Provisional Translation (as of July 2020)*

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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"), defined in the notification entitled, "Ensuring the Quality and Safety of Gene Therapy Drugs" (PFSB/ELD Notification No. 0701-4, by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated July 1, 2013; hereinafter, the "old notification issued by the ELD Director").

To facilitate development of innovative drugs, medical devices, and regenerative medical products, MHLW worked with academic and research institutions studying and developing state-of-the-art techniques to develop guidelines that aim to establish procedures for quality and safety assessment based on regulatory science, and also conducted a project to personnel exchange between those institutions and the Pharmaceuticals and Medical Devices Agency (PMDA) or the National Institute of Health Sciences (NIHS), from 2012 fiscal year (FY) to 2016 FY.

Based on the draft of revised guideline proposed in this project, and taking into account the advancement of science and technology and the status of clinical trials, we reviewed the old guideline. In addition, taking into account the trends of overseas regulatory authorities, we have now presented the basic technicalities to ensure the quality and safety of, among regenerative medical products, *in vivo* gene therapy products and *ex vivo* genetically gene modified human cell therapy products (including investigational products; hereinafter, "gene therapy products") as the new guideline, "Guideline on Ensuring the Quality and Safety of Gene Therapy Products", shown in the Appendix and have decided its handling as described below. Please notify the related organizations under your jurisdiction of this Notification to utilize as a reference for the marketing authorization application.

The old notification issued by the ELD Director is withdrawn together with the issuance of this notification.

* This English translation of the Japanese Notification is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail.

REMARKS

1. Note that Chapter 1 of Guideline on Clinical Studies of Gene Therapy (MHLW Ministerial Announcement No. 344 of 2015) will be applied also to clinical trials of gene therapy products. It is advisable to use also the remaining part of the guideline for reference.

2. It is still necessary to submit applications for approval and confirmation regarding the use of living modified organisms based on the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No. 97 of 2003). It is therefore advisable to discuss with the Office of Cellular and Tissue-based Products, PMDA before submitting the application and submit the application taking into account the normal time required for the relevant paperwork.

End

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 the patient's quality of life (QOL) by compromising the function and/or morphology of their body to a certain degree who cannot overcome the disease because of limitations in conventional treatments. The decision of whether or not the treatment is applied will be left to the patients after all information on these points is disclosed to them. In other words, it is also important to make evaluations taking into account that patients themselves will make a decision whether they participate in the clinical trial after all information on risks and expected benefits is disclosed to them. In submitting a clinical trial notification, the applicant therefore will not necessarily be asked to meet all the requirements and conditions presented in this guideline regarding the submitted data.

Instead, appropriate data that match the intent of this guideline and are reasonably prepared should be submitted at the initiation of the clinical trial, assuming that the data ensuring the quality and safety of the product that will be submitted at marketing authorization application will be augmented and organized according to this guideline as the clinical development program of the product advances.

3. Technical requirements for the testing procedures, criteria, and others described in this guideline are intended to be considered, selected, applied, and evaluated according to the purpose and other factors of the test, and they are not always required to be interpreted and operated at the same or highest level. The developer should therefore justify the background of the consideration as well as what it selected, applied, and evaluated and the level at which it did so by showing that they match the purpose of each test.

Table of Contents

Chapter 1. GENERAL PROVISIONS 7
1. Objective 7
2. Scope 7
3. Definitions 7
Chapter 2. DESCRIPTION AND DEVELOPMENT HISTORY OF GENE THERAPY PRODUCTS 8
1. Description and Development History 8
2. Status of Clinical Trial 8
Chapter 3. QUALITY 9
1. Expression Construct 9
(1) Structure of Expression Construct 9
(2) Construction of Expression Construct 9
(3) Functional Characteristics of Transgene 9
(4) Structure and Functional Characteristics of Regulatory Elements for Gene Expression 10
(5) Structure and Functional Characteristics of Expression Product from Transgene 10
(6) Configuration and Functional Characteristics of Other Components and Open Reading Frames 10
2. Structure and Characteristics of Vector and Production Procedure 10
(1) Viral vector 10
(2) Non-Viral Vector 15
3. Target Cells 18
(1) Gene Therapy Products 18
(2) Genetically Modified Human Cell Therapy Products 18
4. Characterization and Specifications 20
(1) General Principles 20
(2) Characterization and Controls of *in vivo* Gene Therapy Products 21
(3) Characterization and Controls of Genetically Modified Human Cell Therapy Products 24
5. Product Development 26
6. Process Evaluation/Validation 27
7. Stability Testing 27
Chapter 4. NONCLINICAL STUDIES 28
1. Studies Supporting the Potency or Efficacy 28
2. Biodistribution 28
3. Nonclinical Safety Studies 29
(1) General Toxicity 29
(2) Gene Integration Risk 32
(3) Tumorigenic Potential and Carcinogenic Risk 32
(4) Reproductive and Developmental Toxicity 33
(5) Immunotoxicity 33
(6) Potential Emergence of Replication-Competent Viruses 33

Chapter 5. CONSIDERATIONS IN CLINICAL TRIALS 34
1. Justification of the Conduct of Clinical Trial 34
2. Clinical Trial Protocol 34
3. Long Term Follow-Up..........34
Chapter 6. EVALUATION OF THE RISK OF TRANSMISSION OF GENE THERAPY PRODUCTS TO THIRD PARTIES..........35

Chapter 1. GENERAL PROVISIONS

1. Objective

   The objective of this guideline is to show basic technicalities required to ensure the quality and safety of gene therapy products.

2. Scope

   This guideline cover the products belonging to the category of *in vivo* gene therapy products and *ex vivo* genetically modified human cell therapy products (*ex vivo* gene therapy products) among regenerative medical products.

   In the case that this guideline is referred to for a pharmaceutical or another product that is not included in the scope of this guideline such as genetically modified animal cell therapy products or an expression construct-containing pharmaceutical for a prophylactic purpose, developers should consult the Ministry of Health, Labour and Welfare (MHLW) and PMDA for each individual case.[1]

3. Definitions

(1) “Gene therapy products” mean *in vivo* gene therapy products and *ex vivo* genetically modified human cell therapy products (*ex vivo* gene therapy products), among regenerative medical products.

(2) “Gene transfer” means delivery of an expression construct to a cell.

(3) “Genetically modified cell” means a cell to which an expression construct has been delivered.

(4) “Expression construct” means a construct containing a genetic regulatory elements and regulatory elements for gene expression such as promoter or enhancer.

(5) The “transgene” refers to the base sequence that codes the protein or nucleic acid having a specific function and is essential for the indication(s) or performance of the product.

(6) “Vector” is a vehicle used to deliver an expression construct to a cell.

(7) “Viral vector” is a virus used as a vector in which a transgene and/ or regulatory elements is/ are packaged in the genome of the wild-type virus by using genetic modification technology.

(8) “Non-viral vector” is a vector that is not a viral vector, and refers to a plasmid itself or a complex of a plasmid and a carrier such as a liposome.

(9) “Carrier” is an entity that is used to efficiently deliver an object such as a plasmid to a cell.

(10) “Bank system” refers to the Master Cell Bank (MCB), Working Cell Bank (WCB), Master Virus Bank (MVB), or Working Virus Bank (WVB) prepared to properly produce a vector.

(11) The “MCB” is an aliquot of a single pool of cells which generally has been prepared from the selected cell clone under defined conditions, dispensed into multiple appropriate containers and stored under defined conditions.

(12) The “WCB” is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions. The aliquots are stored in appropriate containers under defined conditions.

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[1] When developing gene modified animal cell therapy product or a prophylactic pharmaceutical that contains an expression construct, the RS strategy consultation service should be adequately utilized. (Reference: Repeal of the System of Verification Application in the Development of Gene Therapy Drugs [PFSB Notification No. 0701-13, dated July 1, 2013])

(13) The "MVB" is the original source from which the viral vector is manufactured, and it is prepared from the selected virus clone under defined conditions, dispensed into multiple appropriate containers and stored under defined conditions.
(14) The "WVB" is prepared from aliquots of a homogeneous suspension of viruses prepared from the MVB under defined conditions. The aliquots are stored in appropriate containers under defined conditions.
(15) "Helper virus" is a virus that needs to be present when the intended viral vector is produced in a cell. The helper virus is used to complement the production of the viral vector by having it infect the viral vector-producing cells.
(16) "Packaging cell" is a cell to which part of the genes needed for the production of the viral vector has been transferred; the packaging cell alone is not enough to produce the viral vector.
(17) "Virus-producing cell" is a cell capable of producing the viral vector and is prepared by introducing the complementary genes necessary for the production of the viral vector to the packaging cell.
(18) "Final product" is the product finally administered to humans.

Chapter 2. DESCRIPTION AND DEVELOPMENT HISTORY OF GENE THERAPY PRODUCTS

1. Description and Development History

The target disease and current therapies should be first described, and the characteristics and usefulness of the product belonging the category of gene therapy products intended to be developed should be outlined based on the results from fundamental studies of the product. Then, the rationale for believing that the target disease can be treated with the product should be presented.

The method of gene transfer to humans should be outlined. The route of administration and dosing procedure (e.g., whether a viral vector or non-viral vector is used, whether the vector is directly administered or genetically modified cells produced outside the human body are administered, etc.) should be outlined, and after the characteristics and usefulness of the method of gene transfer are elucidated, the reason of its use for the target disease should be explained. When using a replication-competent vector or a vector that selectively replicates in specific conditions, the mechanism of replication, justification of the proposed therapeutic mechanism, and appropriateness of the clinical use of the vector should be explained.

The structure, manufacturing method, and characteristics of the product administered to humans should be clarified.

2. Status of Clinical Trial

If clinical trials have been conducted in Japan or overseas using the same intended product, descriptions of the studies including the target disease(s) , information on efficacy and safety , and differences from the proposed trial should be presented.

Even if there is no clinical experience for the intended product, in the case that clinical trials of a similar product that may be used as a reference have already been conducted in Japan or overseas, descriptions, results, and relationship to the intended product should be presented.

The overseas status of marketing authorization and clinical experience of the intended product should be presented.

Chapter 3. QUALITY

The structure, characteristics, production procedures, and quality control of the gene therapy product should be detailed for each of the points described below in this chapter.

It is vital for developers to keep it in mind in advancing the product that production procedures and quality control to ensure the consistent and stable production of the final product comparable to the investigational product must have been established by the time of marking authorization application. To achieve more rapid development, therefore, it should be noted that increasing the quality assurance level is important during the course of development by reviewing the production method and quality control of the investigational product and constructing the control method in a step-by step manner that allows for more stably and consistently producing the product with the intended quality based on the extensive knowledge on quality obtained as the clinical trial advances.

1. Expression Construct

An expression construct suitable for the expression of the transgene needs to be developed to establish a production process for the gene product that is manufactured using genetic modification technology. The appropriateness of the expression construct chosen should be explained with respect to the following points.

(1) Structure of Expression Construct

A schematic representation for the structure of the expression construct should be presented. In addition, main restriction sites and configuration of the components (transgene, regulatory elements for gene expression such as promoter or enhancer, replication origin, drug selection marker gene, among others) should be clarified, and the entire base sequence of the expression construct should be shown.

(2) Construction of Expression Construct

Construction of the expression construct should be explained by detailing the acquisition, history, structure, etc. of the transgene so that its origin is clarified. The expression construct should be analyzed using the guidelines, "Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products" (PMSB/ELD Notification No. 3, dated January 6, 1998; hereinafter, the "ICH Q5B Guideline") as reference.

(3) Functional Characteristics of Transgene

The mechanism of action of the transgene on the target disease should be elucidated. The difference of structure and base sequence between the target sequence and the wild one, e.g. a substitution, addition, or deletion, or homologies, should be determined. If a gene sequence that does not exist in humans is introduced or if a base sequence coding RNA with a certain function such as small interfering RNA (siRNA) is transferred using a vector, the expected mechanism of action and biological activity of the transferred base sequence should be clarified.

(4) Structure and Functional Characteristics of Regulatory Elements for Gene Expression
The structure and function of any element involved in the regulation of transgene expression such as promoter or enhancer should be clarified. If the transgene expression is designed to be regulated by a certain mechanism, it should be explained that the designed mechanism does properly work.

(5) Structure and Functional Characteristics of Expression Product from Transgene
Depending on the characteristics of the expression product from the transgene, the following points should be clarified.
If clinical effects are to be expected by the expression of a certain protein from the transgene, available findings on the characteristics of the expressed protein should be clearly described, and in addition, the expected biological activity of the expressed protein in the human body be determined, and the application of the intended product to the target disease be justified. Clinical experiences of the expressed protein, if any, should be described. If a gene coding an entity such as a protein that does not exist in humans is transferred, it should be explained whether relevant risks for safety exist, including those due to the expected immunogenicity or biological activity of the protein.
If clinical effects are to be expected by the expression of a certain nucleic acid from the transgene, available findings on the characteristics of the expressed nucleic acid should be clearly described, and in addition, the expected biological activity of the expressed nucleic acid in the human body be determined, and the application of the intended product to the target disease be justified. Clinical experiences of a similar nucleic acid, if any, should be described, and whether the nucleic acid from the transgene expression has any relevant risk for safety should be elucidated.

(6) Configuration and Functional Characteristics of Other Components and Open Reading Frames
The open reading frames (i.e., base sequences of the transgene and other genes in the expression construct that have the ability to be translated) of the transgene should be identified. It should be confirmed that no oncogene or gene associated with pathogenicity is included in the components of the expression construct, by searching the available database, literature, and other materials for information; if there is any base sequence that can be adverse, the reason for its use should be explained.

2. Structure and Characteristics of Vector and Production Procedure

(1) Viral vector

1) Structure of Viral vector

(i) Selection and characteristics of the method of gene transfer
The developer should outline the method of gene transfer selected and clarify its characteristics and usefulness to explain the rationale for its application to the target disease.

(ii) Particle structure of the viral vector
The particle structure of the viral vector should be clarified. Differences from the wild-type virus particle, if any, should be described.

(iii) Gene structure

The whole sequence of the viral vector should be determined as far as technically possible and evaluated to determine whether there is any risk for safety. The sequence analysis should be performed using a validated method, and the method used should be explained. The sequencing should be performed at least for the transgene, flanking regions (the non-coding sequences that are adjacent to the 5' and 3' ends of the coding sequence of the target product), promoter, enhancer, splicing sequences, and regions modified to change the virus characteristics. Among the other sequences, those with unknown functions should be evaluated for their safety by searching for homology with other sequences previously reported or by another method of sequence analysis. Whether the transgene sequence is stably maintained as is designed should be assessed, and the stability of the whole gene of the viral vector should also be confirmed. Furthermore, the stability during the whole production process should also be evaluated. In particular, when using RNA viruses, it is advisable to evaluate the genetic stability during production, considering the high susceptibility of genome alteration.

2) Origin and Characteristics of the Viral Vector

For the wild-type virus from which the viral vector is derived, its name, structure, life cycle, host range, physicochemical stability, pathogenicity, and cytotoxicity should be identified to explain the rationale for selecting the virus. In particular, if the original virus has not previously been used in any clinical trial, the developer should elucidate its pathogenicity, immunogenicity, and cytotoxicity to humans and sustainability in the human body to evaluate its safety in humans.

Based on the type of the viral vector adopted, the properties of the viral vector should be delineated by clarifying the following points:

(i) specificity of the virus infection, e.g., species specificity, tissue specificity, and cell cycle-dependency;
(ii) possible range of the infection if the vector is modified to change the viral tropism;
(iii) efficiency of the gene transfer and expression of the transgene;
(iv) status of the presence of the viral vector in a cell, e.g., incorporated in a chromosome or exist outside as episome. The integration site is specific or not in the former case, and the extrachromosomal replication occurs or not in the latter case. In addition, the intracellular stability of the transgene;
(v) replication-competency of the viral vector. If so, it occurs in a replication-selective manner or not. In addition, sustainability of the transgene expression; and
(vi) experiences and relevant recent status of clinical use, if any, of the viral vector as information on its efficacy and safety.

3) Viral vector construction and cells used for the production

(i) Plasmids or viruses for production of the viral vector

The rationale for the use of all plasmids or viruses in the production of the viral vector should be explained and the suitability of them for the intended product should be clarified. In addition, the quality control method for them should be determined. These should include presentation of all sequences and restriction site maps for the plasmids or virus genomes, configuration of all components, origin and function of

each base sequence, and in addition, description of the cloning method and production procedure including the gene modification process and preparation of the host used.

If a helper virus is used in the production of the viral vector, the rationale for its selection should be explained with its properties including name, structure, life cycle, host range, physicochemical stability, pathogenicity, and cytotoxicity.

Items of the quality control for the plasmid or virus used for production should be determined based on the results of characteristic analyses.

The quality control using the bank system is also recommended to constantly maintain the quality level of the final product.

(ii) Cells used for production of viral vector

For the cells used to produce the viral vector, the rationale of their use and the reason that they are suitable in obtaining the target viral vector should be explained. In addition, appropriate quality controls for the cells should be determined. These should include the properties of the cells including name, origin, pathogenicity/safety, replication potential, growth factor dependence, phenotype, tumorigenic potential and gene stability, and the rational for the use of them should be explain by presenting the results of characteristic analyses for, e.g., virus safety when used for production. In addition, differences from the original cells should be identified in terms of properties including potential emergence of replication-competent viruses, replication potential, growth factor dependence, phenotype, tumorigenic potential, and stability

If a packaging cell or virus-producing cell is used for the virus production, the characteristics of the original cell and the details of the transferred gene to modify the original including the whole sequence and restriction site map showing the configuration of all components along with the origin and function should be explained. In addition, the cloning and preparation method for these modified cells including the host information and genetic changes during the process (e.g., culture condition, genetic modification, clone selection) should be clarified.

The quality control using the bank system is also recommended to constantly maintain the quality level of the final product.

(iii) Bank system

In case of use of the bank system for cell substrates including packaging cell, virus producing cell, and feeder cell, or for the cell substrates for preparation of the plasmid, or for the viral vector itself and/or helper virus, the developer should clarify the character of each bank such as MCB, WCB, MVB, or WVB and the preparation procedure for these materials including culture condition, culture medium and raw materials/ supplements. In addition, they should determine the quality control for the duration of preservation and renewal process properly based on the results of characteristic analyses for comparability, purity and stability during preservation under the proper test items, analysis method, and its quality control. The developer must show that cell banks and biological materials among primary reagents used in

the banks meet the Standards for Biological Raw Materials (MHLW Ministerial Announcement No. 210 of 2003).

The developer should refer to the ICH Q5B Guideline and the notification, "Notification on Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products" (PMSB/ELD Notification No. 873, dated July 14, 2000; hereinafter, the "ICH Q5D Guideline") when establish, characterize, and manage the bank system and also evaluate the virus safety according to the notification, "Notification on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin" (PMSB/ELD Notification No. 329, dated February 22, 2000; hereinafter, the "ICH Q5A Guideline"). Note particularly the following points for the characterization and management of the bank system.

(A) Cell Bank

Purity tests should include at least ones for sterility, absence of mycoplasma, and viruses. Tests for replication-competent virus should also be done for the final product, if necessary.

Virus tests for cell banks should, as a general rule, be performed according to the ICH Q5A Guideline for the MCB, WCB, and cells at the limit of in vitro cell age used for production. In particular, when using cells of human origin, considering expected virus safety risks, in addition to tests for human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), human hepatitis B virus (HBV), human hepatitis C virus (HCV), and human T-cell leukemia virus type 1 (HTLV-1) and type 2 (HTLV-2), tests for other viruses such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), and parvovirus B19 should also be performed, as necessary. With virus safety risks being evaluated, virus tests for the cell bank control should be determined. In particular, when producing virus vectors other than retrovirus-derived ones, it should be considered to determine for the MCB or WCB whether it is contaminated with retroviruses, using, e.g., a reverse transcriptase test or electron microscopy.

If bovine- or porcine-derived materials such as serum, serum-derived ingredients, trypsin, or growth factors are used for culture, their safety should be investigated and determined using appropriate tests for possible contamination with bovine- or porcine-derived infectious agents. Appropriate controls in cell banks should also be determined, as necessary.

For cell banks of organisms used for plasmid production, tests for bacteriophage should be considered, in addition to identification of cell strain and testing for the drug resistance used as a selection marker.

(B) Virus Bank

Purity tests should include at least ones for sterility, absence of mycoplasma, absence of adventitious/ contaminating virus and replication-competent virus. In addition, possible risks to viral safety should be clarified and what virus tests to control the virus bank should be determined. When producing a viral vector derived from one other than a retrovirus, a test of absence of retroviruses

contaminated in the MVB or WVB should be considered, using, e.g., a reverse transcriptase test or electron microscopy.

If bovine- or porcine-derived materials such as serum, serum-derived ingredients, trypsin, or growth factors are used in the culture, their safety should be investigated and determined using appropriate tests for possible contamination with bovine- or porcine-derived infectious agents. Appropriate controls for issues above in virus banks should be determined, as necessary.

4) Materials Used in Viral Vector Production

All materials used in production must be identified. In particular, the materials used in the culture medium, including its ingredients and other additives such as serum, growth factors, and antibacterial/antifungal agents should be identified. It should also be clarified in what step each material is used during the production process. In addition, necessity of those materials should be explained, and specifications for acceptance testing should be presented for key materials that may influence the quality of the final product. Appropriateness of the controls for those key materials should also be explained.

In particular, the following points should be clarified to ensure the safety in humans.

(i) If materials that contain human- or animal-derived ingredients are used, all those ingredients should be confirmed to meet the Standards for Biological Raw Materials.

(ii) Measures to prevent contamination with infectious agents should be presented including the inactivation/removal procedures and tests performed.

5) Production of Viral Vector and Process Control

The production method of the viral vector should be clarified using flow diagrams of the whole production process and showing an outline of all steps and their controls set to constantly produce the viral vector with proper quality. This should include presentation of the information on: the culture method for the cells used for production; the entity such as a plasmid or virus introduced to cells to produce the viral vector; approximate number of passages and cell seeding densities during viral vector production; and the steps for purification such as centrifugation, column purification, and density gradient centrifugation.

An outline of the process controls performed on each production step, including action limits/acceptance values, should be presented and the purpose and rationale of the process controls including the action limits/acceptance values should be explained.

Process parameters should be identified to ensure the product quality. In particular, critical processes and their parameters influencing the quality should be identified with their control margins. If critical intermediates arise in the production process, the preservation procedures as their controls, if necessary, including action limits etc. should be defined.

Tests for quality control required for safety assurance, for viral vectors including ones derived from replication-competent viruses may be performed as part of the process control because of their characteristics and capability of virus detection. The timing and method of each test for safety assurance should therefore be presented.

6) Assessment of Process-Related Impurities

The residual levels of process-related impurities in the final product should be measured and no safety concerns on humans at the residual levels should be demonstrated.

Appropriateness of the process control for materials as source on the impurities, as well as the rationale for the determination of the specifications for acceptance testing should be explained, as necessary. Furthermore, a production process with minimized residual risk should be established by means of, e.g., evaluation on the capacity of removal process and review of the production procedure. In the case of materials that have not previously been administered to humans and are considered to be poisonous deleterious, biologically active, or sensitizing substances, in particular, the residual levels of them in the final product should be carefully evaluated with safety concerns in humans.

(2) Non-Viral Vector

1) Structure of Non-viral vector

(i) Selection and characterization of the gene transfer method

The outline of the gene transfer method adopted should be presented and the rational of the application for the target disease should be explained by clarifying the character and usefulness of it.

(ii) Composition of the non-viral vector

Composition of the non-viral vector should be clarified, with the information of a plasmid used alone or packaged in a carrier such as a liposome.

(iii) Gene structure

The whole sequence of the non-viral vector should be determined and analyzed to determine whether there is any risk for safety. The sequence analysis should be performed using a validated method, and the method used should be explained.

Whether the transgene sequence is stably maintained as is designed should be evaluated, and the stability of the whole gene of the non-viral vector should also be confirmed. Furthermore, stability during the whole production process should also be evaluated.

2) Origin and Characteristics of the Non-Viral Vector

For the plasmid from which the non-viral vector is derived, its name, structure, physicochemical stability, pathogenicity, and cytotoxicity should be identified to explain the rationale for selecting the plasmid. In particular, if the plasmid has not previously been used in any clinical trial, the developer should elucidate its pathogenicity, immunogenicity, and cytotoxicity to humans and sustainability in the human body to evaluate its safety in humans.

In addition, for each of the components (proteins, carbohydrates, lipids, etc.) of the carrier used for the non-viral vector, its name, structure, physicochemical stability, pathogenicity, and toxicity should be identified to explain the rationale for selecting them. In particular, if they have not previously been used in any clinical trial, the developer should elucidate their pathogenicity, immunogenicity, and toxicity to humans and disposition in the human body to confirm that no safety concern will arise in humans.

Properties of the non-viral vector should be explained by clarifying the following points in relation to the type of the non-viral vector adopted and method of gene transfer:

(i) with the method of gene transfer being clarified, into what kind of cells the method can transfer genes, whether species specificity or tissue specificity exits, and cell cycle-dependency;
(ii) efficiency of gene transfer and expression efficiency of the transgene;
(iii) properties of the non-viral vector in terms of whether it is incorporated into a chromosome or exits outside the chromosomes as an episome, including whether the transgene is incorporated in a site-specific manner (if the former is the case) or whether extrachromosomal replication is involved and whether the transgene is stable in the intracellular environment (if the latter is the case);
(iv) sustainability of the transgene expression; and
(v) experiences of clinical use and relevant recent status.

3) Cells Used for Development and Production of Non-Viral Vector

(i) Construction of the plasmid used for production of the non-viral vector
The method to construct the plasmid transferred to humans should be clarified. This should involve explanation of the amplification and purification methods performed to obtain the plasmid, and in addition, the whole sequence and restriction site map of the plasmid, configuration for all components, origin and function of each base sequence, and the cloning and production method of the plasmid including the procedure of genetic modification of the host gene with their information.

(ii) Organisms used for the production of non-viral vector
For the organisms used for the non-viral vector production, their suitability in obtaining the intended product should be justified, and appropriate quality controls for the organisms should be determined. These should include presentation of the properties of them, including name, origin, pathogenicity/safety, replication potential, phenotype, gene stability, and infectious agents generated in the production and the rationale for the use of the organisms in production should be explained with these pieces of information.
In order to constantly maintain the quality of the final product, quality control using bank systems is recommended.

(iii) Bank system
In the case of using bank system of the organisms used for production of the non-viral vector, the developer should clarify the origin of the organisms used for preparation of the MCB and WCB, the preparation procedures and process (e.g. culture conditions, media used for culture, and source materials and additives), preservation method, and their characteristics. In addition, they should determine tests for identity and purity, analytical methods, and proper control and its standard for preservation, stability, and renewal for the bank. The biological raw materials in materials used for preparation of the cell bank must be shown to meet the Standards for Biological Raw Materials. The establishment, characterization, and management of a bank system should be conducted in reference to the ICH Q5B and ICH Q5D Guidelines. If human- or animal-derived cells are used, virus safety should be evaluated according to the ICH Q5A Guideline.

4) Materials for Non-Viral Vector Production

All materials used for production must be identified. In particular, the culture medium used should be characterized for its ingredients and other additives such as serum, growth factors, and antibacterial/antifungal agents. Also, the non-viral vector should be characterized for its components and constituents. Furthermore, it should be clarified in what step each material is used during the production process. In addition, necessity of those materials should be explained, and specifications for acceptance testing should be presented for key materials that may possibly affect the quality of the final product. Appropriateness of the controls for those key materials should also be explained.

In particular, the following points should be clarified to ensure the safety in humans.

(i) If materials that contain human- or animal-derived ingredients are used, including when those materials are used for production of a carrier etc., all those ingredients should be confirmed to meet the Standards for Biological Raw Materials.

(ii) Measures to prevent contamination with infectious agents should be presented including the inactivation/removal procedures and tests performed.

5) Production of Non-Viral Vector and Process Control

An outline of the procedure and its control for consistent production of non-viral vector with proper quality should be clarified by using flow diagrams presenting the whole production process. This should include information on culture for the organisms used in the production, transfection of the plasmid to the organisms and centrifugation, purification with columns, and so on, and preparation of the carrier components and its purification.

An outline of the process controls performed for each step in the production of the non-viral vector, including action limits/acceptance values, should be presented and the aim or rationale of the process controls with the action limits/acceptance values should be explained.

Appropriate process controls to ensure a certain level of constancy in quality should be performed in the production of the carrier, etc.

Process parameters necessary for the controls to ensure product quality should be identified. In particular, critical processes affecting the quality together with critical process parameters should be identified with their control margins. If critical intermediates exit in the processes, the preservation method as the control and action limits, etc. as necessary, should be defined.

Tests for quality control required for safety assurance, including ones for infectious agents, can be more appropriately performed as part of process control, in view of increasing the safety. The timing and method of each test regarding safety should therefore be presented.

6) Assessment of Process-Related Impurities

The residual levels of process-related impurities that can remain in the final product should be measured, and no safety concerns on humans at the residual levels be demonstrated when the product is administrated into humans. Appropriateness of the process control for materials as source of the impurities as well as the rationale for the approach of the specifications for acceptance testing should be provided, as necessary. Furthermore, a production process with minimized residual risk should be established by means of, e.g., evaluation on the capacity of removal process and review of the production procedure. In

the case of materials that have not previously been administered to humans and considered to be poisonous, deleterious, biologically active, or sensitizing substances, in particular, the residual levels of them in the final product should be carefully evaluated with safety concerns in humans.

3. Target Cells
   (1) Gene Therapy Products
   The intended method, site, and instruments/devices used for administration to humans should be clarified. Human- or animal-derived substances contained in the instruments/devices should be confirmed to meet the Standards for Biological Raw Materials.
   The target cells or tissues should be biologically characterized. In particular, if the target cells or tissues lack the transgene, changes in their characteristics caused by gene transfer should be evaluated in detail. Furthermore, advantages and disadvantages compared with the cases where the gene is transferred to other cells or tissues should be identified to explain the rationale for selecting the target cells or tissues.
   (2) Genetically Modified Human Cell Therapy Products
      1) Origin and Characteristics of Target Cells and Rationale for Selecting Them
      If human-derived cells are used as the target of gene transfer, their origin (including the type of the cells/tissues and autologous or allogeneic cell) and biological characteristics (e.g., morphological characteristics, growth potential, biochemical markers, immunological markers, characteristic products, HLA types, and other relevant genotypic or phenotypic markers) should be identified.
      In the case of use of cell substrates such as cell lines as the target cell, the developer should clarify the origin and show the information on the preparation or establishment process of them including culture conditions with medium, additives, and source materials, and the stability during preservation. In addition, they should set the proper control such as tests for identity and purity, their methods, preservation methods, and renewal methods. Virus safety should be evaluated according to the ICH Q5A Guideline, and the cells must be shown to meet the Standards for Biological Raw Materials. Furthermore, advantages and disadvantages compared with the cases where the gene is transferred to other cells should be identified to explain the rationale for selecting the target cells. In order to constantly maintain the quality of the final product, quality control using bank systems is recommended.
      2) Donor Eligibility
      In the case of use of human cells collected from donors as a target cells of gene transfer, the confirmation of proper eligibility for the donor should be shown. In particular, the issue that the donor be selected ethically through an appropriate procedure should be shown. Inclusion or eligibility criteria should be determined and their rationale be explained, considering factors such as age, sex, ethnic characteristics, genetic characteristics, medical history, heath status, collected cells, tests for infectious diseases that can infect through tissues, and immunological compatibility.
      In the case of use of allogenic cells, considering possible safety risks related to expected infectious agents such as viruses, the donor screening using serological and/or nucleic acid

amplification tests for HBV, HCV, HIV-1, HIV-2, HTLV-1, HTLV-2, EBV, CMV, parvovirus B19, and for other viruses as necessary, should be done.

Using the results of past serological tests, diagnostic history, and medical history of the donor as much as possible, the rationale for the use of the donor cells as a target cell should be explained.

The rationale for the use of allogeneic cells should be clarified by analyzing, as necessary, genetic polymorphism and HLA matching. Even in the case of use of autologous cells (patient's own cells) , the donor (patient) should be tested for infectious diseases, considering the possibility of infection through the collected cells/tissues, in view of ensuring the safety of the patients, production personnel, and medical staff.

3) Procedure for Tissue Collection

In the case of collection of human tissues from a donor as a target cell of gene transfer, the type of collected tissue, collection site and method, collection quantity, the frequency and intervals of collection, and instruments/devices used for collection should be specified. The collection method should be detailed if the cells are collected from the donor after administrating medical drugs such as a cytokine to recruit the target cells.

4) Materials or Instruments/Devices Used for Production of Genetically Modified Cells

Materials used in the production of transgenic cells such as culture medium, serum, and additives including growth factor, and anti-bacterial/anti-fungal drugs, and the components and constituents of carriers, if used, should be clarified. The instruments/devices used for gene transfer should be demonstrated to be equipped with the required performance and all materials, source, and devices used in the instruments should be clarified. It should also be clarified in what step each material or instrument/device is used during the production process. The necessity of the materials and instruments/devices in the production of the genetically modified cells should be clarified and, in particular, for critical materials that can affect the quality of the final product, their specifications for acceptance testing should be determined and the appropriateness of their controls be explained.

In particular, in view of ensuring human safety in administering the product to humans, the following points should be clarified:

(i) If the materials contain human- or animal-derived ingredients or instruments/devices use such materials in the production, all those ingredients should be confirmed to meet the Standards for Biological Raw Materials

(ii) Measures to prevent contamination with infectious agents should be presented including the inactivation/removal procedures and tests performed.

(iii) If any carriers or instrument/device are used for gene transfer, the safety and rationale of their use should be clarified.

5) Production of Genetically Modified Cells and Process Control

An outline of all steps in the production of the genetically modified cells and their control set to constantly produce those cells with proper quality should be explained using the flow diagrams. This should include presentation of the information on: culture method for the target cells; number of days in culture, number of passages, and seeding densities; method of gene transfer; and method of cell washing. If the target cells need a process of

concentration, selection, or extended culture, or a process of irradiation following gene transfer, or if genetically modified cells are used following cryopreservation and thaw, those processes should also be explained.

An outline of the process controls performed for each production step, including action limits/acceptance values, should be presented to explain the aim or rationale of the process controls, with the rationale of the action limits/acceptance values. In particular, in administering the product to humans, it should be confirmed that gene transfer causes no safety concerns in cells including unwanted changes in cell phenotype, and controls of them should be determined as necessary.

Process parameters that need to be controlled to ensure product quality should be identified. In particular, critical processes affecting the quality and critical process parameters should be identified with their control margins. The controls for critical intermediates if generated should be defined by determining preservation methods and, as necessary, action limits/acceptance values, etc.

6) Assessment of Process-Related Impurities

In the case that process-related impurities that can remain in the final product of human cell processed products consisting of genetically modified cells, the residual levels in the final product should be determined, and it should be shown that no safety concern will arise even if they are administered at each residual level to humans. Appropriateness of the process control for the source and other materials including the impurities as well as the rationale for the determination of their specifications for acceptance testing should be explained, as necessary.

If the genetically modified cells need a further preparation procedure such as washing before being administered to humans, the rationale for determining the specifications for the amount of residues in the final product may be shown considering the steps in the procedure and their efficiency for removing the process-related impurities.

Furthermore, a gene transfer process with minimized residue risk should be established by means of, e.g., process evaluation regarding removal capacity and review of the production procedure. In particular, residual levels in the final product should be carefully determined for materials containing substances with safety concerns in humans such as chemicals that have not previously been administered to humans, poisonous and deleterious substances, biologically active substances, and sensitizing substances.

7) Evaluation of Vectors remaining on Genetically Modified Cells

In the case that genetically modified cells are produced using a viral vector, the residual potential of infectivity of the viral vector should be evaluated. This evaluation should be performed taking into account the physicochemical properties of the viral vector, culture duration of cells after gene transfer, washing, etc.

4. Characterization and Specifications

(1) General Principles

Details of characterization need to be determined for each product on a case-by-case basis. Based on the principles presented in the notification, “Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products” (PFSB/ELD Notification No.

571, dated May 1, 2001), characterization for gene therapy products should be performed in accordance with the features of each product, and its characteristics including structure, physicochemical properties, biological properties, purity, and impurities should be identified. Based on the results of the characterization, specifications for investigational and commercial products to ensure the quality of individual products should be determined, and quality control including controls for source materials and intermediates should be performed to ensure homogeneity for each production.

For commercial products marketed as gene therapy products, specifications for each bulk material and product should be determined so that final products with the quality comparable to that of the lots used for clinical trials are constantly supplied. As the clinical development program advances, an appropriate procedure of lot control for the bulk material and the product should be established based on results from characterization and multiple lot tests so that homogeneity and constancy are ensured for the final product. Its justification should include a rationale for the number of the test lots used. An appropriate control strategy for the bulk material should be developed considering the necessity of its control.

As a general rule, specifications for an investigational product are recommended to be made more appropriate as the trial phase advances so that a quality level that can ensure the intended efficacy and safety in humans is achieved, rather than maintaining the specifications intact through the clinical development program. To initiate clinical trials, controls for the investigational product should be determined considering at least the following points:

(i) Tests and acceptance values in specifications for the investigational product should be determined so that the quality is ensured to be consistent with that of the samples and products used in the nonclinical studies except for studies of infectious agents.

(ii) Specifications represent the tests performed, assays used, and corresponding acceptance criteria, and should be determined regarding, in general, description, identity test, purity, general tests (referring to measurements of osmolality, pH, extractable volume, insoluble particulate matter, foreign insoluble matter, etc.), infectious agents, biological activity, potency, and quantity. Appropriate specifications should be determined depending on what stage of development the product is in. Acceptance values should be determined with their rationale being presented. The testing procedures with validated performance should be adopted.

(iii) For testing items that are not appropriate to be tested in the final product in view of the characteristics of the test sample and/or the test sensitivity, it is more reasonable to perform controls for intermediates. In this case, it should be shown that reasonable procedures for in-process control and sample preservation, reasonable action limits, etc. have been determined. If the final product is genetically modified cells, the way to control the viral vector or non-viral vector used to produce those cells should be determined, as necessary, based on the results of characterization and quality tests of the vector.

(2) Characterization and Controls of *in vivo* Gene Therapy Products

1) Characterization

In the case of a direct application of gene therapy product (*in vivo* gene therapy product), gene sequencing of the vector should be done carefully, because structural characteristics

of the vector affect the safety of the product. For example, in the case of a viral vector, the sequence of the transgene, sequence of its flanking region, sequences of the promoter and enhancer, and whole sequence of the vector should be determined. Even if the whole sequence is not determined, the overall structure of the vector should be confirmed to coincide with its designed structure by analyzing a detailed restriction site map. Furthermore, data on what level of transgene expression is expected in the target cells should be obtained by conducting, e.g., *in vitro* studies on the expression level and sustainability. In addition, data on the expression of the transgene in cells other than the target cells should be obtained, as necessary.

2) Identity

The purpose of identity testing is to confirm that the product obtained is the intended gene therapy product, and identity tests are required to be highly specific. An appropriate identity test(s) should be planned based on the results of the characterization of the product.

3) Purity and Impurities

The purpose of purity and impurity tests is to ensure constancy of the inhomogeneity level and control the impurity content. Impurity content is in general controlled by setting specifications for the intermediates and final product. Impurities that have been shown to be constantly removed to an acceptable level during the purification process, however, may be controlled only with in-process controls without setting tests for the final product. Impurities that have been confirmed to be constantly removed at a high level may need no setting of specifications for the intermediates or final product or in-process controls.

Impurity tests should be appropriately set and performed on the materials used for vector production, including DNA or RNA, proteins, peptides, medium additives, solvents, and serum. In the case of a plasmid vector, it should be considered to set quantitative tests for total DNA or RNA content, tests for size and structure, homogeneity tests for plasmid description (e.g., supercoiled, open circular, or linear), and tests for contamination with DNAs from plasmid-producing cells and host proteins. In the case of a viral vector, it should be considered to assay residual levels of the nuclease used for production, plasmid DNA, helper virus, proteins and DNAs from the vector-producing cells, and non-infectious particles, derived from the viral vector, as part of impurity testing.

4) Infectious Agents

In view of the purpose of increasing the safety against infectious agents as much as possible, reasonable controls of infectious agents should be planned for the bank system, in-process control, intermediates, or final product, whichever is appropriate, after extensive analysis on the source materials, cell banks, virus banks, crude bulks, intermediates, and final product, based on risk evaluation on infectious agents that can cause contamination, considering the points listed below.

Safety may be assured even if virus testing or mycoplasma testing is performed on crude bulks or intermediates, provided that it is justified, e.g., for the reason that, after the process of culturing, amplification of virus or mycoplasma is not expected or can be detected with high sensitivity.

Sterility testing should be performed for the final product administered to humans as much as possible.

(i) Sterility testing (Testing for absence of bacteria and fungi)

Sterility testing should be performed according to the requirements of the Sterility Test <4.06>, Japanese Pharmacopoeia (JP), if it is applicable to the vector used for the clinical trial. If it is difficult to prepare a sufficient amount of sample because of the characteristics of the gene therapy product, technically feasible and appropriate testing procedures should be designed. In this case, the tests used should be appropriately validated to ensure their reliability.

(ii) Mycoplasma testing (Testing for absence of mycoplasma)

Mycoplasma testing should be performed according to Mycoplasma Testing for Cell Substrates Used for Production of Biotechnological/Biological Products included in the General Information, JP.

(iii) Virus testing

Virus safety should be evaluated according to the ICH Q5A Guideline. Based on the results of the evaluation, virus tests for the quality control should be set. In doing this, it is recommended to perform infectivity tests to detect adventitious viruses such as *in vitro* virus tests. If a packaging cell derived from mouse or another rodent is used to produce a retroviral vector, testing for ecotropic retroviruses, which can contaminate the MCB at a low level, should be performed.

If human-derived cells are used for vector production, it should be considered to perform tests for viruses that may be infectious or pathogenic to humans. For example, if HEK293 cells are used to produce an adenoviral vector, besides the aforementioned viruses, those that may be infectious or pathogenic to humans, including adenoviruses and adeno-associated viruses, should be considered to be tested.

(iv) Testing for replication-competent virus (for the case a viral vector is used)

If a replication-incompetent retroviral vector or lentiviral vector is used, testing and evaluation for absence of replication-competent retroviruses and replication-competent lentiviruses should be performed on samples of the bank system, postproduction crude bulks, and final product. Controls on samples should be planned at appropriate steps, as necessary. If another type of replication-incompetent viral vector is used, testing and evaluation for absence of replication-competent viruses should be performed on samples of the bank system and final product. Controls on samples should be planned, as necessary. In addition, an outline of replication-competent virus tests should be presented, with their detection sensitivity being shown to be appropriate and suitable for the test purpose.

If other viruses are used to produce the viral vector, contamination with the viruses other than the viral vector in the final product should be denied using a test with an appropriate sensitivity.

If a replication-competent or restricted replication-competent viral vector is used, viral vectors lacking the transgene that exist in the final product, and unintended replication-competent viruses including revertants that exist in each cell bank system or the final product should be tested and denied using an approach with an appropriate sensitivity.

(v) Endotoxin test

Endotoxin test should be performed according to the requirements of the Bacterial Endotoxins Test <4.01>, JP, if it is applicable. If the application of the Bacterial Endotoxins Test, JP, is not justified because of the amount or characteristics of the sample, the test should be performed using an appropriate approach in reference to the Bacterial Endotoxins Test, JP. In this case, the testing procedure used should be validated to ensure its reliability.

5) Biological Activity or Potency

All evaluation results from the studies conducted to characterize the biological activities of the expression product of the vector should be presented. The relationship between the evaluation results for the biological activities closely related to the intended clinical effects and the expected clinical effects should be clarified based on results from the characterizations, pharmacological studies, and/or clinical trials. It is preferable that these studies on biological activities have a quantitative nature. Acceptance ranges should be determined for the biological activities, as necessary.

In the case of a viral vector, the ratio of infectious and non-infectious particles should be determined to measure the virus titer and set an appropriate acceptance range, or the infectivity titer per virus particle be measured to set an appropriate acceptance range.

6) Content

For vector content, an acceptance range for a physical quantity relevant to administration, such as number of virus particles or virus titer (for viral vectors) or plasmid DNA concentration (for non-viral vectors), should be determined.

7) Other Tests Performed Depending on Product Characteristics

Characteristics specific to the viral vector or non-viral vector used, such as particle size distribution, should be determined and, as necessary, their acceptance range should be set. If the gene therapy product has some particular characteristics, consideration regarding the potential of aggregate formation may be needed, and the amount of aggregates should be controlled by setting an appropriate testing procedure.

(3) Characterization and Controls of Genetically Modified Human Cell Therapy Products

1) Characterization

The genetically modified cells should be characterized in terms of cell surface markers and cytokine production capacity, and identification of the type of the genetically modified cells, determination of the copy number of the transgene per cell, and analysis of inserted genes should be performed to confirm that there are no inserted genes at particular sites.

The potential of gene transfer to unintended cell populations should be evaluated from the viewpoint of safety. For example, if genes are intended to be transferred to T cells, the possibility for the collected cell population to contain hematopoietic stem cells and the efficiency of the gene transfer to the hematopoietic stem cells should be elucidated. In addition, tumorigenicity caused by insertional mutagenesis should be evaluated using appropriate test systems.

If gene transfer is performed to induce *in vitro* differentiation, in order to determine the duration of *in vitro* culture and justify the determined time, the changes in cell characteristics caused by varied time in culture should be evaluated by characterizing the

growth, viability, and gene expression of cells cultured beyond the determined time. In addition, the remaining vector used for the gene transfer should be evaluated, as necessary.

2) Identity

The purpose of identity testing is to confirm that the product obtained is the intended genetically modified cells, and identity tests are required to be highly specific. An appropriate identity test(s) should be planned based on the results of phenotyping studies of the genetically modified cells.

3) Purity and Impurities

The purpose of purity and impurity tests is to ensure constancy of the inhomogeneity level and control the impurity content. Appropriate impurity tests should be performed regarding the proteins and peptides that remain after being used for activation or processing of cells, as well as materials used for production such as cytokines, growth factors, antibodies, and serum. In addition, impurity tests for cells with an unintended character should be considered.

4) Infectious Agents

Testing for infectious agents should be performed in reference to the subsection “4) Testing for Infectious Agents, (2) Characterization and Controls of Gene Therapy Products, 4. Characterization and Specifications, Chapter 3. QUALITY” of this document, taking into account the remarks described below.

For sterility testing (See (i) in the subsection mentioned above), if it is difficult to prepare a sufficient amount of sample because of the characteristics of the human cell processed product, or if it is difficult to apply the Sterility Test, JP for the reason of time constraint on testing, a technically feasible and appropriate test should be performed, with reference to the Microorganism Fast Method explained in the General Information, JP. In this case, the test used should be validated to ensure its reliability.

For virus testing (See (iii) in the subsection mentioned above), if autologous cells are used, necessity of virus safety evaluation should be examined, considering the virus contamination risk related to the materials used for production and, as necessary, the potential of virus replication in production process, using “Quality and Safety Assurance of Human (Autologous) Cell or Tissue Processed Pharmaceuticals and Medical Devices “ (PFSB Notification No. 0208003, dated February 8, 2008) for reference. Even if autologous cells are used, risk of serious infections due to adventitious viruses should be assessed, considering the characteristics of the administration route or transplant site (e.g., intracerebral administration). If allogeneic cells are used, appropriate virus testing and, as necessary, evaluation of the potential of virus replication in the production process should be performed, considering the characteristics of the cells/tissues collected, using “Quality and Safety Assurance of Human (Allogeneic) Cell or Tissue Processed Pharmaceuticals and Medical Devices” (PFSB Notification No. 0912006, dated September 12, 2008) for reference. If allogeneic cells are banked or stocked for use, virus safety evaluation also should be performed according to the ICH Q5A Guideline. After gene transfer, effects on virus safety such as activation of endogenous viruses are of concern; cells after gene transfer should be evaluated for their virus safety, as necessary. Based on the results of the

virus safety evaluation mentioned above, virus tests for quality control should be determined.

For testing for replication-competent virus (See (iv) in the subsection mentioned above), if genetically modified cells are cultured for a long period of time, testing for absence of replication-competent viruses should be performed, as necessary.

5) Biological Activity or Potency

All evaluation results from the studies conducted to characterize the biological activities of the genetically modified cells should be presented. The relationship between the evaluation results for the biological activities closely related to the intended clinical effects and the expected clinical effects should be clarified based on results from characterizations, pharmacological studies, and/or clinical trials. It is preferable that these studies on biological activities have a quantitative nature. Acceptance ranges should be determined for the biological activities, as necessary.

6) Cell Count and Cell Viability

Acceptance ranges for the number of live cells and the number of cells with the intended function should be determined as physical quantities for genetically modified cells. If an upper limit has been set for the number of cells administered, the rationale for it should be presented. To ensure that there remains a sufficient number of live cells when the product is administered, a lower limit for the number of live cells should be determined, considering the results of stability assessments.

7) Other Tests Performed Depending on Product Characteristics

If genetically modified cells are cryopreserved and then thawed before use, a testing procedure for identity and a shelf life for cryopreservation should be determined, as necessary, based on evaluation results including cell viability after cryopreservation.

If the product contains genetically modified cells at a high concentration, or if the genetically modified cells have some particular characteristics, consideration regarding the potential of aggregate formation may be needed, and the amount of aggregates should be controlled by setting an appropriate testing procedure.

5. Product Development

The plan to design the gene therapy products composed of the primary ingredient (e.g., vector or genetically modified cells) and secondary one in order to acquire the intended quality must be explained. The composition of the contents (e.g., solution) of the final product administered to humans should be clearly presented, e.g., in the form of a listing. The necessity of each secondary ingredient should be explained and justified, and its safety, past usage, stability, etc. be evaluated.

The primary container and its closure system must be proved to be free from defects in design so that the quality specifications are ensured over the shelf life and that the safety is ensured when the product is properly used and administered. Toxicological aspects of the container and closure system attributable to their materials, appropriateness of the sterilization procedure, integrity for preventing contamination, etc. should be evaluated, referring to the General Information, JP. In addition, the secondary container devised to prevent contamination due to breakage when transferred, if necessary, should be explained.

For gene therapy products that require special instruments/devices to administer to humans, the information on the instruments/devices usage and safety should be presented.

6. Process Evaluation/Validation

For marketing authorization application, as process evaluation/validation, it should be demonstrated that expected results (e.g., that the results of in-process controls, other critical process parameters, and the status of impurity removal during production process agree with prespecified criteria) are obtained from the production at commercial scale based on the results from generally multiple lots, to show the constancy of the production procedure. As in the case of human cell processed products consisting of genetically modified cells, if it is difficult to perform process validation for a reason such as quantitative restriction on the sample imposed for ethical reason, or technical limitations, evaluation by means of verification may be accepted.

In performing the evaluation, the critical quality characteristics identified, the related critical process parameters and their control margins, the control margins for source materials, etc. should be justified. The process evaluation/validation should include the evaluation performed on the measures that are taken for the production process to ensure its sterility. As for the process evaluation/validation for impurity removal, the target substances that are to be detected as contaminants and degradants and the reason for having selected them, and the assay procedures used and their justification including information on detection sensitivity should be presented.

During development, changes are often made in production procedure to improve or scale up the production process. In particular, in some cases, those changes are repeated, because of, e.g., changes in cell substrates during development or requirement of mass production. If changes are made in production procedure, the comparability of the products before and after changes should be assessed to use the data from the nonclinical and clinical trials conducted using the product before changes as data for the product after changes. Changes in production procedure may occur after marketing approval as well, because of, e.g., adoption of new technologies about the production of gene therapy products or new information about infectious substances. In this case, comparability of the products before and after changes must be assessed, as well. The comparability exercise should be performed in reference to "Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process "(PFSB/ELD Notification No. 0426001, dated April 26, 2005). Challenges in comparability exercise for gene therapy products include assessment of inhomogeneity and impurity removal, as well as biological characterization. If differences in quality characteristics are found for the products before and after changes, what effects those differences have on the efficacy and safety of the product has to be determined in nonclinical and/or clinical trials in some cases. Note that if comparability is not demonstrated for the products before and after changes, the characterization and nonclinical data obtained using the product before changes may need to be obtained again using the product after changes.

7. Stability Testing

The stability of the product during the period until administration to humans must be assessed to determine appropriate storage conditions and times. If the vector or genetically modified cells are stored for a certain period of time or shipped to another site, a protocol for the temporary storage

or shipping should be prepared, and their effects on the efficacy and safety of the vector or genetically modified cells be verified.

For the purpose of marketing authorization application, appropriate stability testing must be performed, taking into account the storage conditions and times, in reference to "Stability Testing of Biotechnological/Biological Products" (PMSB/ELD Notification No. 6, dated January 6, 1998), to determine storage requirements and a shelf life and justify them. The rationale for the production scale, storage condition, and number of lots should also be presented for the production lots used to justify the storage requirements and shelf life.

Chapter 4. NONCLINICAL STUDIES

The primary objective of nonclinical studies of gene therapy products is to predict their pharmacological and toxicological effects on humans in the case that the product is administered to humans, and should be conducted not only before initiating clinical trials but, as necessary, during a clinical trial.

1. Studies Supporting the Potency or Efficacy

The developer is required to obtain data from *in vitro* and *in vivo* studies to support the intended efficacy in humans. Conduct of *in vitro* studies is required to obtain data on the expression level of the transgene and the regulation and sustainability of gene expression, but *in vitro* studies alone are often inadequate for predicting pharmacological and toxicological effects in humans. It is therefore recommended to obtain data from *in vivo* studies to predict efficacy in humans as far as possible.

To obtain evidence supporting that the intended gene therapy product or human cell processed product consisting of genetically modified cells has efficacy in humans, it may be useful to select an animal model of the target disease in which the transgene from the product or the genetically modified cells administered is expected to show pharmacological effects similar to those in the case that the product is administered to humans. If an appropriate animal model in which the transgene carried by the vector induces pharmacological effects similar to those in humans is not available because of species differences, it should be considered to conduct studies that use a vector expressing a gene derived from the model animal that is homologous to the intended human gene. In this case, it should be justified that the results obtained from those studies can be extrapolated to humans.

The objectives of *in vivo* studies include (a) exploration of the minimal dose showing pharmacological activity and of optimal doses, (b) establishment of an optimal administration route, (c) exploration of optimal dosing schedules, and (d) clarification of the mechanism of action and expected biological activities of the product.

2. Biodistribution

Biodistribution data for the intended gene therapy product or human cell processed product consisting of genetically modified cells must be obtained in appropriate animal species as fundamental data to evaluate the safety and efficacy of the product. Organs that should be focused on for evaluation of the safety and unintended integration risks in humans may be identified by analyzing the biodistribution data and by determining not only the distribution to the target tissues

but to off-target tissues and germ cells. Information on appropriate evaluation timing in humans will be obtained by determining the vector distribution and characteristics about persistency and elimination. Biodistribution data may also be useful for investigation of the toxicological significance of tissue-specific abnormal findings in toxicity studies.

If the developer has no plan to conduct biodistribution studies before initiating clinical trials of a new gene therapy product or human cell processed product consisting of genetically modified cells, the reason for not doing biodistribution studies must be justified.

To evaluate the biodistribution, tissue or blood samples should be collected and stocked at regular time intervals after administration of the product, and copy number of the vector be assayed using an appropriate technique such as quantitative polymerase chain reaction (PCR). Furthermore, information on the decline of the vector with time will be obtained by analyzing temporal changes in copy number of the vector. If the expression construct is found in certain tissues or fluids, expression of the transgene by the expression construct should be analyzed, as necessary.

3. Nonclinical Safety Studies

The purpose of nonclinical safety studies is to determine possible toxicological effects in humans, and they should be conducted in a timely manner, not only before initiation of clinical trials but when advancing to higher stages of clinical development, to ensure the safety in humans participating clinical trials. The objectives of nonclinical safety studies include (a) determination of the initial dose and determination of dose escalation and the maximal dose in clinical trials, (b) identification of toxicological target organs, (c) identification of markers that help grasp adverse reactions in clinical trials, and (d) determination of discontinuation criteria for clinical trials. Based on the results from nonclinical safety studies, it should be determined what adverse reactions are suspected to occur in humans and how large the safety margin will be.

The data from nonclinical safety studies submitted at marketing authorization application for gene therapy products must be collected and prepared according to the standards defined in the "Ministerial Ordinance on Good Laboratory Practice (GLP) for Nonclinical Safety Studies of Regenerative Medical Products" (MHLW Ordinance No. 88 of 2014; hereinafter, "GLP"), as in the case of other regenerative medical products. In some cases, however, conduct of GLP-compliant studies may be practically impossible because of their study procedures or study conditions; if this is the case, the applicant should clarify what part of the study is GLP-noncompliant and evaluate what effects the GLP-noncompliant part has on the overall evaluation of the nonclinical safety.

(1) General Toxicity

1) Selection of Animal Species

(i) General principles

In conducting nonclinical safety studies, selection of suitable animal species is important; animal species that are considered to show the same pharmacological activities that the product is expected to show in humans should be selected so that data that can be extrapolated to humans are obtained. Considerations for the selection of animal species include (a) whether the transgene loaded on the expression vector expresses in the target cells, (b) whether the nucleic acid or protein derived from the transgene exerts the pharmacological action expected in humans, (c) if using a viral

vector, whether the corresponding wild-type virus infects the animal and targets specific tissues/cells in the animal in a manner similar to that in humans, and (d) whether the same administration method proposed for the clinical use can be applied. The selection of animal species in nonclinical safety studies should be justified on the basis of the results of these considerations. In some cases, nonclinical safety of gene therapy products can be evaluated also in studies conducted to predict efficacy using an animal model of the target disease in which safety endpoints are included, other than toxicity studies conducted in normal animals. If the gene therapy product is designed to selectively target certain tissues/cells, the developer should confirm the tissue specificity and duration of gene expression and biological activity of the expression product in appropriate animal models, in addition to conducting biodistribution studies.

(ii) Number of animal species

While nonclinical safety of drugs is usually evaluated using two animal species, that of gene therapy products may adequately be evaluated using only one appropriate animal species because of the characteristics of the product in some cases, e.g., in the case that biological characteristics of the vector or transgene are fully understood or only one appropriate animal species is available for the nonclinical safety evaluation. If only one animal species is used for the evaluation of nonclinical safety, it should be justified. If nonclinical safety studies using the proposed clinical application route cannot be conducted in small animal species such as a rodent using the proposed clinical application route, systemic effects of the product may be evaluated in small animals using an alternative route, although effects on the clinical application site may need to be evaluated in a large animal species such as a non-rodent.

(iii) Use of alternative methods

If no appropriate animal species can be selected from the conventional nonclinical species, alternative methods that use genetically modified animals or animal-derived transgenes may be considered. Note, however, that although nonclinical safety studies that use those methods may be useful to detect adverse outcomes or to identify biomarkers for clinical trials, they are not always suitable for a quantitative risk assessment.

2) Study Design

(i) General principles

Toxicity studies should be designed considering the target disease and other factors including (a) information on the *in vivo* and *in vitro* biological activity of similar gene therapy products or similar products, (b) biological characteristics and safety information of the expression protein of the transgene, (c) information on the proposed method of administration in clinical trials and information on the usage of the instruments/devices used for administration, (d) biological reactions in the animal species used for the nonclinical safety studies of the product, and (e) the proposed mechanism of action of the product.

General toxicity studies of gene therapy products must be designed considering the characteristics of the product, and using the Appendix, Guidelines for Toxicity Studies of Drugs, in "Guidelines for Toxicity Studies Required for Manufacturing (Import) Approval Application of Pharmaceuticals" (PAB/ELD1 Notification No. 24, dated September 11, 1989) for reference.

(ii) Dose selection

Doses for gene therapy products should be selected taking into account not only the dose-response relationship for the pharmacological activity but factors such as the gene transfer efficiency, expression efficiency, and species difference in pharmacological activity, and multiple treatment groups should be included in the study along with an appropriate control group to confirm the response is dose dependent. The highest dose in a toxicity study should be selected taking into account appropriate limit doses such as the clinical dose, dose at which the intended pharmacological activity is maximized, maximum tolerated dose, and maximum feasible dose, and the rationale for the selected highest dose must be presented.

(iii) Study duration

The duration of toxicity studies should be determined considering factors such as the expression duration of the vector, immunogenicity of the product, and proposed clinical indications. For toxicity studies of gene therapy products, the longest duration may be determined to be a period of up to 6 months, in reference to the notification, "Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals" (PFSB/ELD Notification No. 0323-1, dated March 23, 2012), because the pharmacological action is exerted by the nucleic acid or protein derived from the transgene.

(iv) Observations and tests

As in repeat-dose toxicity studies of drugs, safety endpoints in toxicity studies of gene therapy products should include animal death, clinical observation, body weight, food consumption, hematology, chemistry, urinalysis, ophthalmology, organ weight, necropsy, and microscopic examination. The microscopic examination should cover not only the tissues/organs in which distribution is confirmed from biodistribution studies but also at least major organs including the brain, lung, heart, liver, kidney, and spleen, the testes/ovaries, and the administration site. Furthermore, on the basis of the characteristics of the product, any effects on the major physiological systems (e.g., cardiovascular, respiratory, renal, and central nervous systems) should be revealed, and other endpoints related to the pharmacological activity (e.g., immunological function tests, behavioral tests, neurological examination, and markers for cell proliferative activity) may also be evaluated.

(v) Reversibility

If serious adverse effects that raise concerns for clinical safety are found in toxicity studies, reversibility of the toxicity should be evaluated in reversibility studies or by scientific assessments (e.g., assessment of the extent and severity of the lesion, the regenerative capacity of the body system affected, and findings of the existing drugs that show the same effect).

(2) Gene Integration Risk

1) General Principles

The potential that the vector may be integrated into chromosomes should be investigated. If the product is, however, intended to be used in patients with a life-threatening disease such as a cancer whose life expectancy may not be long, investigation of this potential will not necessarily be required. As indicated by this, investigation of the integration potential should be done taking into account the target disease. For example, if the vector is a retroviral or lentiviral vector that is inserted into a chromosome, the copy number of the vector per cell and the potential of integration at a particular site should be investigated. Furthermore, it is also important to evaluate the carcinogenic potential attributable to the activation of a specific gene caused by insertional mutagenesis. If a vector that has no capability of integration into chromosomes, such as an adenoviral vector or a plasmid DNA, is used, a high sensitivity test that can detect insertions occurring at a low frequency should be considered. If the target cells are hematopoietic stem cells or other cells that are relatively undifferentiated compared with differentiated cells such as T cells or muscle cells, it should be noted that the risk of insertional mutagenesis may be high.

2) Risk of Unintended Gene Integration into Germ Cells

If the vector is directly administered to the body, and biodistribution studies demonstrate distribution of the vector to germ cells, the risk of integration into chromosomes of germ cells should be assessed using a scientifically appropriate approach, although if the product is intended to be used in patients with a life-threatening disease such as a cancer whose life expectancy may not be long, assessing the risk of integration into germ cell chromosomes may not necessarily be required. It is recommended that the risk assessment will be done in reference to the notice, “ICH Considerations: General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors” (Administrative Notice issued by the Office of Counsellor for Medical Devices/Regenerative Medical Products, ELD, PFSB, MHLW, on June 23, 2015).

(3) Tumorigenic Potential and Carcinogenic Risk

The risk of tumorigenesis and carcinogenesis associated with gene therapy products needs to be assessed by investigating the potential carcinogenicity of the vector (e.g., due to the promoter activity of the expression product, or potential insertional mutagenesis in chromosome genes) or the tumorigenic potential associated with the preparation of genetically modified cells for an *ex vivo* dosing procedure (e.g., due to the cytokine used) or with possible mutations resulting from vector insertion into chromosomes. The assessment results should be adequately informed to patients and be reflected to the determination of an appropriate risk management plan.

1) Carcinogenic Risk

Although standard carcinogenicity studies used for assessing the risk of chemical drugs may not be suitable for gene therapy products, carcinogenic risk should still be assessed for those products, as well. Appropriate procedures for a product belonging the category of gene therapy products to assess the carcinogenic risk should be considered taking into

account factors such as the duration of clinical use, patient population, characteristics of the target cells/tissues for gene transfer, characteristics of the vector and its components (e.g., characteristics of the expression product derived from the transgene and gene transfer), and carrier used, in reference to the notification, "Revision of the Guideline for Carcinogenicity Studies for Pharmaceuticals" (PFSB/ELD Notification No. 1127001, dated November 27, 2008). The carcinogenic risk should be assessed using a weight of evidence approach, including a review of relevant data including published data, characteristics of the wild-type virus associated with the viral vector, information on analogous products, biological characteristics and mechanism of action of the expression product, *in vitro* study data, general toxicity study data, and clinical trial data.

2) Tumorigenic Potential

Any human cell processed product consisting of genetically modified cells should be first evaluated for the carcinogenic potential of the vector used for the preparation of the genetically modified cells, and then evaluated for the potential to cause proliferative changes in cells and formation of tumor tissues according to the relevant guidance for quality and safety assurance to assess the carcinogenic risk associated with the product.

(4) Reproductive and Developmental Toxicity

If adverse effects on reproductive organs are suspected based on microscopic findings obtained from general toxicity studies, a study(s) of fertility and early embryonic development to implantation should be conducted. Whether it is necessary to conduct a study for effects on embryo-fetal development or on prenatal and postnatal development, including maternal function should be determined taking into account the patient population and relevant biological characteristics of the product (e.g., general toxicity, pharmacological action, exposure level, biodistribution, placental transfer, and tissue/cell tropism).

(5) Immunotoxicity

The potential that the vector and the expression product from the vector may cause adverse effects on the immune system should be determined. In animal studies, specific immune responses may be induced, and study results should be interpreted taking into account the effects of those responses.

(6) Potential Emergence of Replication-Competent Viruses

If a replication-incompetent viral vector is used, it should be assessed whether replication-competent viruses appear during the vector production in the packaging cell. Furthermore, for a human cell processed product consisting of genetically modified cells, it should be assessed whether replication-competent viral vectors appear from the genetically modified cells. The assay method used for the detection of replication-competent viruses must have been validated regarding appropriate aspects including the detection sensitivity for them. In addition, in the case of gene therapy products, potential emergence of replication-competent viruses from the gene therapy product administered should be investigated using an animal model, if possible.

Potential emergence of replication-competent viruses due to mutation events or genetic recombination with an endogenous virus fragment should be assessed.

Chapter 5. CONSIDERATIONS IN CLINICAL TRIALS

1. Justification of the Conduct of Clinical Trial

   In addition to presenting currently available findings regarding the target disease including those on its etiology, epidemiology, pathology, clinical course, therapies, and prognosis, the sponsor or initiator of the proposed clinical trial should explain the rationale for the proposed gene therapy on the basis of the mechanism in which the product may exert the expected treatment effects, the points for which the proposed therapy may be superior to the conventional therapies, and the predicted risks.

2. Clinical Trial Protocol

   A specific protocol for the proposed clinical trial must be presented. The selection of the proposed dose, number of doses, and administration site should be justified. If a special pretreatment is required before administering the product, the possible effects on subjects and safety profile should be elucidated and in addition, it should be clarified what actions will be taken if adverse events occur because of the pretreatment.

   In clinical trials, unexpected immune responses may occur. With this in mind, an appropriate study plan must be developed to follow the patients' condition. If a viral vector is administered multiple times, it should be noted that antibodies to the viral vector may be produced. It should be clarified whether the expression of the transgene needs to be controlled, and if not, it should be justified. If a vector that expresses a protein corresponding to a defective gene is administered, it should be noted that immune responses to the expressed protein might be induced. In particular, considering the possibility that production of antibodies to the vector or expressed protein or unexpected immune responses occurs or can occur, a study plan that allows for appropriate evaluation must be developed to follow the patients' condition.

3. Long Term Follow-Up

   The study schedule for the following observation must be presented: The biodistribution of the vector or genetically modified cells administered to subjects, viability of the genetically modified cells, mode of expression of the transgene, emergence of replication-competent viruses, and infusion (of the product)-related clinical symptoms. The duration of subject follow-up should be appropriately determined taking into account relevant factors including the type of the vector and characteristics of the target disease. If the vector is integrated into a chromosome, the observation to evaluate the sustainability of the transgene and, if feasible, the clonality of the genetically modified cells should be done at least once a year. It should be taken into account that the observation duration might have to be prolonged depending on the results of the follow-up. Preservation of the final product containing the vectors or genetically modified cells during the period until the end of follow-up should be considered to allow for investigation of the cause of adverse events.

Chapter 6. EVALUATION OF THE RISK OF TRANSMISSION OF GENE THERAPY PRODUCTS TO THIRD PARTIES

Effects of the vector administered to patients on humans, including the risk of the vector to transmit to third parties other than the treated patients, should be investigated in reference to the notice, “ICH Considerations: General Principles to Address Viruses and Vectors Shedding” (Administrative Notice issued by the Office of Counsellor for Medical Devices/Regenerative Medical Products, ELD, PFSB, MHLW, on June 23, 2015). In the case of genetically modified human cell therapy products, the possibility that the viral vector administered remains in the final product consisting the treated cells should be investigated, and if the existence of the remaining vector cannot be ruled out, the risk of transmission to third parties should be assessed on the basis of the characteristics of the vector used for the gene transfer.