

Quality-Related Considerations for Human Cell-Derived Extracellular Vesicle (EV) Products at the
Time of Initial Clinical Trial Notification
(Early Consideration)

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1. Introduction

Extracellular vesicles (EVs) are vesicles with a lipid bilayer structure that are secreted from cells. They contain various proteins and nucleic acids and are involved in various life phenomena and diseases by delivering functional molecules to other cells.¹⁾

In recent years, therapeutic product development of EVs derived from human cells, such as mesenchymal stromal cells, has been progressing with the aim of treating specific diseases through anti-inflammatory effect, immunomodulatory effect, tissue repair effect, and other effects.^{2), 3)} Preparations for clinical trials of EV products are underway in Japan.

The Pharmaceuticals and Medical Devices Agency (PMDA) compiled the Science Board report entitled “Report on Therapeutic Products Based on Extracellular Vesicles (EVs) Including Exosomes,”¹⁾ which addresses quality, safety, and other considerations for therapeutic products based on EV research and published the report on January 17, 2023. In addition, under the Fiscal Year 2025 Regulatory Science Research Program for Pharmaceuticals and Medical Devices of the Japan Agency for Medical Research and Development (AMED) (research title: Regulatory Science Research for Ensuring the Quality and Safety of Extracellular Vesicle (EV) Products, Including Exosomes), the “Draft Guideline on Ensuring the Quality of Medicinal Products Using Human Cell-Derived Native Extracellular Vesicles (EVs)”⁴⁾ was compiled and published on March 17, 2026.

Based on the draft guideline, this document presents PMDA’s current regulatory perspective on quality-related considerations for the development of EV products as investigational medicinal products. It should be noted that the considerations outlined in this document have been developed based on currently available knowledge and may change in the future due to newly obtained knowledge and scientific progress.

2. Scope of Application

This document applies to EV products containing purified EVs derived from human cells as the active substance and intended for marketing authorization as therapeutic drugs. This document may also be applied to engineered EV products in which EV producer cells are modified by gene transfer or other means, or EVs are processed by the conjugation of peptides, encapsulation of specific molecules, or

other means, for such purposes as controlling biodistribution or conferring pharmacological activity.

3. Quality-Related Considerations at the Time of Initial Clinical Trial Notification

3.1. Basic Principles

To initiate a clinical trial of an EV product, the safety of the EV product must be ensured for study participants. Biological raw materials are used in the manufacture of EV products, including in cell substrates. In addition, because EVs have similar physicochemical properties to those of viruses, such as size and charge, it may be difficult to establish a purification process with virus clearance capability. Therefore, in ensuring the safety of EV products, it is particularly important to establish an appropriate control strategy for adventitious agents.

It is also important to evaluate the extent of impurity removal and to establish specifications and analytical procedures relating to safety. In an initial clinical trial notification for an EV product, information sufficient to support the safety of the investigational medicinal product should be provided, taking into account the matters specified in Q&A No. 27 of the “Revision of Questions and Answers (Q&A) regarding the Notification of Clinical Trial Plans and Implementation of Clinical Trials for Drugs” (Administrative Notice dated August 31, 2022).⁵⁾

If there are any concerns regarding the safety of a product under development, it is recommended that applicants consult PMDA through face-to-face consultation meeting before submitting the initial clinical trial notification.

3.2. Control of Adventitious Agents

Effective virus removal and inactivation may be difficult in the manufacturing process for EV products. Consequently, it is particularly important to select and test raw materials for the absence of viruses and to conduct virus testing at appropriate step(s) of the manufacturing process, in accordance with the principles of the “Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin” (ICH Q5A(R2) Guideline)⁶⁾. A control strategy for adventitious agents should be established with reference to the principles described above and items (1) through (3) below.

(1) Prevention of Adventitious Agents from Raw Materials

Biological raw materials used in the manufacturing process must comply with the Standards for Biological Raw Materials. When a cell substrate is prepared from tissue collected from a donor, the appropriateness of the donor interview, medical examinations, tests, and other procedures should be confirmed. When establishing a master cell bank or working cell bank, virus testing of cell substrates should be conducted in accordance with the ICH Q5A(R2) Guideline. For the cell substrate, in addition to confirming by testing that the cell substrate is not contaminated with at least hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV), which are infectious to humans,

testing for viruses other than those listed above should also be conducted according to the level of infection risk, taking into account the tissue of origin of the cell substrate. It should also be confirmed that biological raw materials used in manufacture, including culture medium components and medium additives, are not contaminated with bacteria, fungi, mycoplasma and viruses.

(2) Testing for Adventitious Agents in the Manufacturing Process

Generally, testing for bacteria, fungi, mycoplasmas, and viruses should be conducted at the step of the manufacturing process that provides the highest sensitivity, and it should be confirmed that the process fluid is not contaminated. For therapeutic products consisting of recombinant proteins, virus testing using unprocessed/unpurified bulk provides the highest sensitivity. For EV products, however, viruses may be concentrated by ultrafiltration or other processes. Therefore, consideration should also be given to the possibility that virus testing may provide the highest sensitivity in a purification-process intermediate, such as a post-filtration intermediate.

(3) Establishment of Virus Removal Steps and Evaluation of Virus Clearance Capability

Purification methods capable of separating viruses from EVs are being developed, and virus removal may therefore be expected during EV purification.⁷⁾ Because viral safety should be ensured to the greatest extent possible in the manufacture of EV products, virus clearance studies should be conducted during manufacturing process development to evaluate the virus clearance of the manufacturing process. If there is no purification step from which virus removal is expected, modification of the process or addition of a step having the capability to remove viruses should be considered.

3.3. Other Safety Considerations

The removal of process-related impurities, including host cell proteins (HCPs), host cell DNA, medium additives, and column packing materials used in the purification process should be characterized. For proteins contained in the drug substance or drug product, it is difficult to distinguish HCPs from EV-derived proteins. One possible approach is to monitor the protein-to-particle ratio as an indicator of impurity levels. In addition, non-vesicular extracellular particles and other particles derived from the cell substrate and extrinsic particles introduced during the manufacturing process should be identified through characterization of the manufacturing process and drug substance, and their impact on safety should be evaluated.

As necessary, tests for impurities should be established as part of the specifications and analytical procedures for the drug substance or drug product. Depending on the dosage form, tests such as the test for sub-visible particles, inspection for foreign insoluble matter, sterility test, and bacterial endotoxins test should be established in accordance with the General Rules for Preparations of the

Japanese Pharmacopoeia.

When immortalized primary cultured cells are used as the cell substrate, nucleic acids and proteins derived from the immortalizing gene may contaminate the EV product, potentially causing the EV product to exhibit carcinogenicity. The contents of nucleic acids and proteins derived from the immortalizing gene in the EV product should be evaluated, and these impurities should be removed to the greatest extent possible.

4. Considerations Other Than Safety

At the time of the initial clinical trial notification, the mechanism of action of an EV product may not have been fully elucidated, and the biological activities of the EVs and active components (components responsible for the biological activity, such as proteins and microRNAs) may not yet have been identified. Therefore, it is desirable to conduct a multidimensional assessment of quality attributes related to the efficacy of the EV product using multiple analytical methods and to broadly collect information on the mechanism of action and active components.

In addition, when a cell substrate is changed, such as by immortalization of the host cells or a change of donor, it is highly likely that comparability before and after the change cannot be sufficiently demonstrated. Therefore, the cell substrate should be selected at an early stage of development including before non-clinical studies are conducted.

5. References

- 1) Report on Therapeutic Products Based on Extracellular Vesicles (EVs) Including Exosomes. (PMDA Science Board. January 17, 2023).
<https://www.pmda.go.jp/files/000268368.pdf>
- 2) Therapeutic Products Using Extracellular Vesicles (EVs), Including Exosomes. (Division of Biological Chemistry and Biologicals, National Institute of Health Sciences)
<https://www.nihs.go.jp/dbcb/exosome.html>
- 3) Mizenko RR, Feaver M, Bozkurt BT, Lowe N, Nguyen B, Huang KW, Wang A, Carney RP. A critical systematic review of extracellular vesicle clinical trials. J Extracell Vesicles. 2024;13:e12510. doi:10.1002/jev2.12510.
<https://pmc.ncbi.nlm.nih.gov/articles/PMC11428870/pdf/JEV2-13-e12510.pdf>
- 4) Draft Guideline on Ensuring the Quality of Medicinal Products Using Human Cell-Derived Native Extracellular Vesicles (EVs) (March 17, 2026).
<https://public-comment.e-gov.go.jp/pcm/detail?CLASSNAME=PCMMSTDETAIL&id=495250482&Mode=0>
- 5) Revision of Questions and Answers (Q&A) regarding the Notification of Clinical Trial Plans and Implementation of Clinical Trials for Drugs (Administrative Notice dated August 31, 2022).

<https://www.pmda.go.jp/files/000248031.pdf>

- 6) ICH Q5A(R2); Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

<https://www.pmda.go.jp/files/000265997.pdf>

- 7) McNamara RP, Dittmer DP. Modern Techniques for the Isolation of Extracellular Vesicles and Viruses. J Neuroimmune Pharmacol. 2020;15(3):459-472. doi:10.1007/s11481-019-09874-x.

<https://pmc.ncbi.nlm.nih.gov/articles/PMC7065924/pdf/nihms-1539689.pdf>