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Quality Considerations in the Development of AAV Vector Products

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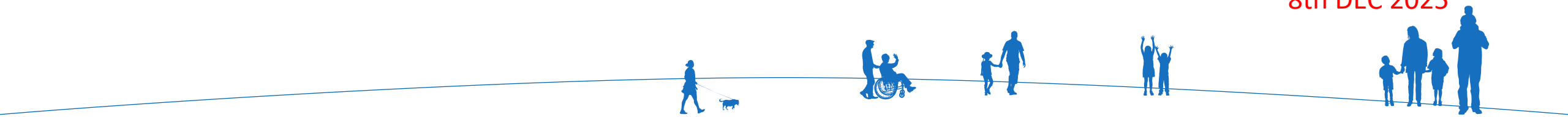
Reviewer
Office of Cellular and Tissue-based Products
Pharmaceuticals and Medical Devices Agency (PMDA), Japan

8th DEC 2025



Disclosure

The speaker is affiliated with Pharmaceuticals and Medical Devices Agency.

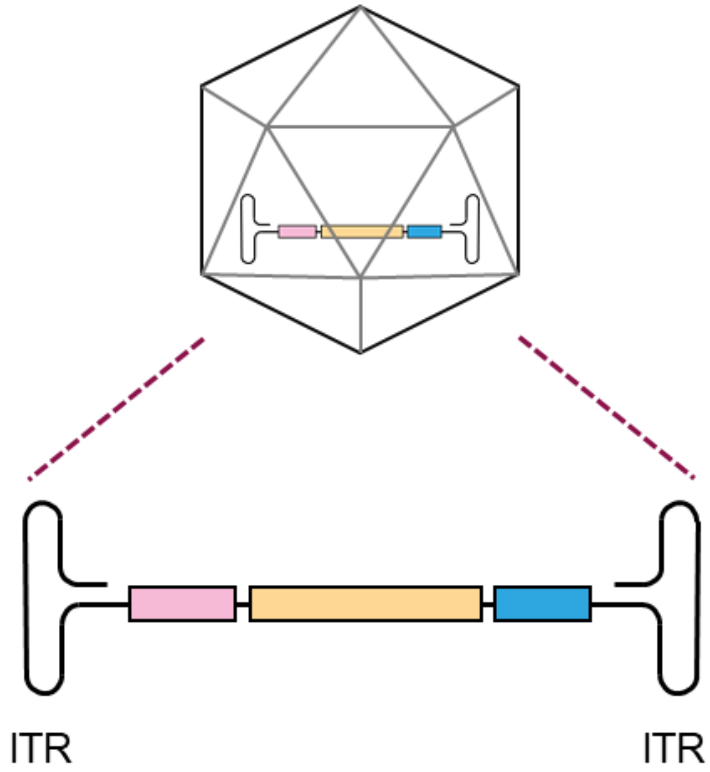
The views expressed in this presentation are those of the author and do not necessarily reflect the official views of Pharmaceuticals and Medical Devices Agency.

Outline

- Key Quality Review Points for AAV Products

- Comparability Assessment

Characteristics of AAV vectors



- AAV is a dependovirus and does not replicate unless a helper virus, such as adenovirus, is present for co-infection.
- In the process of generating the viral vector, AAV is engineered to be replication-deficient, ensuring that it does not proliferate even when helper viruses are present.
- It remains in cells as an episomal form without integrating into the host genome.
- Because it is a non-enveloped virus, it exhibits resistance to surfactants and acidic conditions.
- Due to its very small size, it is able to pass through nanofilters with larger pore sizes.
- Its amenability to purification allows for the effective removal of other viruses.

Provisional Translation (as of July 2020)*

PSEHB/MDED Notification No.0709-2
July 9, 2019

To: Commissioner of Prefectural Health Department (Bureau)

Director of the Medical Device Evaluation Division,
Pharmaceutical Safety and Environmental Health Bureau,
Ministry of Health, Labour and Welfare
(Official Seal Omitted)

Ensuring the Quality and Safety of Gene Therapy Products

Regarding basic requirements to ensure the quality and safety of pharmaceuticals used for gene therapy including investigational products (hereinafter, "gene therapy drugs"), the Ministry of Health, Labour and Welfare (MHLW) previously issued "Guideline on Ensuring the Quality and Safety of Gene Therapy Drugs" (hereinafter, the "old guideline"

Gene Therapy Drugs" (PFSB/E

Division, Pharmaceutical and

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Guideline on Ensuring the Quality and Safety of Gene Therapy Products

Introduction

1. This guideline is to lay down basic technicalities required to ensure the quality and safety of *in vivo* gene therapy products and *ex vivo* genetically modified human cell therapy (products including investigational products; hereinafter, "gene therapy products") among regenerative medical products.
   It may, however, not always be appropriate to apply this guideline in a uniform way or to treat it as if it includes all necessary information, because types, characteristics, and clinical applications of gene therapy products are diverse, and rapid strides are being made in the scientific advancement and accumulation of experience in this area. Therefore, when conducting tests or evaluations of individual gene therapy products, things should be done flexibly on a case-by-case basis, based on reasonable grounds reflecting the status of academic progress at that point, with reference to the intent of this guideline.

2. In developing a product belonging to the category of gene therapy products, the proposed clinical trial plan might not be approved when a clinical trial notification specified in the "Act on Securing the Quality, Efficacy and Safety of Products Including Pharmaceuticals, Medical Devices" (Act No. 145 of 1960) is submitted, if available data on quality and safety are not sufficient for the product to be administered to humans. It is therefore advisable to, before clinical trial notification, adequately utilize the regulatory science (RS) strategy consultation offered by the Pharmaceuticals and Medical Devices Agency (PMDA) to confirm the product has eligible quality and safety for administering to humans in clinical trials so that the proposed clinical trial will be smoothly initiated.
   Basic considerations in initiating clinical trials of a product belonging to the category of gene therapy products include whether there is any evident problem with quality and/or safety that rises when the product is applied to humans, whether the quality characteristics of the product are well understood so that their relationship to clinical findings can be verified, and whether constancy of the quality within a certain range is ensured. In confirming these points, any apparently expected risk of the product should be excluded by taking advantage of the present science and technology, and its scientific rationale should be clarified first. After that, it is also necessary to have the following viewpoint; i.e., the still remaining unknown possible risks should be compared with the "possible risks caused by losing a new treatment opportunity" in patients with a serious and life-threatening disease, a significantly disabling disease, or a disease that significantly compromises

Ensuring the Quality and Safety of Gene Therapy Products

Key guidelines on the quality and safety of gene therapy products

URL : https://www.pmda.go.jp/files/000235607.pdf

Key Issues Specific to AAV Vectors

- ✓Control of Vector Genome Content
- ✓Potency Assessment and Standardization
- ✓Management of Empty Capsids
- ✓Compliance with the Cartagena Act (Japan)
- ✓Strategies to Address Limited Manufacturing Yield

Common Issues for Gene Therapy Products

- ✓Assessment of Comparability and Product Consistency
- ✓Strategies for Ensuring Viral Safety and Control of Biological Raw Materials
- ✓Determination of Applicability as a Specified Regenerative Medicine Product (Japan)

Examples of Product Characterization and Release Testing Items

Category	Test Item
Identity	Capsid protein profiling, Vector genome sequence confirmation
Purity	Capsid Proteins（VP1, VP2, VP3 composition）, Full/empty capsid ratio, Process-Related Impurities, Host Cell–Derived Impurities（HCP, HCD）, Residual plasmid DNA
Safety	Endotoxin, Microbial limit testing for Drug Substance, Sterility Testing for Drug Product, Testing for Bulk Harvest（*in vitro* virus assay, assay for replication-competent viruses, mycoplasma testing)
Potency	Infectivity titer Expression level or activity of the carried protein
Content	Genome copy number, potency
Others	Physical properties, osmotic pressure, pH, insoluble particulate matter, insoluble foreign matter, etc.

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Control of Vector Genome content

- Because the measured copy number and potency serve as the basis for the dosage and administration, ensuring reliable quantification performance is essential from early development.
- If the assay method must be changed during development (not preferable), bridging of assay results before and after the change is required.

Vector Genome Copy Number

- Represents the number of genomes within full capsids
- A key indicator of vector content for AAV and other viral vectors
- Measured using ddPCR or qPCR with transgene-specific primers
- Infectious titer or potency assays may also be used as measures of content

Control of Infectious Titer and Potency Assays

Infectious Titer

- An indicator of the infectivity of a viral vector
- Quantified using in vitro assay systems with permissive cell lines

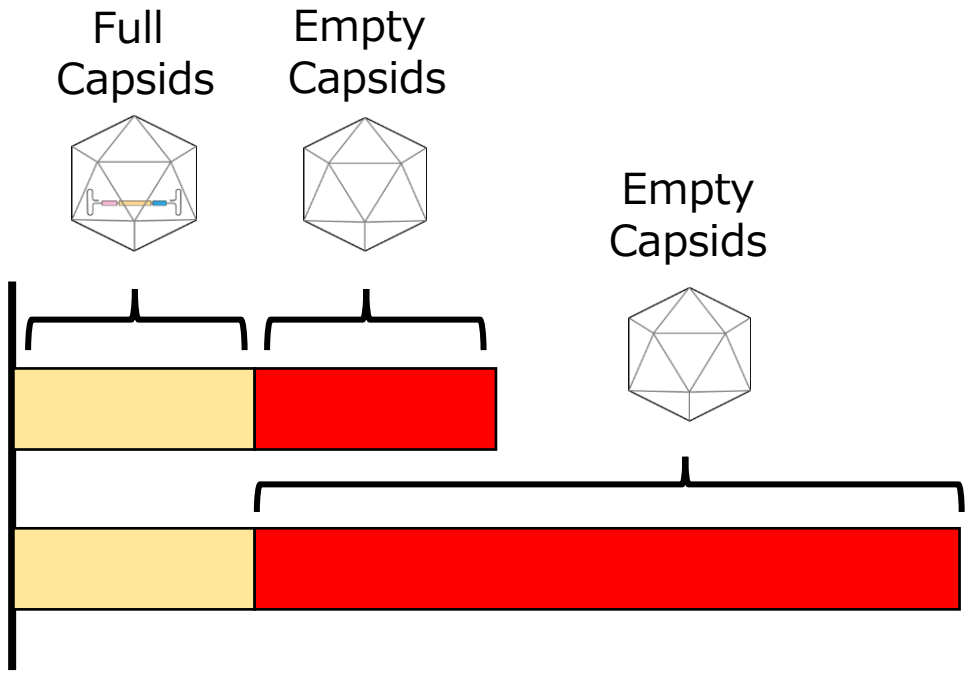
Potency

- An indicator of the functional efficacy of a viral vector
- Quantified by measuring expression or activity of the transgene product
- Evaluated using in vitro or in vivo assay systems that reflect the vector's mechanism of action

Control of Empty Capsids

Capsid Ratio

- Managed using the ratio of full to empty capsids
- Measured by analytical ultracentrifugation (AUC) or similar methods



- A high proportion of incomplete particles can reduce overall efficacy relative to the total particle count.
- Increased total capsid load may heighten the risk of adverse effects.
- Therefore, minimizing incomplete particles is necessary to the greatest extent possible.

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Summary

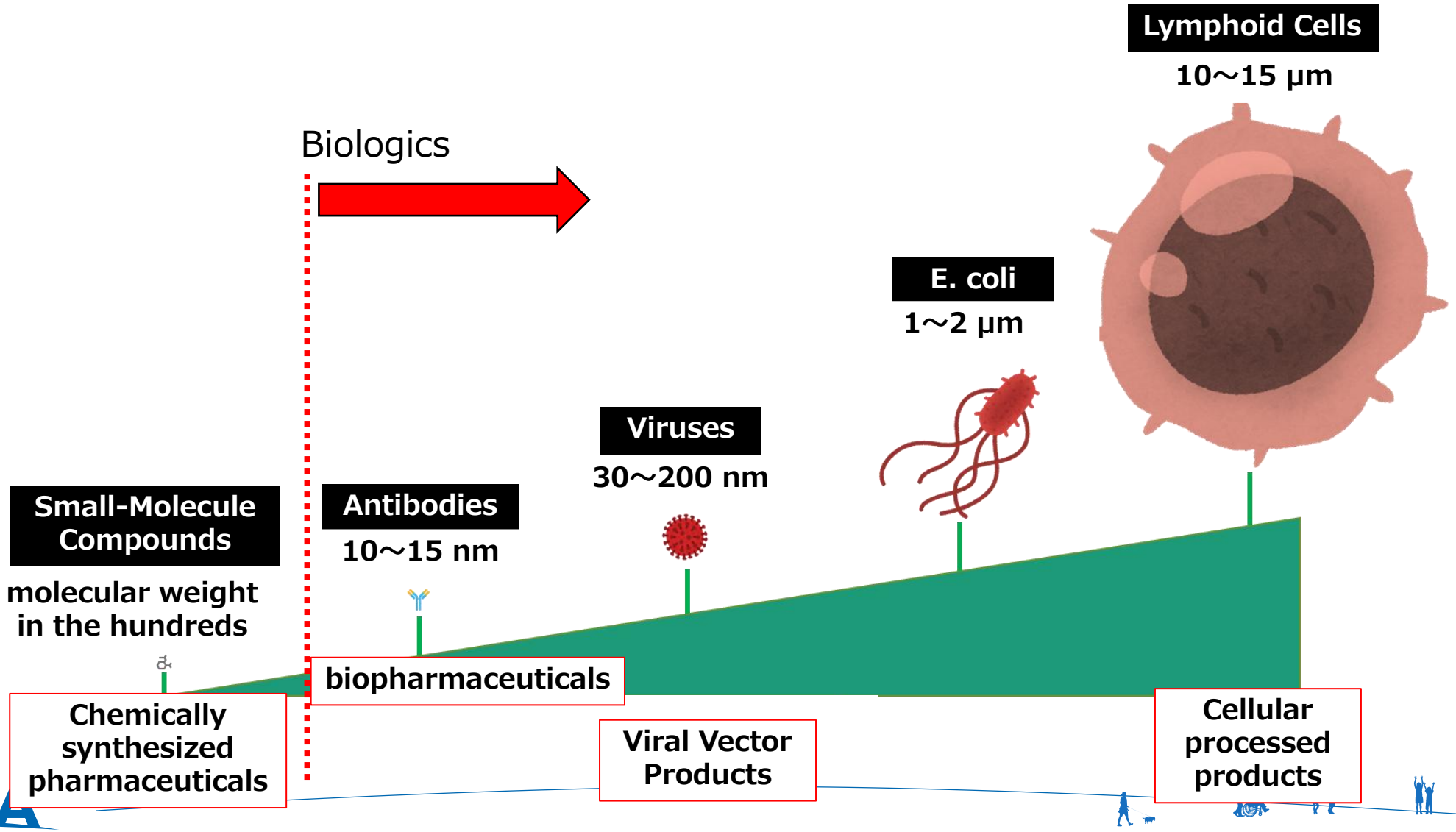
- Ensuring the Quality and Safety of Gene Therapy Products serves as an important reference for the development of AAV products.
- Among quality specifications, content, empty capsids, and potency are particularly important, as these parameters can influence dose selection and justification in clinical trials.
- The Cartagena Act applies broadly—from manufacturing through product release—and must be considered throughout the process.
- Due to the small manufacturing scale, the available sample volume is limited, requiring highly precise and well-planned testing strategies.

Outline

- Key Quality Review Points for AAV Products

- Comparability Assessment

Size of Active Pharmaceutical Ingredients



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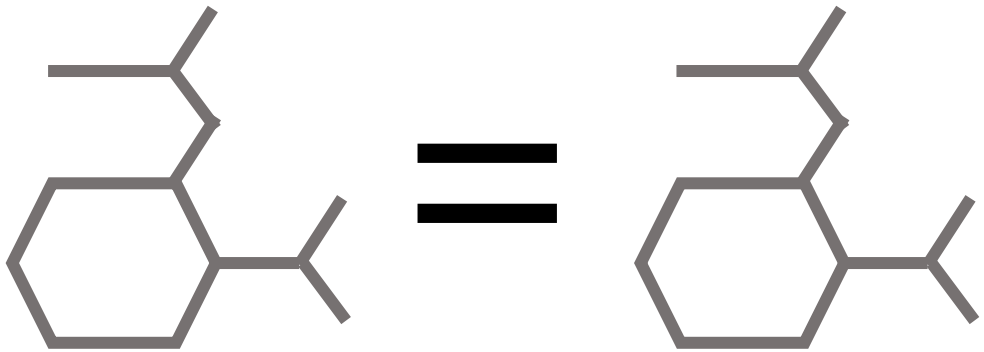

For a single molecule, demonstrating complete identity is not difficult.

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2025

ICH Guideline Q5E: Comparability of Biotechnological/Biological Products

ICH Q5E: COMPARABILITY OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

1.0 Introduction

1.1 Objectives of the Guideline

The objective of this document is to provide principles for assessing the comparability of biotechnological/biological products before and after changes are made in the manufacturing process for the drug substance or drug product. Therefore, this guideline is intended to assist in the collection of relevant technical information which serves as evidence that the manufacturing process changes will not have an adverse impact on the quality, safety and efficacy of the drug product. The document does not prescribe any particular analytical, nonclinical or clinical strategy. The main emphasis of the document is on quality aspects.

1.2 Background

Manufacturers[1] of biotechnological/biological products frequently make changes to manufacturing processes[2] of products[3] both during development and after approval. Reasons for such changes include improving the manufacturing process, increasing scale, improving product stability, and complying with changes in regulatory requirements. When changes are made to the manufacturing process, the manufacturer generally evaluates the relevant quality attributes of the product to demonstrate that modifications did not occur that would adversely impact[4] the safety and efficacy of the drug product.

The same fundamental concepts apply to gene therapy products.

Comparability does not require that the quality attributes of the pre-change and post-change products be completely identical.
Rather, it means that the products are highly similar, and that any observed differences in quality attributes can be sufficiently justified—based on existing knowledge—to ensure that they do not impact the safety or efficacy of the final product.

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ICH Q5E is useful for establishing comparability of AAV products; however, it should be applied together with the Guideline on Ensuring the Quality and Safety of Gene Therapy Products.

Thank you for your attention !

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