

I. Objective

The present guidelines outline basic technical elements for ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of human allogenic somatic stem cells (excluding autologous somatic stem cells). These products are hereafter referred to as allogenic human somatic stem cell-based products or merely as the “desired cell products.”

II. Definitions

The definitions of the technical terms used in this guideline are as follows:

1. “Human somatic stem cells”: Cells that are collected from humans or cells that are obtained from such cells through cell division and that possess multipotency and maintain the ability to self-renew or a similar ability. In other words, tissue stem cells (e.g., hematopoietic stem cells, neural stem cells, mesenchymal stem cells [including bone marrow stromal stem cells and adipose tissue-derived stem cells], corneal stem cells, skin stem cells, hair follicle stem cells, intestinal stem cells, hepatic stem cells, and skeletal muscle stem cells) or cell groups that have abundant populations of these cells (e.g., whole bone marrow cells that include hematopoietic stem cells), including vascular precursor cells, umbilical cord blood, and bone marrow stromal cells. “Human somatic stem cells” also include cells obtained by culturing these cells in vitro. Human embryonic stem (ES) cells, human induced pluripotent stem (iPS) cells, human induced pluripotent stem-like

(iPS-like) cells, human embryonic germ (EG) cells, human multipotent germline stem (mGS) cells, human parthenogenesis stem cells, human nuclear transplant stem cells, human cancer cells, human cancer stem cells, and cells derived from these cells are not included. (Note: The definitions for human ES cells, human iPS cells, and human iPS-like cells are provided in other guidelines, specifically in “Guidelines on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Human ES Cells” and “Guidelines on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Allogenic/Autologous Human iPS(-Like) Cells,” respectively.)

2. “Processing of cells and tissues”: Any processing of a cell or tissue, such as propagation and/or differentiation, production of a cell line, activation of a cell by pharmaceutical or chemical treatment, alteration of a biological characteristic, combination with a noncellular component, and manipulation by genetic engineering, with the aim of preparing desired cell products to treat a patient or repair or regenerate tissue.

Isolation of tissue, disintegration of tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by gamma irradiation or other methods, freezing, thawing, and other such procedures regarded as minimal manipulations are not considered to be processing.

3. “Manufacture”: Actions undertaken before the final product (an allogenic human somatic stem cell-based product) is released to market. This includes, in addition to the processing of cells and tissues, minimal manipulations such as separation of tissue, disintegration of tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by gamma irradiation or other methods, freezing, thawing, and other procedures that do not change the original properties of the cells or tissues.

4. “Phenotype”: A morphological or physiological characteristic that is expressed by a certain gene under constant environmental conditions.

5. “HLA typing”: Specifying the type of HLA (human leukocyte antigen), a human primary histocompatibility antigen.

6. “Donor”: Persons who donate their own cells or tissue, which serve the raw material for an allogenic human somatic stem cell-based product.

7. “Transgenic construct”: A construct that contains a vector for introducing a target gene (a specific gene encoding a desired protein or RNA) into a target cell, the target gene itself, and the coding sequences of the elements essential for the expression of the target gene.

Chapter II Manufacturing Methods

Describe all important and relevant information concerning the manufacturing method, taking into

account the items listed below. This information will help ensure the quality, safety, and efficacy of the final products, and it is important for guaranteeing consistency in quality from a manufacturing perspective. It should be noted that quality, safety, and consistency are assured by mutual complementary measures throughout the manufacturing process. It is most important that the measures are rational and that they serve the intended purpose. It may be acceptable to omit a portion of the items listed below, if the quality, safety, and constancy of the final products can be established by suitably chosen quality tests, control of the final or intermediate products, or control of the manufacturing process.

I. Raw Materials and Materials Used in Manufacturing

1. Human cells and tissues used as raw materials

(1) Source and origin, justification of their selection

Explain the source and origin of the cells and/or tissues used as raw materials and justify the reasons for selecting these cells and/or tissues.

(2) Characteristics and eligibility of cells and/or tissues used as raw materials

(i) Features of biological structure and function, selection criteria

Explain and justify the reasons for selecting the cells and/or tissues used as raw materials with reference to characteristics of their biological structure and function, such as morphological characteristics, growth characteristics, biochemical indicators,

immunological indicators, specific substances produced, HLA typing, and other suitably chosen and appropriate genotype or phenotype indicators (or markers). In particular, demonstrate that the somatic stem cells used as a raw material possess clinically useful stemness. Stemness in this case does not necessarily indicate the potential for multilineage differentiation, but refers to the ability to differentiate into cells that have expected functions in vivo. In addition, although demonstrating differentiation in vitro is desirable, it may suffice to show differentiation in vivo if a rational explanation is provided. For example, when using myocardial stem cells, which are somatic stem cells, as a raw material, it is acceptable to show that myocardial stem cells can differentiate into cardiomyocytes. This should lead to the identification of the main cell characteristics that will be employed when preparing the somatic cells as raw materials.

It is recognized that quantitative technological limits to sample analysis will affect the extent to which such studies can be performed.

(ii) Donor selection criteria and eligibility

Indicate that the donor was selected in an appropriate and ethical manner and that the proper procedure was followed. Establish selection criteria and eligibility criteria that take into consideration age, sex, ethnic characteristics, genetic characteristics, disease history, health conditions, test parameters related to any type of infection that may occur via cell and/or tissue samples, immunological compatibility, etc. and justify their

appropriateness. If donor genome or gene analysis is undertaken, they shall be performed in accordance with “Ethical Guidelines for Human Genome/Gene Analysis Research,” issued jointly on December 28, 2004 and revised on December 1, 2008 by the Japanese Ministry of Education, Culture, Sports, Science, and Technology, Ministry of Health, Labor, and Welfare, and Ministry of Economy, Trade, and Industry.

Infection with hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), adult human T-lymphotropic virus (HTLV), and parvovirus B19 shall be ruled out by physician-donor interviews and clinical laboratory tests, such as serological tests and nucleic acid amplification tests. Infection with cytomegalovirus, Epstein-Barr (EB) virus, and West Nile virus shall also be ruled out, if necessary, by performing the appropriate clinical laboratory tests.

In addition, further investigate and determine the eligibility of donors by examining the past medical history (mentioned below) of the donor through physician-donor interviews etc., and establish if they ever received a blood transfusion or underwent a transplantation procedure.

- Bacterial infections, such as syphilis (*Treponema pallidum*), chlamydia, gonorrhea, and tubercle bacillus
- Sepsis or suspected sepsis
- Malignant neoplasm
- Serious metabolic or endocrine disease
- Collagen and blood diseases

- Hepatic diseases
- Confirmed or suspected transmissible spongiform encephalopathy (TSE) or other cognitive disorders
- Specific genetic disease or a family history of a specific genetic disease

(3) Records related to the donor

Retain complete records related to the donor in order that any information necessary to ensure the safety of cells and tissues used as raw materials can be verified. Concrete measures shall be described. For patients and donors of test samples, it is sufficient to prepare and retain only specific information that relates to the intended use of the cells.

(4) Collection, storage, and transport of cells and tissues

(i) Eligibility of personnel and medical institutions collecting samples

Describe the technical requirements for personnel and medical institutions that collect the cells and tissues.

(ii) Suitability of the sampling site and sampling method

Describe the rationale for selecting the cell and tissue sampling sites and the sampling method. State how the selected sites selected are scientifically and ethically appropriate. For cell and tissue sampling methods, indicate the suitability of the equipment and drugs used and the measures adopted to prevent microbial contamination, erroneous sampling (mix-ups), and cross contamination.

(iii) Informed consent from donors

Describe the details of the informed consent, including the clinical application, provided by the donor of the cells or tissue.

(iv) Protection of donor privacy

Indicate the measures adopted to ensure the protection of the donor's privacy.

(v) Tests to ensure donor safety

If tests such as those to confirm the state of the sampling site need to be performed in order to ensure the safety of the donor at the time of cell or tissue sampling, describe the details of the tests, as well as any interventions taken when test results indicated a problem existed.

(vi) Storage method and measures to prevent erroneous sampling (mix-ups)

If the cells and/or tissues need to be stored for a definite period of time, set the storage conditions and storage period and justify their appropriateness. Describe in detail the measures and procedures to be taken to prevent erroneous sampling (mix-ups).

(vii) Transportation methods

If cells and/or tissues need to be transported, set the containers used for transport and the transportation procedure (including temperature control, etc.) and justify their appropriateness.

(viii) Preparation of records and record-keeping procedures

Prepare written records for (i) through (vii) above and describe the record-keeping procedures in detail.

2. Raw materials other than the target

cells and tissues, materials used in manufacturing

Describe raw materials other than target cells and tissues, as well as other materials used in the manufacturing process; indicate their appropriateness for the intended use; and, if necessary, establish their specifications (set of acceptance criteria and analytical procedures). Proper quality control for these materials should be implemented.

When so called “Biological Products: or “Specific Biological Products” (refer to Article 2.9 and 2.10 of the Pharmaceutical Affairs Law) are used as raw materials, use the minimum amount required and strictly obey the relevant laws and notifications, such as “Standards for Biological Raw Materials” (Notification No. 210, Japanese Ministry of Health, Labor, and Welfare, 2003; note that a new version will soon be issued). It is particularly important to sufficiently evaluate information related to the inactivation and elimination of viruses and to indicate measures for ensuring retrospective and other studies.

(1) When culturing cells

(i) Indicate the appropriateness of all media components, additives (serum, growth factors, antibiotics, etc.), and reagents, etc. used in the treatment of cells and set specifications if necessary. Give consideration to the route of clinical application, etc. of the final product when setting specifications concerning the appropriateness of each component.

(ii) Take into consideration the following points with respect to

media components:

(a) The ingredients and water used in media should be of high quality and high biological purity, and their quality should be controlled at standards equivalent to those used with pharmaceuticals and pharmaceutical raw materials.

(b) Provide information on all ingredients used in the media, as well as the rationale for their selection, and, if necessary, the quality control and other procedures. However, widely known and commercially available media products such as DMEM, MCDB, HAM, and RPMI are regarded as a single raw material set.

(c) Conduct sterility tests and performance tests on media that contain all components in order to determine whether they are suitable as target media. Set specifications for any other relevant parameters expected to be controlled in the process and perform proper quality control.

(iii) Heterologous serum or components derived from heterologous or homologous serum shall not be used unless they are essential for processes such as cell activation or cell growth. In particular, for products that may be used repeatedly, investigate, to the extent possible, ways to avoid using these serum components. If the use of serum or other such material is unavoidable, consider the following points and investigate ways to prevent the contamination of serum and other products and the spread of bacteria, fungi, viruses, and abnormal prions,

as well as treatment methods for their elimination, to the extent possible, from the final product.

- (a) Clarify the origin of the serum or other component.
- (b) Make strenuous efforts to minimize the risk of prion infection, such as by strictly avoiding the use of serum from areas or regions with known outbreaks of bovine spongiform encephalopathy (BSE).
- (c) Only use these sera after confirming that they are not contaminated with viruses or other pathogens by conducting appropriate tests to prove the absence of specific viruses and mycoplasma that originate in animal species.
- (d) Perform appropriate procedures to inactivate and eliminate bacteria, fungi, and viruses to an extent that does not impact the activation and growth of the cells. For example, to avoid the risks associated with latent viral contamination, perform combinations of heat treatment, filtration, irradiation, and/or UV treatment, if needed.
- (e) Preserve and store a portion of the serum used in order to monitor for viral infections in cultured cells, monitor for outbreaks of viral diseases in the patient, and measure antigen production in response to a component of the heterologous serum used.

(iv) When using feeder cells, conduct a quality evaluation while referring to “Derivation and Characterization of Cell Substrates Used for the Production of

Biotechnological/Biological Products” (Pharmaceutical Notification No. 873, Ministry of Health, Labor, and Welfare, issued July 14, 2000), “Guidelines on Public Health Infection Issues Accompanying Xenotransplantations” (Notification No. 0709001, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labor, and Welfare, issued July 9, 2002), and “Guidelines on Epithelial Regenerative Therapy Using 3T3J2 Strain or 3T3NIH Strain Cells as Feeder Cells” based on “Guidelines on Public Health Infection Issues Accompanying Xenotransplantations” (Notification No. 0702001, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labor, and Welfare, issued July 2, 2004) in order to prevent the contamination feeder cells and the spread of bacteria, fungi, viruses, and abnormal prions. Indicate methods for the inactivation of cell division potential and conditions such as cell density when using the feeder cells. However, if, for example, the feeder cells or equivalent cells are being used in the manufacture of a cell or tissue product that has already been used clinically and whose characteristics and microbiological safety have already been assessed and confirmed, it may be possible to omit the virus tests or parts of other tests by demonstrating the appropriateness of using these cells.

(v) Avoid the use of antibiotics as much as possible. However, if the use of antibiotics in the initial stages of processing is deemed indispensable, attempt to decrease their use in

subsequent steps as much as possible, and clearly indicate the appropriateness of their use from perspectives such as the scientific rationale, estimated residual amounts in the final product, and the effects on the patient. If it has been verified that an antibiotic can be adequately eliminated, its use need not be restricted. On the other hand, if a patient has a past history of allergy to the antibiotic used, in principle, this therapeutic method should not be used. If there is no way to avoid the use of antibiotics, administer them very carefully and obtain informed consent from the patient.

(vi) If growth factors are used, show the appropriate quality control methods using relevant parameters, such as purity and potency, for which established acceptance criteria and assay methods are employed, in order to guarantee the reproducibility of the cell culture characteristics.

(vii) For media components and other components that are used in processing and that may contaminate the final product, choose components that do not have any harmful biological effects.

(2) When combining with noncellular components

(i) Quality and safety of noncellular raw materials

If the final product consists of cells and noncellular components such as matrix, medical materials, scaffolds, support membranes, fibers, and beads, clearly describe in detail the quality and safety of the noncellular components.

Provide any relevant information concerning the noncellular raw materials, taking into consideration their type and characteristics, form and function in the final product, and an evaluation of the quality, safety, and efficacy from the perspective of the presumed clinical indication. If using materials that are absorbed by the body, perform the necessary tests on the degradation products to assess safety concerns.

With respect to the tests that should be carried out, refer to “Basic Views on Biological Tests Necessary for Regulatory Approval for Manufactured or Imported Medical Devices” (Notification No. 0213001, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labor, and Welfare, issued February 13, 2003), describe the test results, and justify the use of such raw materials. Use of information obtained from the literature is encouraged.

(ii) Interactions with target cells

Demonstrate the validity of the test methods used and justify the results obtained for the following three items with respect to the interactions between noncellular components and cells in the final product as well as in any intermediate products.

(a) The noncellular components do not have any deleterious effects on the function, growth capability, activity, or stability of the cells in the final product required for the presumed clinical indication or the cells in any intermediate products.

(b) Evaluate to the extent possible

any potential interactions between the cells and noncellular components, taking into consideration, for example, the mutation, transformation, and/or dedifferentiation of cells in the final product or cells in intermediate products.

- (c) Show there is no loss of the expected properties of the noncellular components in the presumed clinical indication as a result of any interactions between the noncellular components and the cells in the final and intermediate products.

(iii) When using noncellular components to isolate the desired cell products from the application site

Upon using noncellular components with the objective of segregating the cells from the application site, confirm their usefulness and safety by referring to (a) through (e) below.

- (a) When immunological segregation is the objective, describe its level.
- (b) Membrane permeability kinetics and the pharmacological effects of target physiologically active substances derived from the cells in the final product.
- (c) Diffusion of nutritional components and excretory products.
- (d) Effects of noncellular components on the area near the application site.
- (e) When a pharmacological effect of a target physiologically active substance derived from a desired cell product is anticipated and the objective is segregation of the application site and the desired cell product or

undifferentiated cells, confirm that the cells do not leak out, which might result from the degradation, etc. of noncellular components.

(3) When cells undergo genetic modifications

When genes are introduced into cells, provide the following details:

(i) For the target gene (specific gene encoding a desired protein or RNA), information related to its structure and origin, the method by which it was obtained, cloning methods, and methods of cell bank preparation, control, and renewal, etc.

(ii) Nature of the transgene.

(iii) Structure, biological activity, and properties of the desired protein or RNA derived from the target gene.

(iv) All raw materials, properties, and procedures (transgenic method, origin and properties of the vector, and method of obtaining the vector used for gene introduction) needed to produce the transgenic construct.

(v) Structure and characteristics of the transgenic construct.

(vi) Control and preparation methods for cell and virus banks needed to prepare vectors and transgenic constructs.

For manufacturing methods for transgenic cells, refer to Chapter 2 and other sections of "Guidelines for Ensuring the Quality and Safety of Gene Therapy Pharmaceuticals," which is an appendix in "Concerning Guidelines for Ensuring the Quality and Safety of Gene Therapy

Pharmaceuticals” (hereafter referred to as “Gene Therapy Pharmaceutical Guidelines”), published as Notification No. 1062 by the Ministry of Health and Welfare on November 15, 1995. In addition, state the appropriateness of the establishment in accordance with the appendix of the same notification.

On the basis of the law (Law No. 97, 2003) implemented to ensure biodiversity by regulating the use, etc. of genetic recombination organisms, etc., a separate application procedure for evaluation will be required when living organisms, including certain cells, “viruses,” and “viroids,” are genetically modified. The following cells are not regarded as living organisms: “human cells, etc.” or “cells that have the ability to differentiate, or differentiated cells that are not viable when alone under natural conditions.”

Regardless of the guidelines mentioned above, if a gene introduced into cells is used as a reagent in the manufacturing process and does not either chemically or functionally contribute to the final product, it is acceptable to describe, on the basis of current knowledge, how the quality and safety of the gene conform to the intended use.

II. Manufacturing Process

When manufacturing pharmaceuticals and medical devices derived from the processing of allogenic human somatic stem cell (i.e., allogenic human somatic stem cell-based products), describe in detail the manufacturing method and verify, to the extent possible, the

appropriateness of the method, using the items listed below, in order to maintain consistency in the quality of the product.

1. Lot composition and lot control

Indicate whether a lot comprises final products and intermediate products. If a lot comprises both final and intermediate products, establish standardized procedures for the composition and control of the lot.

2. Manufacturing method

Provide an outline of the manufacturing method, from the receipt of the cells and tissues through to the isolation of somatic stem cells and the establishment of the final product. Describe the technical details of the process and necessary process control and product quality control.

(1) Tests upon receipt

Establish a battery of tests as well as acceptance criteria with which to assess the appropriateness of the cells and tissues that will serve as the raw materials, taking into account the nature of the cells and their intended use. These may include, for example, visual tests, microscopic examination, recovery factor of target cells, cell viability assays, characterization of cells and tissues, microbiological tests, etc. At the stage of initiating clinical trials, provide the actual measured values obtained up until that point with test samples, and propose a provisional set of acceptance criteria based on these values.

(2) Inactivation and elimination of bacteria, fungi, viruses, and other microorganisms

In cells and tissues that serve as raw

materials, inactivate bacteria, fungi, viruses, and other microorganisms, if needed and whenever possible, to the extent possible without affecting the characteristics (cell viability, phenotype, genetic traits, specific functions, etc.) and quality of the cells and tissues serving as raw materials. State the appropriateness of the measures, procedures, and evaluation methods used, if any.

(3) Tissue disintegration, cell separation, isolation of specific cells, etc.

Describe the methods for the disintegration of tissue, separation of cells, and isolation of somatic cells, as well as methods for washing, etc. cells and tissues, that are performed in the early stages of manufacturing somatic stem cell-based products from collected cells and tissues. Upon isolating somatic stem cells, establish methods of cell identification.

(4) Preparation of cells that are a principal component and active ingredient in the final product

Describe the methods used to collect human cells and tissues, to isolate somatic stem cells, and to obtain the cells that serve as the active ingredient in the final product. The methods to be described include the induction of differentiation, the isolation and culture of the desired cells, and the media, culture conditions, culture period, yield, etc. at each step. Describe the appropriateness of each method.

(5) Establishment and use of cell lines
Establish cell lines after having determined to the extent possible the genetic background of the donor.

Describe the method of establishment and its appropriateness to the extent possible.

To ensure that the quality of the established cell line remains stable and consistent, identify the critical quality attributes of the cells (e.g., cell purity, morphological features, phenotypic markers, karyotype, cell growth properties, pluripotency, etc.) and set acceptance criteria for them. In addition, demonstrate the potent number of passages through which the cells remain stable.

Discuss the possibility of tumorigenicity and malignant transformation, using an appropriate animal model.

(6) Establishment of cell banks

When a cell bank is established at any stage during the manufacture of allogenic human somatic stem cell-based products, describe the rationale for preparing the cell banks; the methods used to prepare the cell banks; the characteristics of the cell banks, and the storage, maintenance, control, and renewal methods, as well as any other processes and tests performed. Justify the appropriateness of each. Refer to “Derivation and Characterisation of Cell Substrates Used for the Production of Biotechnological/Biological Products” (Pharmaceutical Notification No. 873, Ministry of Health, Labor, and Welfare, issued July 14, 2000) and other documents.

(7) Measures to prevent erroneous sampling (mix-ups) and cross contamination during the manufacturing process

It is extremely important to prevent erroneous sampling and cross contamination during the manufacturing process when manufacturing allogenic human somatic stem cell-based products. Therefore, describe preventative measures in the process.

3. Characterization of cells that are a principal component and active ingredient in a final product

For cells that are a principal component of the final product, analyze their attributes, such as cell purity (to control contamination by non-target cells), cell viability, morphological characteristics, cell growth characteristics, biochemical markers, immunological markers, distinctive substances produced by the cells, karyotype, and other appropriate genotypic and phenotypic markers. In addition, characterize their biological functions, if necessary. Furthermore, to evaluate the appropriateness of the culture period and the stability of the cells, use appropriate cell characteristic markers to demonstrate that there have been no unintended changes in cells cultured longer than the proposed culture period. It may be acceptable to perform these studies preliminary, using test samples obtained from donors in place of the real products that will be prepared for clinical trial. On the basis of these test results, identify the critical cell characteristics that should be used when applying the product to a patient. Although comprehensive cell characterization is always desirable, it may not always be possible to characterize the cells fully because there are quantitative and limits to sample analysis. Thus, it

is acceptable to perform the studies to the extent possible. When cell processing, such as growth within the body, is anticipated after clinical application, clearly demonstrate the functions expected by describing the specified criteria with respect to the passage number or number of cell divisions.

4. Form and packaging of the final products

The form and packaging of the final product shall ensure the quality of the final product.

5. Storage and transport of the final product

If intermediate or final products need to be stored and transported, the storage procedure and duration, the containers used for transport, and the transportation procedure (including temperature control, etc.) shall be stated and their appropriateness clearly indicated (refer to Chapter III).

6. Consistency of the manufacturing procedure

When manufacturing allogenic human somatic stem cell-based products, assess during the manufacturing process and for each individual product (or each lot, if any) whether differ significantly with respect to the number of cells, cell viability, and cell characteristics (such as relevant markers of phenotype and genotype, functional characteristics, and the percentage content of the desired cells) and from the point of view of the clinical application methods and the intended clinical use of the product. It is acceptable to use test samples

obtained from donors who are not patients in place of the real product that will be prepared for clinical trial. Evaluation of intermediate products may provide insight into the appropriateness of cells and tissues used as raw materials and the validity of the manufacturing process up until the intermediate product stage and provide an appropriate guidepost en route to the final product. Therefore, it may be reasonable to adopt such an approach, where necessary and appropriate.

When the manufacturing process involves long cryopreservation periods or cell cultivation periods, perform sterilization tests at constant intervals to confirm sterility has been maintained.

7. Changes in the manufacturing process

If the manufacturing process is altered at some point during development and test results obtained using products manufactured before the change in manufacturing method are to be used in the application for clinical trial or regulatory approval, demonstrate that the products manufactured before and after the change to the manufacturing process are comparable.

III. Quality Control of the Final Product

1. Introduction

The overall quality control strategy for products manufactured using human somatic stem cells include specifications (a set of acceptance criteria and analytical procedures) for the final products, quality control of

raw materials for each different application to each individual patient, verification of the appropriateness of the manufacturing process, maintenance of consistency, and proper quality control of any intermediate products.

Specifications will differ for each final product depending upon the type and properties of the desired cells and tissues, manufacturing methods, intended clinical use, method of clinical application, stability, and test methods. These differences shall be taken into consideration when setting the acceptance criteria and test procedures. In addition, specifications shall be set and justified from the perspective of achieving the purpose of quality control as a whole, by taking into consideration the mutually complementary relationships between 1) the verification of the suitability of the manufacturing process, 2) the method of maintaining consistency, and 3) quality control of the raw materials and intermediate products. The purpose of the assessment at the initiation of clinical trials is to confirm that the product is deemed to pose no significant quality/safety problems for using investigational clinical trials. Therefore, it may be possible to set provisional specifications with allowances for some variation on the basis of values measured in a few test specimens, as long as one can argue for a relationship between the results of clinical tests and the quality attributes after the clinical trial. However, testing for sterility and the presence of mycoplasma is essential. It should be noted that the quality control strategy, including specifications,

should be enriched and developed as the clinical trial progresses.

2. Quality control of the final product
Refer to the general quality control parameters and tests shown below, set necessary and appropriate specifications for the final product, and justify the rationale for the specifications set.

Set appropriate acceptance criteria and test procedures for individual products that do not make up a lot and for products that do make up a lot, because normally each individual lot is the unit subject to quality control.

(1) Cell number and cell viability

For cells that are an active ingredient in the final product, determine the cell number and viability in the final product or, if needed, in an appropriate intermediate product. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on values measured in a small number of test samples.

(2) Tests of identity

Confirm that the cells are the intended target cells by assessing important cell characteristics, such as morphological characteristics, biochemical markers, immunological markers, characteristic products, and other appropriate genotypes or phenotypes.

(3) Tests of purity

To confirm the purity of the cells in a final product, if necessary, set the test parameters, test methods, and acceptance criteria for evaluating and controlling non-target cells, such as

undifferentiated cells, cells that exhibit abnormal growth, transformed cells, and contaminating cells, taking into consideration the origin of the target cells and tissues, the culture conditions, other parameters of the manufacturing process, quality control of intermediate products, etc. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on values measured in a small number of test samples.

(4) Tests for cell-derived, undesirable, physiologically active substances

Specify appropriate tests for determining the permissible limit of any potential undesirable, physiologically active substances that are derived from the target cells whose presence in the product is presumed to affect the safety of the patient. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on values measured in a small number of test samples.

(5) Tests for process-related impurities

For substances that may be present in the final product as contaminants, residues, or newly generated products or degradation products, etc.; that potentially originate from raw materials, non-cellular components, media ingredients (including feeder cells), chemical reagents, or any other process-related materials; and that may have deleterious effects on quality and safety (for example, albumin derived from fetal calf serum, antibiotics, etc.), it is necessary to 1) prove that the substance is not present in the final product by taking into

consideration the results of process evaluations related to the elimination of the substance or the results of in-process control of the substance or 2) establish appropriate tests with which to control permissible levels of the substance in the final product. When selecting substances to be tested and setting their acceptance criteria, their appropriateness should be explained and justified.

At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on values measured in a small number of test samples.

(6) Sterility tests and tests for the presence of mycoplasma

Sterility should be ensured throughout the entire manufacturing process by evaluating test samples. The sterility (negative for common bacteria and fungi) of the final product should be demonstrated before its use in a patient. Appropriate tests confirming the absence of mycoplasma should also be performed. A validated nucleic acid amplification test can be used. If the results of sterility and other tests on the final product can only be obtained after the product is administered to the patient, methods for dealing with non-sterility detected after administration should be established beforehand. In such an instance, demonstrate by testing that the intermediate products are sterile and that sterility has been strictly controlled in all processes leading to the final product. If a product from the same facility and same process has already been used in patients, its sterility must be confirmed by testing all patients. If complete closure (hermetic seal) of a product that is

part of a lot has been assured, tests on representative samples are sufficient. When each different application needs to be tested and if the test results can only be obtained after administration to the patient, base the decision to administer the product will be based on the most recent data. However, even in such an instance, the final product shall be tested.

It is desirable that every possible effort be made to avoid the use of antibiotics in cell culture systems. However, if antibiotics are used, adopt measures to ensure that they do not influence the sterility tests.

(7) Endotoxin test

Perform the endotoxin test, taking into consideration the impact of the contaminant in the samples. The acceptance criteria do not necessarily depend on the actual measured values. Set acceptance by criteria taking into consideration the safety ranges in the Japanese Pharmacopoeia and/or any other relevant compendia that are based on a single dose of the final product. Endotoxin testing can be established as an in-process control test. However, in such cases, specify the criteria, including the validation results, and justify their appropriateness.

(8) Virus tests

Use titer test to detect viruses in the intermediate and final products, when using cells that are not banked; that are from donors not tested during the infection window; and in which HBV, HCV, HIV, or HTLV can propagate. If materials of a biological origin are used in the manufacturing process, it may be necessary to test the final product for viruses originating from

those components. However, whenever possible, it is preferable to verify there is no contamination by testing at the original component stage or by process evaluation.

(9) Efficacy tests

In some instances, it will be necessary to consider efficacy testing that takes into account the cell type, intended clinical use, or distinctive characteristics of the cells. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on values measured in a small number of test samples.

(10) Potency tests

If a specific physiologically active substance secreted from cells or tissues contributes to the clinical efficacy or effect of a somatic stem cell-based product, establish test parameters and/or acceptance criteria for the substance in order to demonstrate the intended effect. Set acceptance criteria for potency, amount produced, etc. for phenotypic products produced by the desired cells or for an expression product secreted from the cells when a gene has been introduced. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on values measured in a small number of test samples.

(11) Mechanical compatibility tests

For products that require a certain degree of dynamic strength, set acceptance criteria for mechanical compatibility and durability that take into account the site of application. At the beginning of the clinical trial, it is acceptable to set provisional

acceptance criteria that are based on values measured in a small number of test samples.

Chapter III Stability of Allogenic Human Somatic Stem Cell-based Products

Taking into full consideration the storage and distribution periods and the storage form, test the cell viability, potency, etc. of allogenic human somatic stem cell-based products and/or critical intermediate products to establish storage methods and an expiration date. Justify their appropriateness. In particular, when product storage and use involves freezing and thawing, confirm that the freezing and thawing processes do not affect the stability or acceptance criteria of the product. Where necessary and possible, conduct stability studies on products whose manufacturing period or storage period exceeds normal periods in order to confirm, to the extent possible, the limits of stability. This does not apply if a product will be used immediately after its production.

If an allogenic human somatic stem cell-based product will be transported, the relevant transportation vessels and transportation procedures (such as thermal management, etc.) shall be set and their appropriateness justified.

Chapter IV Preclinical Safety Testing of Allogenic Human Somatic Stem Cell-based Products

To the extent that they are scientifically reasonable and technically possible, relevant animal tests and/or in vitro tests may be

performed in order to identify safety concerns associated with an allogenic human somatic stem cell-based product. For noncellular constituents and process-related impurities, safety concerns should be addressed as much as possible by physicochemical analyses, not animal testing.

Testing human specimens is very valuable, and testing products of human origin in experimental animals does not always yield meaningful results. Thus, there may be a scientific rationale for preparing products of animal origin and testing them in appropriate experimental animals if such a test system is expected to generate useful results. In such a case, consider using an animal model that is suitable for the target disease. (For example, monkeys may be suitable for studies of nervous system diseases, and pigs and/or dogs may be suitable for studies of cardiovascular diseases.) However, because cells with characteristics identical to those of cells that constitute an allogenic human somatic stem cell-based product cannot necessarily be obtained from non-human animal species, even if the preparation procedures are the same, and because a product of animal cell origin manufactured by identical processes will not necessarily be comparable to a human cell product, conduct a feasibility study before adopting, conducting, and evaluating such tests. When performing animal experiments using somatic stem cell-based products obtained from non-human animal species, explain how extrapolation to humans is appropriate. Depending on the case, consider test systems that

employ cells, and clearly explain the appropriateness of the test system.

Presented below are items and points to consider and refer to when confirming the preclinical safety of a product. These are examples provided for illustration purposes; they are not intended to prescribe tests for which there is no rational basis. Taking into consideration that the cells are allogenic-derived, the characteristics of the product, the intended clinical use, etc., conduct necessary and appropriate tests, and evaluate and discuss the results in a comprehensive manner.

1. For cells expanded beyond the limit set for routine cultivation (defined by period of time, the population doubling level, or the passage level), demonstrate that transformations other than those intended have not occurred.
2. It may be necessary to quantify special physiologically active substances produced by the cells and tissues and discuss their effects when administered to patients. In some cases, significant amounts of active substances including cytokines and growth factors would be produced by the cells, potentially resulting in undesirable effects on the patients.
3. From the aspect of product safety, examine and discuss the potential effects of the product on a patient's normal cells and tissues and the consequences.
4. Depending on the type of product, investigate and discuss the possibility of ectopic tissue formation by the cells in the

product and the potential safety consequences thereof when the product is administered to the patient.

5. Investigate and discuss the possibility of undesirable immunological reactions caused by the product and/or the expression product of a transgene and the consequences thereof.

6. Discuss in a comprehensive manner the possibility of tumor formation, including benign tumors and/or malignant transformations, taking into consideration the type and characteristics of the product, number of cells and route of administration, mode of application (e.g., cell sheet, cell suspension etc.), cell engraftment site, target diseases, appropriateness of the tests systems, etc. If necessary, conduct studies using a suitable animal model. If tumorigenicity or malignant transformation is a possibility, provide justification and rationale for the use of the product in question, taking into consideration the anticipated efficacy. (Note: For tumorigenicity studies, it is most important to assess accurately the tumorigenicity of the final product that will be used in patients.

However, tumorigenicity may have to be evaluated using cells from the intermediate product if, for various reasons, such as insufficient cell number, the cells in the final product cannot be used. Furthermore, when conducting tumorigenicity tests in animal models, variables such

as cell dispersion, cell adhesion to scaffolding, cell density, and administration site may not be the same as for the final product. The species, strain, and immunological state of the animal may also affect its sensitivity. The tumorigenicity of the final product should be evaluated in a comprehensive manner taking these factors into account.) The risks to the patient arising from tumorigenicity of the final product should be evaluated by weighing the risks of treatment against the benefits of treating the disease.

7. If an exogenous gene is introduced into certain cells during the manufacturing process, conduct tests in accordance with "Gene Therapy Pharmaceutical Guidelines," published as Notification No. 1062 by the Ministry of Health and Welfare on November 15, 1995. In particular, if viral vectors are used, perform quantitative tests to determine if any replication-competent viruses are present, and justify the appropriateness of the test method employed. Describe the safety of the transgene and its products based on their characteristics. For cells, discuss the possibility of changes in cell growth and tumor formation, including benign tumors and malignant transformations. When using a vector that can insert into a chromosome, consider the necessity of evaluating abnormal proliferative characteristics and/or tumorigenicity and implementing long-term

follow-up.

8. Consider conducting rationally designed general toxicology tests if the product, including an animal-derived product, is easy to obtain and if doing so will generate useful information regarding its clinical application. When conducting general toxicology tests, refer to "Guidelines for Toxicology Studies on Pharmaceuticals," which is an appendix in the document entitled "Guidelines on Toxicology Studies Required for Regulatory Approval for the Manufacture or Import of Pharmaceuticals" (Drug Evaluation Notification 1:24, Ministry of Health and Welfare, issued September 11, 1988).

Chapter V Studies Supporting the Potency or Efficacy of Allogenic Human Somatic Stem Cell-based Products

1. A well-designed study with experimental animals and/or cells should be performed to demonstrate, to a scientifically reasonable and technically possible extent, the functional expression, the sustainability of effect, and/or the anticipated clinical efficacy (proof of concept) of an allogenic human somatic stem cell-based product.
2. For transgenic cells, demonstrate the expression efficiency, sustainability of expression and biological activity of the desired products from the transgene. Discuss the anticipated clinical efficacy (proof of concept) of the allogenic human somatic stem

cell-based product in question

3. Where appropriate products derived from the processing of animal somatic stem cells and/or disease model animals are available, use them to study the potential therapeutic efficacy of the product.
4. At the beginning of the clinical trial, detailed experimental studies will not necessarily be required if scientific literature and/or other information supports the prediction that the potency or efficacy of the product in question will be markedly superior to that of a different therapeutic method.

Chapter VI Pharmacokinetics of Allogenic Human Somatic Stem Cell-based Products

1. Pharmacokinetic studies of the internal behavior of transgene expression products or cells/tissues that constitute the final products, which may include assessment of their absorption and distribution in experimental animals, should be performed to an extent that is technically possible and scientifically reasonable. Thereby, it is expected to estimate the survival of cells/tissues administered to patients and the duration of their effect, and determine if the intended efficacy is sufficiently achieved. (Note: Testing methods may include histological studies, Alu-PCR, MRI, PET, SPECT, and bioimaging.)
2. For autologous human somatic

stem cell-based products, clarify, through animal studies, the rationale for the administration method. In particular, extrapolate from animal experiments the systemic distribution of cells after systemic administration and discuss the distribution from the point of view of clinical usefulness. (Note: Although it is unclear exactly where cells adhere with each administration route, local administration is presumed to be preferable to systemic administration. However, if the benefits to patients can be explained, it may be acceptable to use systemic administration. In any case, an administration method that minimizes the distribution of a somatic stem cell-based product to organs other than target organ would be a rational choice. Even if cells do localize to a site other than the intended transplantation site, the administration method may be used if no effects on patients result. Arrhythmia caused by osteogenesis of mesenchymal stem cells that ectopically locate to the heart is an example of an adverse effect that could result from ectopic differentiation.)

3. When the cells or tissues are directly applied or targeted to a specific site (tissue, etc.) where they are expected to act, clarify the localization and discuss the effect of the localization on the efficacy and safety of the product.

Chapter VII Referring to Clinical Trials

The main purpose of the present guideline is to outline points to consider for evaluating the quality and safety of allogenic human somatic stem cell-based products, at the time of application for marketing authorization or at the beginning of an investigational clinical trial. In the latter case, it is necessary to evaluate, while taking into consideration the clinical usefulness, whether there are any quality and/or safety problems that might impede the initiation of human clinical trials. Thus, quality and non-clinical safety evaluations for determining to initiate the investigational clinical trials of the product in question should refer to the points outlined below. Any presumed risk factors associated with product quality and safety should be eliminated, as much as possible, using up-to-date scientific and technological methods, and the scientific appropriateness should be clearly described. Any remaining risks should be weighed against the risks associated with not performing the trials in patients that suffer from diseases that are serious and life-threatening, that involve marked functional impairment or a marked decrease in quality of life (QOL) resulting from the loss of physical function or form, or for which existing therapies have limitations and do not provide cures. Furthermore, it is critical to entrust to the patient the right to make a decision after providing all of the information available, including all information on identified/unidentified risks and anticipated benefits.

1. Target disease

2. Target subjects and patients who should be excluded as participants.
 3. Details of the therapy to be performed in the subjects, including the application of allogenic human somatic stem cell-based products and drugs used concomitantly. (Note: If it is anticipated that drugs will be co-administered in order to maintain, enhance, and/or induce the function of administered or transplanted cells, verify the intended activity of the drugs either in vitro or in vivo.)
 4. Appropriateness of conducting the clinical trials in light of existing therapeutic methods.
 5. Plan for explaining the clinical trial to the patients, including the currently known risks and benefits of the product.
1. Takao Hayakawa, Takashi Aoi, Akihiro Umezawa, Keiya Ozawa, Yoji Sato, Yoshiki Sawa, Akifumi Matsuyama, Shinya Yamanaka, and Masayuki Yamato: Study on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from Processing of Autologous Human Somatic Stem Cells. submitted to *Regenerative Therapy*, 1(2014)
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Clinical trials should have an appropriate study design and specified endpoints. They should be designed in light of the desired cells/tissues, target disease, and method of application.

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