the active substance being delivered. However, it is questionable whether an exosome can be completely emptied of its natural components and it will be important therefore to demonstrate the safety of the exosome itself, as well as in combination with the delivered active substance. Further complications in defining the "drug substance" and "drug product" for regulatory purposes could arise if the exosome itself, or its natural contents, contribute in any way to the mechanism of action of the product (i.e. have an additive therapeutic effect compared to the drug being delivered alone). In this case, the exosome could be considered a "combined drug substance" alongside the loaded protein, nucleic acid or small molecule and the contribution of both components to the safety and efficacy of the drug product would need to be established. This would be an important discussion point with regulators early in the development of such a product.

If the exosomes are being used to deliver a known (already approved) active substance, the nonclinical programme (pharmacology, safety pharmacology, toxicology) would be designed to address possible differences in exposure/pharmacokinetics to the active substance(s) compared to existing formulations of the same active substances. This would result in an abbreviated development programme, with use of some of the regulatory mechanisms that are in place to avoid the need to repeat testing for drugs that are off-patent and outside their regulatory data exclusivity period.

For exosomes delivering known drug substances, the potency assay would be based on measuring the content of the active substance being delivered, as in the case of conventional medicines. If the exosome contributes to the mechanism of action however, a specific potency assay relating to the exosome component would also be required.

Where new active substances are being delivered (small molecules, proteins, nucleic

acids), the nonclinical programme will include the usual studies required for novel medicinal products (single and repeat dose toxicology, local tolerability (if applicable), safety pharmacology, immunotoxicity and toxicokinetics/biodistribution). Similar issues as described for native, unmodified exosomes would arise in terms of how the exosome component of the product is characterized and purified, how it effectively delivers the active component to the desired target and whether it may have its own adverse effects or contribution to efficacy. This will result in studies being required to evaluate the combination of drug and exosome together and the need for a potency assay that measures the activity of the exosome component.

Exosomes contain a large number of miRNAs, growth factors, cytokines and other components and therefore the mechanism of action, nature of the human target and the relevance of animal models may be less clear than with other types of medicine. This adds to the risks of initial human trials and measures to reduce this risk such as those described in the EMA/CHMP Guideline on Strategies to Identify and Mitigate Risks for First in Human Clinical Trials with Investigational Medicinal Products will apply [11]. Furthermore, the characterisation of the exosome product is critical to allow appropriate analytical tests to be developed to control the quality of the product, to ensure that batches to be used in humans are representative of those used in animal studies and to allow comparability following future process changes. Scientific advice from key regulatory agencies on the proposed development plan is essential considering the novel nature of these products and the resulting lack of precedent.

CONCLUSION

Exosomes represent a novel potential treatment option in a wide range of therapy areas, potentially as cell-free regenerative medicines, as treatments for cardiovascular, CNS and oncological indications, as vectors for gene therapy, as immune modulators and as drug delivery vehicles. Their novelty and range of uses means that there will be specific regulatory classification and jurisdiction issues to be clarified to enable development plans to be established. For a completely new class of product they are also unusual in that their possible use as drug delivery vehicles means that a regulatory strategy and development plan that merges established concepts applicable to highly innovative new drugs with those applicable to abbreviated drug development of already approved drug substances may be required. They will therefore represent some interesting regulatory challenges over the coming years as they progress from concept, through to clinical trials and ultimately, to market.

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