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Review

Guidance on the clinical application of extracellular vesicles☆

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ABSTRACT

Therapies using extracellular vesicles (EVs), exosomes, or cell culture supernatants containing EVs or exosomes (referred to as "EV therapies" in this Guidance) have garnered an increasing amount of interest. However, pharmaceutical products containing EVs as their main active ingredient are yet to receive regulatory approval.

The "Basic points to consider regarding the preparation of extracellular vesicles and their clinical applications in Japan," was announced by the Japanese Society for Regenerative Medicine (JSRM) on March 10, 2021, with a specific focus on exosomes among EVs to promote high safety standards in this evolving field and facilitate the application of EV clinically. The Scientific Committee of the Pharmaceuticals and Medical Devices Agency and the Japanese Society for Extracellular Vesicles have issued reports and statements regarding treatment, resulting in a growing momentum toward treatment in Japan.

This article summarized the basic items that should be recognized comprehensively for clinical practice in three categories: (1) risk profiling, (2) preparation (manufacturing) process and quality, and (3) verification of EVs checking items and effectiveness. This guideline will be revised in the future with technological innovations and new findings; nevertheless, it will serve as a guide for the development of treatments.

Abbreviations: EVs, extracellular vesicles; DDS, drug delivery system; ISEV, International Society for Extracellular Vesicles; JSRM, Japanese Society for Regenerative Medicine; JSEV, Japanese Society for Extracellular Vesicles; PMDA, Scientific Committee of the Pharmaceuticals and Medical Devices Agency; ASRM, The Act on the Safety of Regenerative Medicine; FCM, nano-flow cytometry; NTA, Nanoparticle Tracking Analysis; TRP, Tunable Resistive Pulse; DLS, Dynamic Light Scattering; FFF, Field Flow Fractionation; FBS, fetal bovine serum; CQAs, critical quality attributes.

☆ This guidance was written by the Japanese Society for Regenerative Medicine in cooperation with the Japanese Society for Extracellular Vesicles.

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[2] https://jsev.jp/

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Contents

1. Current status leading to the creation of this guidance

Therapies using extracellular vesicles (EVs), exosomes, or cell culture supernatants that contain EVs or exosomes, referred to as "EV therapies" in this Guidance, have garnered an increasing amount of attention in recent years. EVs are vesicles secreted by cells that comprise exosomes and other vesicles containing growth factors that stimulate tissue regeneration and intercellular signaling molecules (Fig. 1). EVs can be classified as unmodified natural EVs and modified EVs. Unmodified natural EVs are collected from cell culture supernatants, whereas modified EVs are EVs that are conferred with certain functional characteristics. Modified EVs can be obtained through two methods. The first method involves purifying and collecting EVs from the culture supernatant fluid of specific cell lines, specific animals, and plants, or their cultured cells and then modifying them, for example, through linkage to peptides. The second method involves genetically modifying cells or animals and plants that are then used as the source for collecting modified EVs. Modified EVs also include EVs that encapsulate specific therapeutic drugs; thus, they can be used as drug delivery system (DDS) carriers.

EVs can be applied to a wide range of potential fields and diseases in the future, and this potential for further development creates high expectations for this field. However, pharmaceutical products containing EVs as the main active ingredient are yet to receive regulatory approval. Furthermore, no guidelines or guideline documents have been set forth by the International Society for Extracellular Vesicles (ISEV) or individual countries.

The "Basic points to consider regarding the preparation of extracellular vesicles and their clinical applications in Japan" [1,2] was announced by the Japanese Society for Regenerative Medicine (JSRM) on March 10, 2021. This document specifically focused on

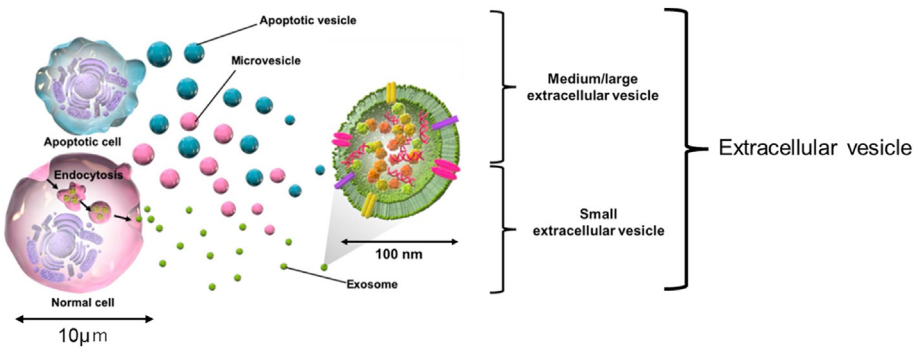

Fig. 1. Extracellular vesicles (EVs) are substances secreted by cells such as exosomes and other vesicles. These substances contain growth factors that stimulate tissue regeneration and intercellular signaling molecules. EVs play an important role in intercellular communication and can be applied to various diseases.

exosomes among EVs to promote high safety standards in this evolving field and facilitate the application of EVs. Advances in knowledge and technology in the past 3 years and concrete initiatives have led to the application of EVs to clinical trials aimed at clinical use. However, the global trend of unapproved products and preparations claiming to contain EVs and exosomes being prescribed to patients by physicians in private clinics remains unchanged, leading to concerns about their safety and efficacy. The "Position of the Japanese Society for Extracellular Vesicles on Medical Practice Using Extracellular Vesicles," [3] expressing the same concerns as those of our Society, was published by the Japanese Society for Extracellular Vesicles (JSEV) on December 25th, 2023.

This position paper aimed to provide and explain "current status check items" and "recommended items" that personnel involved in administering EV therapies should confirm to ensure the development and expansion of safe and secure EV therapies, with the goal of outlining a sound direction for the future advancement [4]. The guidelines are divided into three parts: (1) risk profiling, (2) preparation (manufacturing) process and quality, and (3) verification of EVs, including checklist items and effectiveness. The recommendations provided herein are prepared based on a review of the currently available literature, the "Report on Preparations for Therapeutic Applications Using Extracellular Vesicles (EV) Including Exosomes" [5] published by the Scientific Committee of the Pharmaceuticals and Medical Devices Agency (PMDA), as well as the understanding of the situation and experience of each member involved in the preparation of this Guidance.

A diverse range of cellular sources can be used as sources for creating EVs. Thus, modified EVs, in addition to natural EVs, will also be developed in the future. This diversity and progress is expected to lead to further development of this domain in the future. Moreover, revision of the current guidelines to be consistent with research developments is also expected. In preparing this Guidance, we therefore focused on natural EVs derived from mesenchymal stem cells, the type of EVs that is currently expected to be used in clinical applications [6], and we have focused on the three key items listed above.

This Guidance is based on the data available at present. Consequently, it will be revised as necessary in light of future scientific advances, developments, or actual applications in clinical trials.

2. Risk profiling

2.1. Outline of risk profiling

2.1.1. Current status-check items

Any medical intervention using EVs prepared (manufactured) under the direction of a physician or dentist and administered to patients at the medical institution wherein the physician or dentist practices does not fall under the scope of the "The Act on the Safety of Regenerative Medicine" (ASRM) [7] in Japan. Hence, the Certified Special Committee for Regenerative Medicine or Certified Committee for Regenerative Medicine does not review these therapies.

(Explanation).

Several clinical risks and risk factors contributing to these risks are inherent to EV therapies. All steps of the process, from the collection of cellular source materials to the preparation (manufacturing) of the EVs, transport, storage, clinical administration, and post-treatment management, are associated with certain risk factors. "EVs as the final drug product (pharmaceutical formulation)" (referred to as "EV preparations" throughout this document) contain complex and heterogeneous EV components that may comprise infectious factors that are difficult to inactivate or remove. Thus, specific risks are likely to be related to multiple risk factors. Possible risks must be identified before using a specific EV preparation in a clinical application. Furthermore, the causative risk factors of each risk must be identified, the magnitude of the contribution of each risk factor in the respective risk must be evaluated (these steps are also called "risk profiling"), and methods to mitigate and manage the risk factors must be determined and implemented.

Any medical intervention utilizing EV preparations that have not received marketing approval according to the "The Act on Pharmaceuticals and Medical Devices" (PMD Act) but are prepared (manufactured) under the direction of a physician or dentist and administered to patients at the medical institution wherein the physician or dentist practices does not fall under the scope of the "The Act on the Safety of Regenerative Medicine" (ASRM) in Japan. Consequently, the Certified Special Committee for Regenerative Medicine or Certified Committee for Regenerative Medicine does not review these therapies. Thus, physicians or dentists who prescribe EV therapies using EVs (EV preparations) that have not received regulatory approval must possess a sufficient understanding of the quality and risk profile of the EV preparation and must take measures to ensure the safety of the patients. Physicians or dentists must also confirm that all risks are sufficiently low compared with the potential benefit of the treatment before commencing treatment.

2.2. Risk profiling methods

2.2.1. Recommended items

The risks, risk factors, and measures to mitigate them must be identified during the early stages of the development of EV therapy. The risks associated with EV preparations do not differ significantly from those associated with biopharmaceuticals developed in accordance with the PMD Act. Nevertheless, risk factors related to a specific risk may be present at various stages, such as the properties or composition of the EV preparation, procurement of source materials, manufacturing process, transport, storage, preclinical evaluation, clinical evaluation, clinical administration method, and handling of the EV preparation at the medical institution where it is used. EVs are complex, heterogeneous, and non-uniform. Thus, identifying and managing all critical quality attributes (CQAs) related to risk factors that may interfere with the safety or efficacy of the product would be difficult even when all possible quality attributes are listed.

2.2.2. Recommended items

An inventory of the specimens (e.g., EV preparations or biological source materials from the same lot as that used in clinical applications) as well as preparation (manufacturing) and administration records must be maintained as part of the risk management of EV therapy to identify the cause of adverse events.

(Explanation).

(1) Identification of the risks associated with clinical use

The risks and measures to mitigate them must be identified during the early stages of the development of EV therapy. The risks associated with EV preparations do not differ significantly from those associated with biopharmaceuticals developed in accordance with the PMD Act. Similarly, risks associated with EV preparations do not differ significantly from those associated with regenerative medical products developed in accordance with the PMD Act or specified processed cells used for the provision of regenerative medicines or cell therapies in accordance with the ASRM, with the exception of the risk of tumor formation due to abnormal proliferation of living cells in the product (tumorigenicity). Common safety

risks related to the clinical use of EV preparations include (a) transmission of infectious diseases (e.g., viruses, bacteria, and fungi); (b) undesirable immunoreactions; (c) contamination with impurities; (d) unintended changes in the properties of EVs; (e) undesirable biodistribution of EVs; (f) major organ, germ line, or developmental toxicities; (g) formation of patient cell-derived tumors or undesirable tissues; and (h) failure to demonstrate the expected efficacy. These risks can be attributed to the properties or quality of EVs, which are the main components in the EV preparation (final administration product), or components other than EVs.

(2) Identification of risk factors related to each risk

Risk factors related to a specific risk may be present at various stages, such as the properties or composition of the EV preparation, procurement of source materials, manufacturing process, transport, storage, preclinical evaluation, clinical evaluation, clinical administration method, and handling of the EV preparation at the medical institution where it is used. The following are examples of risk factors.

(a) Origin of cell-substrate [8] (or cell bank) [9] (e.g., autologous, allogeneic, and xenogeneic)
(b) Cell type, culture conditions, and passage number of the cell substrate (or cell bank)
(c) Molecules that are immune system targets and intensify an immunoreaction
(d) EV purification method
(e) EV stabilization and storage method
(f) Toxicity of components other than EVs
(g) Method and site of administration (e.g., local vs. systemic)
(h) Administration period and frequency of administration (e.g., short-term vs. long-term and single vs. repeated)
(i) Robustness of quality controls and preparation (manufacturing) controls (e.g., GMP [10] /GCTP [11] -compliant vs. non-compliant)
(j) Structural facilities and equipment used at the preparation (manufacturing) site or medical institution (e.g., sterility)
(k) Proficiency of the workers at the preparation (manufacturing) sites or medical institutions (e.g., aseptic processing)
(l) Availability of clinical data of the product or experience with similar products

Each risk factor may be related to several risks, and their effects on a specific risk may interact. Thus, the process of identifying risk factors related to EV preparation and its clinical application must be continued throughout the process, spanning from the procurement of the source materials to product development, throughout clinical trials, and during the provision of therapy.

(3) Understanding of the correlation between risk and risk factors [12]

Qualitative or quantitative data or information for each risk factor can be mapped onto a two-dimensional matrix, with risk factors on the vertical axis and risks on the horizontal axis, or vice versa, to evaluate the relationship between each risk factor and a specific risk. A systematic overview of the correlation between risk and risk factors can be obtained using such a matrix. It is recommended to follow steps (a)–(f) to gain an understanding of the correlation between risk and risk factors.

(a) Identify the combinations with an evident correlation between risk and risk factor
(b) Evaluate the scientific background of this correlation using available knowledge
(c) Evaluate the effect of risk factors, such as contamination with specific bacteria or fungi, on the correlated risks
(d) The appropriateness of not performing steps (b) or (c) must be evaluated based on the balance between the seriousness of the risk and the potential benefit if these steps cannot be completed.
(e) Identify and implement procedures to reduce or control the risk factors to mitigate the risk
(f) Formulate and implement a management plan for any remaining risks after controlling for the risk factors. An inventory of the specimens (for example, EV preparations or biological source materials from the same lot as that used in the clinical application), as well as preparation (manufacturing) and administration records, must be maintained during risk management planning to identify the cause of adverse events.

3. Preparation (manufacturing) process and quality

3.1. Risks related to preparation (manufacturing)

3.1.1. Recommended items

Aseptic processing (including processing using aseptic techniques) and measures to prevent contamination with various components must be implemented throughout the process, beginning with the acquisition of the source material. The condition of the cells used for manufacturing can degrade. Thus, process control is very important as the quality, efficacy, and safety of the EVs secreted from these cells are greatly affected by the manufacturing process.

(Explanation).

Among the concrete risks for EV preparations used in clinical applications, the following are closely related to the preparation (manufacturing) process.

(a) Contamination with infectious factors such as viruses, bacteria, or fungi from source cells or other source materials
(b) Contamination with substances other than EVs, which can induce allergies or rejection of the source materials
(c) Contamination with substances other than EVs, such as particulate matter or leachable substances from the processing materials
(d) Contamination with biological or chemical contaminants during the process
(e) Contamination with residual accumulation of cells or EVs generated during the process
(f) Variability between the efficacy or quality of consecutive batches
(g) Quality deterioration during storage of the EV preparations
(h) Cross-contamination or mix-up of autologous EV preparations

EVs are originally secreted by cells; consequently, the preparation (manufacturing) processes for cellular products and the risks related to ensuring safety and efficacy are very similar.

Ensuring high purification of EVs and the inactivation or elimination of all viruses during the preparation (manufacturing) process is difficult. Therefore, aseptic processing and measures to prevent contamination with a variety of components must be implemented consistently throughout the process, beginning with the acquisition of the source material. The condition of the cells used for manufacturing can degrade. Thus, process control is very important as the quality, efficacy, and safety of the EVs secreted from these cells are greatly affected by the manufacturing process.

Poor storage conditions after preparation (manufacturing) may negatively affect the quality of EVs. However, the complex properties of EV preparations, such as processed cells [13], make it difficult to understand all quality aspects through specifications and characterization. Therefore, the quality of EV preparations must be ensured by controlling their quality through specifications and characterization and through quality control of source materials and preparation (manufacturing) process control.

The quality attributes of EV preparations can be assessed only to a limited extent at present. Thus, whether product comparability can be evaluated sufficiently by comparing the recognizable quality attributes before and after a preparation (manufacturing) process changes remains unclear. Flexible implementation of changes to the preparation process to achieve scale-up or cost reductions will be difficult. An *in vitro* titer assay system to measure EV preparation potency and continuously explore and identify critical quality attributes can be established to avoid conducting additional preclinical and clinical studies evaluating changes in the preparation method to solve this problem [14].

3.2. Basic considerations on preparation (manufacturing)

3.2.1. Recommended items

Quality control and preparation (manufacturing) control measures must be implemented in accordance with the regenerative medicine provision standards set forth by the ASRM, and the manufacturing control and quality control standards for regenerative medical products [15] (cell-processed products) set forth by the PMD Act to ensure the quality, efficacy, and safety of EV preparations.

(Explanation).

Quality control and preparation (manufacturing) control measures must be implemented in accordance with the regenerative medicine provision standards set forth by the ASRM, and the manufacturing control and quality control standards for regenerative medical products (cell-processed products) set forth by the PMD Act to ensure the quality, efficacy, and safety of EV preparations.

With the exception of manufacturing under the PMD Act, aseptic processing must be implemented during the preparation (manufacturing) process of EV preparations for clinical use in accordance with standards similar to those set forth by the latest version of the “Considerations on Aseptic Processing in Cell Processing Facility under the Act on the Safety of Regenerative Medicine” published by the JSRM.

Freeze-drying (an infrequent part of the preparation [manufacturing] of specific cell-processed products), if conducted, must be designed after accounting for the following risks related to aseptic processing.

- Perform freeze-drying in the aseptic processing area (critical processing zone) as this step is a part of aseptic processing
- Prevent backflow from the vacuum pump, if the process includes a vacuum step, to prevent contamination of the administration product
- Prevent the spread of oil mist from the pump exhaust in the aseptic processing area to preserve the environmental conditions

4. EV preparation checking items and validation of efficacy

4.1. Common specifications for the preparation (product) and its evaluation

4.1.1. Verification of EVs after collection and enrichment

4.1.1.1. Recommended items. Verification of the presence of (1) particles with the shape and size of EVs and (2) EV marker molecules is necessary.

(Explanation).

4.1.1.1.1. Specific example. Methods such as nano-flow cytometry (FCM), immunoelectron microscopy, or single-EV immunodetection can be used to evaluate (1) and (2) simultaneously. (1) Electron microscopy, Nanoparticle Tracking Analysis (NTA), Tunable Resistive Pulse (TRP), Dynamic Light Scattering (DLS), or Field Flow Fractionation (FFF) and (2) proteomics, western blotting, or ELISA can be used for verification if simultaneous verification is difficult.

The PMDA Scientific Committee Report has provided the following reference statement [4].

Tetraspanins (CD9, CD63, and CD81) and the molecules associated with late-stage endosomes (Tsg101 and Alix) are known EV marker molecules. An ISEV position paper [16] recommends analyzing at least three types of EV fraction-specific proteins or other EV-related molecules in a semi-quantitative manner.

4.1.2. Additional comment

Quantifying the RNA or DNA content is preferable, if possible [17]. In addition, the use of the characteristics of EV deterioration or EVs secreted by senescent cells as indicators is recommended if they can be captured.

4.1.3. Evaluation of contaminants other than EVs

4.1.3.1. Recommended items. The use of xeno-free medium without any animal-derived ingredients is recommended. The use of artificially synthesized serum substitutes (knockout serum replacement) should also be considered and assessed.

(Explanation).

The PMDA Scientific Committee Report has provided the following reference statement.

The composition, temperature, oxygen concentration, and CO_2 concentration in the fetal bovine serum (FBS) and culture media containing cytokines and other additives must be monitored stringently. Evaluating the safety of the source materials for the presence of medium additives and determining their presence in the final product is especially important. Notably, risk evaluation of infectious factors is mandatory when using biological source materials such as FBS, which contain animal-derived substances. Furthermore, ensuring the sufficient elimination or reduction of these substances during the purification process is also necessary.

The use of animal-derived materials (including human materials) for the preparation of EV preparations must be avoided. Human- or animal-derived excipients may cause viral contamination. For instance, EVs derived from bovine serum may contaminate the preparation and lead to unforeseen biological activity. Thus, the use of reagents that do not contain animal-derived substances or recombinant proteins is recommended.

4.1.4. Additional comment

Concrete examples of substances present in bovine serum that are a cause of concern include albumin, fibrinogen, fibronectin, filamin, apoB, macroglobulin, hemoglobin, and other blood coagulation factors [17].

4.2. Items specified as critical quality attributes for each target disease and preparation (manufacturing) method, and their evaluation

4.2.1. Evaluation of EV components expected to be related to safety or efficacy in the target disease

4.2.1.1. Recommended items. The quality attributes of EV preparations related to safety or efficacy in the target disease (e.g., proteins or miRNAs present in EVs) must be specified and analyzed as critical quality attributes (CQA).

(Explanation).

CQAs related to the safety of EV preparations include the absence of contamination with pathogenic infectious factors or the absence of contamination with undesirable immunogenic substances. Knowledge regarding the quality attributes identified as CQAs related to efficacy are limited at present.

4.2.1.1.1. Concrete example. Proteomic analysis or ELISA must be conducted to confirm the content of specific EV proteins if the EV proteins or miRNAs are expected to be related to the safety or efficacy of EV preparations. Similarly, comprehensive miRNA analysis or target-specific PCR must be conducted during the preparation (manufacturing) process or at an appropriate stage during preparation (manufacturing) [18] to quantify the content of specific EV miRNAs.

Lai, Lim et al. have reported that the presence of CD59 in EVs is an important CQA for psoriasis [19] as it can suppress the complement C5b9, which in turn results in the suppression of IL-17 produced by neutrophils, thereby improving psoriasis.

The PMDA Scientific Committee Report has provided the following reference statement [4].

Proteins and nucleic acids constitute the majority of the functional molecules of EVs. Thus, an evaluation of the nature of these contents is necessary.

4.2.1.2. Recommended items. In vitro titer assessments or preclinical *in vivo* assessment systems must be established to enable the extrapolation of the efficacy of the target disease.

(Explanation).

4.2.1.2.1. Concrete example 1: an example from the development of an EV therapy for liver cirrhosis. (Background) EVs play an important role in mitigating liver dysfunction caused by liver cirrhosis and tissue repair, such as the improvement of fibrogenesis through macrophages. Previous experiments conducted on mice have revealed that a higher level of EV accumulation was observed in the liver within one day of administering the tail vein injection. In addition, a high accumulation of EVs at the actual site of liver injury was also observed.

(*In vitro*) Evaluate the macrophage reactivity to EV addition using PCR. Macrophagic anti-inflammatory markers, inflammatory markers, phagocytosis-related markers, and fibrolysis-related markers (such as MMP) are the items to be measured using PCR.

(*In vivo*) The activity of EV preparations that results in improved liver fibrogenesis and liver function can be evaluated by administering EVs exhibiting a certain degree of activity *in vitro* to a continuous carbon tetrachloride-induced mouse model of liver dysfunction. The reduction in the levels of ALT and other liver disorder markers following the administration of EV can be evaluated based on indicators such as a smaller fibrosis area or lower collagen content compared with untreated control mice to determine whether fibrosis has improved [20].

4.2.1.2.2. Concrete example 2: example from the PMDA Scientific Committee Report. In addition to anti-inflammatory activity or inhibitory effects on fibrogenesis, MSC-derived EVs are expected to demonstrate functional restoration of various types of injured tissues. Possible evaluation endpoints include inflammatory and anti-inflammatory mediators (e.g., IL-1β, IL-5, IL-10, IL-12, chemokines, and TGF-β1), fibrogenesis markers (e.g., collagen 1, fibronectin, αSMA, and hydroxyproline), and inflammatory cell infiltration (e.g., neutrophil count, eosinophil count, and lymphocyte count) in the tissue, tissue function (e.g., in lung tissue: forced vital capacity [FVC], SpO2 on exercise, and the total Saint George's Respiratory Questionnaire [SGRQ] score), and clinical findings (e.g., acute exacerbation incidence rate). A combination of these endpoints, depending on the target disease, can be evaluated.

4.3. Desirable disease-related evaluations

4.3.1. Pharmacokinetic evaluation

4.3.1.1. Recommended items. Validating whether a certain percentage or more of administered EVs reach the target cells, tissues, or organs in animal models, depending on the administration conditions, is desirable.

(Explanation).

4.3.1.1.1. Concrete example. Kinetic tracking methods include PKH, NIR, DiR, and radioactive labeling. Observation methods include fluorescence microscopy, chemiluminescence detection instruments (e.g., Odyssey and IVIS), and radioisotope imaging. However, concerns regarding the reliability of the biokinetic data obtained with lipophilic dyes such as PKH, NIR, or DiR must be considered as dye dissociation from the EV membrane or micelle formation is possible owing to the long half-life of free dye molecules *in vivo* [21].

4.3.2. Confirmation of safety and therapeutic efficacy and pharmacokinetic evaluation with different dosages

4.3.2.1. Recommended items. In vitro and *in vivo* assessment systems must be used to adequately validate the relationship between the EV preparation dosage and its safety and effectiveness.

(Explanation).

4.3.2.1.1. Concrete example. The effects of various doses administered *in vitro* on target cells must be confirmed. In addition, whether findings such as pulmonary embolism are observed *in vivo* must be determined to validate whether the therapeutic effects vary. Particle number and protein amount are frequently used as indicators for the quantification of EVs for administration; determining a correlation between these indicators is desirable.

5. Summary of important points

Fig. 2 presents the scope of this Guidance.

5.1. Current status check items

Any medical intervention using EVs prepared (manufactured) under the direction of a physician or dentist and administered to patients at the medical institution wherein the physician or dentist practices does not fall under the scope of the “The Act on the Safety of Regenerative Medicine” (ASRM) in Japan. Hence, the Certified Special Committee for Regenerative Medicine or Certified Committee for Regenerative Medicine does not review these therapies. Physicians or dentists who prescribe EV therapies using EVs (EV preparations) that have not received regulatory approval must possess a sufficient understanding of the quality and risk profile of the EV preparation and must take measures to ensure the safety of the patients, as described in this guideline.

5.2. Recommended items

The risks, risk factors, and measures to mitigate them must be identified during the early stages of the development of EV therapy. The risks associated with EV preparations do not differ significantly from those associated with biopharmaceuticals developed in accordance with the PMD Act. Nevertheless, risk factors related to a specific risk may be present at various stages, such as the properties or composition of the EV preparation, procurement of source materials, manufacturing process, transport, storage, preclinical evaluation, clinical evaluation, clinical administration method, and handling of the EV preparation at the medical institution where it is used. EVs are complex, heterogeneous, and non-uniform. Thus, identifying and managing all critical quality attributes (CQAs)

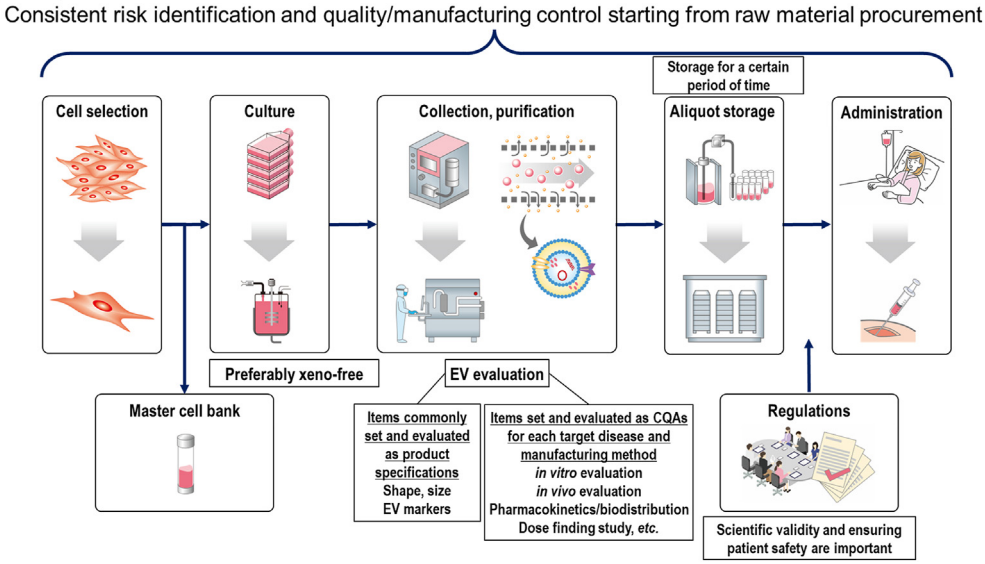

Fig. 2. This figure presents the scope of this Guidance.

related to risk factors that may interfere with the safety or efficacy of the product would be difficult even when all possible quality attributes are listed.

5.3. Recommended items

An inventory of the specimens (e.g., EV preparations or biological source materials from the same lot as that used in clinical applications) as well as preparation (manufacturing) and administration records must be maintained as part of the risk management of EV therapy to identify the cause of adverse events.

5.4. Recommended items

Aseptic processing (including processing using aseptic techniques) and measures to prevent contamination with various components must be implemented throughout the process, beginning with the acquisition of the source material. The condition of the cells used for manufacturing can degrade. Thus, process control is very important as the quality, efficacy, and safety of the EVs secreted from these cells are greatly affected by the manufacturing process.

5.5. Recommended items

Quality control and preparation (manufacturing) control measures must be implemented in accordance with the regenerative medicine provision standards set forth by the ASRM, and the manufacturing control and quality control standards for regenerative medical products [] (cell-processed products) set forth by the PMD Act to ensure the quality, efficacy, and safety of EV preparations.

5.6. Recommended items

Verification of the presence of (1) particles with the shape and size of EVs and (2) EV marker molecules is necessary.

5.7. Recommended items

The use of xeno-free medium without any animal-derived ingredients is recommended. The use of artificially synthesized serum substitutes (knockout serum replacement) should also be considered and assessed.

5.8. Recommended items

The quality attributes of EV preparations related to safety or efficacy in the target disease (e.g., proteins or miRNAs present in EVs) must be specified and analyzed as Critical Quality Attributes (CQA) during the establishment of the preparation (manufacturing) process or at an appropriate stage during preparation (manufacturing).

5.9. Recommended items

In vitro titer assessments or preclinical *in vivo* assessment systems must be established to enable the extrapolation of the efficacy of the target disease.

5.10. Recommended items

Validating whether a certain percentage or more of administered EVs reach the target cells, tissues, or organs in animal models, depending on the administration conditions, is desirable.

5.11. Recommended items

In vitro and *in vivo* assessment systems must be used to adequately validate the relationship between the EV preparation dosage and its safety and effectiveness.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Shuji Terai; ASKA Pharmaceutical Co., Ltd., AbbVie GK., Chiome Bioscience Inc., Takeda Pharmaceutical Company Limited, Cuorips Inc., Otsuka Pharmaceutical Co., Ltd., Masahiro Kino-oka; Cell Exosome Therapeutics, Gaudi Clinical, Shibuya Corporation, Iwatani Corporation, Cell Exosome Therapeutics, Fujimori Kogyo Co., Ltd., Hitachi, Ltd., Nissan Chemical Corporation, TOSOH Corporation, Sekisui Chemical Co., Ltd., SCREEN Holdings Co., Ltd., Mitsubishi Heavy Industries, Ltd., Fujimori Kogyo Co., Ltd., Yuki Takahashi;

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References

[1] Tsuchiya A, Terai S, Horiguchi I, Homma Y, Saito A, Nakamura N, et al. Working Group of Attitudes for Preparation and Treatment of Exosomes of Japanese Society of Regenerative Medicine. Basic points to consider regarding the preparation of extracellular vesicles and their clinical applications in Japan. Regen Ther 2022;21:19–24.

[2] https://www.jsrm.jp/news/news-8031/(Japanese version, last accessed on July 20, 2024).

[3] https://jsev.jp/docs/jsev_ev_treatment_2023122501.pdf (Japanese version, last accessed on July 20, 2024).

[4] https://www.jsrm.jp/news/news-15497/.

[5] https://www.pmda.go.jp/files/000249829.pdf (Japanese version, last accessed on July 20, 2024).

[6] Gimona M, Brizzi MF, Choo ABH, Dominici M, Davidson SM, Grillari J, et al. Critical considerations for the development of potency tests for therapeutic applications of mesenchymal stromal cell-derived small extracellular vesicles. Cytotherapy 2021;23:373–80.

[7] Hirai T, Yasuda S, Umezawa A, Sato Y. Country-specific regulation and international standardization of cell-based therapeutic products derived from pluripotent stem cells. Stem Cell Rep 2023;18:1573–91.

[8] Cell substrate: cell lines derived from microbial cells or human/animal sources that possess the ability necessary for generation of biopharmaceuticals (biotechnology-applied pharmaceuticals/pharmaceuticals derived from biological origins) for human in vivo or ex vivo use.

[9] Cell bank: A collection of appropriate containers, whose contents are of uniform composition, stored under defined conditions. Each container represents an aliquot of a single pool of cells.[6,7]: both as defined in the ICH Q5D Guideline “Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products” https://www.pmda.go.jp/files/000156854.pdf (last accessed on July 20, 2024)].

[10] GMP (Good Manufacturing Practice): Standards for the manufacturing control and quality control for drugs and quasi-drugs as stipulated in the “ministerial ordinance on standards for manufacturing control and quality control for drugs and quasi-drugs” (issued in 2004, MHLW ministerial ordinance No. 179).

[11] GCTP (good gene, cellular, and tissue-based products manufacturing practice): standards for the manufacturing control and quality control for regenerative medical products as stipulated in the “ministerial ordinance on good practices for manufacturing control and quality control for regenerative medical products” (issued in 2014, MHLW Ministerial Ordinance No. 93) and standards for manufacturing control and quality control for specified processed cells as stipulated in Articles 92 to 110 of the “Ordinance for Enforcement of the Act on the Safety of Regenerative Medicine” (issued in 2014, MHLW Ministerial Ordinance No. 110).

[12] EMA/CAT/CPWP. Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to advanced therapy medicinal products (EMA/CAT/CPWP/686637/2011). https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-risk-based-approach-according-annex-i-part-iv-directive-200183ec-applied-advanced-therapy-medicinal-products_en.pdf; 2013 (last accessed on July 20, 2024).

[13] Human cell-processed products or animal cell-processed products as defined in the “Act on securing quality, efficacy and safety of products including pharmaceuticals and medical devices” (issued in 1960, Act No. 145) or specified processed cells as defined in the “Act on the safety of regenerative medicine” (issued in 2013, Act No. 85).

[14] Critical Quality Attribute (CQA): a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality [as defined in the ICH Q11 Guideline “Development and manufacture of drug substances (chemical entities and biotechnological/biological entities)” https://www.pmda.go.jp/files/000156285.pdf (last accessed on July 20, 2024)].

[15] Under the PMD Act, which regulates medicinal/medical products intended for marketing in Japan, human cell-processed products, animal cell-processed products and gene therapeutics for cell or gene therapy are collectively referred to as “regenerative medical products,” a product type that is independent from that of drugs and medical devices. Also see the reference in the footnote 4.

[16] Lener T, Gimona M, Aigner L, Börger V, Buzas E, Camussi G, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles 2015;4:30087.

[17] Silva AKA, Morille M, Piffoux M, Arumugam S, Mauduit P, Larghero J, et al. Development of extracellular vesicle-based medicinal products: a position paper of the group "Extracellular Vesicle translatiOn to clinicaL perspectiVEs - EVOLVE France". Adv Drug Deliv Rev 2021;179:114001.

[18] Miceli RT, Chen TY, Nose Y, Tichkule S, Brown B, Fullard JF, et al. Extracellular vesicles, RNA sequencing, and bioinformatic analyses: challenges, solutions, and recommendations. J Extracell Vesicles 2024;13:e70005.

[19] Lai RC, Tan TT, Sim WK, Zhang B, Lim SK. A roadmap from research to clinical testing of mesenchymal stromal cell exosomes in the treatment of psoriasis. Cytotherapy 2023;25:815–20.

[20] Takeuchi S, Tsuchiya A, Iwasawa T, Nojiri S, Watanabe T, Ogawa M, et al. Small extracellular vesicles derived from interferon-γ pre-conditioned mesenchymal stromal cells effectively treat liver fibrosis. NPJ Regen Med 2021;6:19.

[21] Arifin DR, Witwer KW, Bulte JWM. Non-Invasive imaging of extracellular vesicles: quo vaditis in vivo? J Extracell Vesicles 2022;11:e12241. https://doi.org/10.1002/jev2.12241. Erratum in: J Extracell Vesicles. 2022;11:e12284.