viruses, and to indicate measures for ensuring retrospective and other studies.
The technical requirements described in this paragraph should be taken into consideration when the process of reprogramming or dedifferentiation from the raw materials into iPS(-like) cells, or of directed differentiation from iPS(-like) cells into the final products in question include any relevant elements/concerns.

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(1) Cell Culture

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

(ii) Consider the following points with respect to media components

① The ingredients and water used in media should be of high quality and high biological purity, quality should be controlled at standards equivalent to those for pharmaceuticals and pharmaceutical raw materials.

② Provide information on not only the main ingredients used in media, but all components, 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 individual raw materials.

③ Conduct sterility and performance tests on media that contain all components to determine their suitability as target media. Set specifications for any other relevant parameters thought to be controlled in the manufacturing 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 ways to avoid using these serum components, insofar as possible. If the use of serum or other such material is unavoidable, consider the following points, and investigate ways to prevent contamination and spread of bacteria, fungi, viruses, and abnormal prions from the serum and other products, as well as treatment methods for their elimination, to the greatest 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, for example 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 having 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) Conduct appropriate inactivation and elimination procedures for 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 necessary.

(e) Preserve and store a portion of the serum used to enable monitoring for viral infections in cultured cells, 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 quality evaluation while referring to "Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products" (Pharmaceutical Notification Number 873, Ministry of Health, Labor, and Welfare, July 14, 2000), "Guidelines on Public Health Infection Issues Accompanying Xenotransplantations" (Notification 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 0702001, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labor, and Welfare, issued July 2, 2004) in order to prevent contamination and spread of bacteria, fungi, viruses, and abnormal prions from the feeder cells; indicate the methods used for inactivation of cell division potential and conditions such as cell density. However, for example, if the feeder cells or equivalent cells are being used in the manufacture of a cell or tissue product that has previously 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 portions of other tests by demonstrating the appropriateness of using these cells.

(v) The use of antibiotics should be avoided as far as possible. However, if antibiotics are deemed indispensable in the initial stages of processing, attempt to decrease their use in subsequent steps as far as possible, and clearly state the appropriateness of their use, including the scientific rationale, estimated residual amounts in the final product, and effects on the patient. If it has been verified that an antibiotic can be adequately eliminated from the final product, its use need not be restricted. However, if a patient has a past history of allergy to the antibiotic used, this therapeutic method

should not be used. If use of antibiotics cannot be avoided, administer them carefully and ensure that informed consent is obtained from the patient.

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

(vii) For media components and other components used in processing and those that may contaminated the final product, choose components with no harmful biological effects.

(viii) When using cells derived from a different species (heterologous cells) as feeder cells, ensure that there is no risk of infection from the heterologous-derived cells.

(2) Combination with noncellular components

(i) Quality and safety of noncellular raw materials

If the final product consists of cells combined with noncellular components such as matrix, medical materials, scaffolds, support membranes, fibers, or beads, describe in details 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 evaluation of their quality, safety, and efficacy or the presumed clinical indication. When using materials that are absorbed by the body, perform the necessary tests for the safety of the any degradation products.

To determine which tests are required, refer to "Basic Views on Biological Tests Necessary for Regulatory Approval for Manufactured or Imported Medical Devices" (Notification No. 02013001, 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. Rational use of information from scientific literature is also encouraged.I

(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 cells in the final product required for the presumed clinical indication or the cells in any intermediate products.

(b) Evaluate to the greatest extent possible any potential interactions between cells and noncellular components, taking into consideration for example mutation, transformation, and/or dedifferentiation of cells in the

final or intermediate products.

(c) Demonstrate that there is no loss of the expected properties of the noncellular components in the presumed clinical indication due to any interactions between the noncellular components and the cells in the final and intermediate products.

(iii) Use of noncellular componentsto isolate the desired cell products from the application site.

When 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 pharmacological effect of target physiologically active substances derived from 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 segregation of the application site and the desired cell products and/or undifferentiated cells is the objective, confirm that cells will not leak following degradation, etc., of the noncellular components.

(3) Genetic modification of cells

When genes are introduced into cells, provide details on the following items.

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

(ii) Nature of the transgene.

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

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

(v) Structure and characteristics of the transgene construct

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

For manufacturing methods for transgenic cells, refer to Chapter 2 and other sections of the "Guidelines for Ensuring the Quality and Safety of Gene Therapy Pharmaceuticals", which is an appendix of "Concerning Guidelines for Ensuring the Quality and Safety of Gene Therapy Pharmaceuticals" (hereinafter referred to as "Gene Therapy Pharmaceutical Guidelines"), published as Notification 1062 by the Ministry of Health and Welfare on November 15, 1995. In addition, clearly state the appropriateness of the establishment in accordance with the appendix of the same notification.

Be aware that, based on the law (Law No. 97, 2003) for ensuring the biodiversity by regulating the use, etc. of genetically modified organisms, etc., a separate application procedure for evaluation will be required when living organisms including certain cells, as well "viruses", and "viroids" are genetically modified. The following cells are not regarded as living organism: "human cells, etc." or "cells that have the ability to differentiate, or differentiated cells taht are not viable when alone under natural conditions".

Regardless of the above, if a gene introduced into cells is used as a reagent in the manufacturing process but is neither chemically nor functionally present in the final product, it is acceptable simply to describe how the quality and safety of the gene conform to the intended use, based on the most current knowledge.

(4) Introduction of proteins into cells

When proteins are introduced into cells, provide the details of the items listed below.

(i) Origin, and quality attributes including protein structure, biological activity and physicochemical properties.

(ii) Information concerning the procurement, manufacturing, quality control, and renewal methods for the proteins

(iii) Methods for introducing the proteins into the cells

(iv) Quality attributes including the structure, biological activity, and physicochemical properties of the chemical substances used to introduce the proteins into the cells

(v) When preparing a construct for introducing the proteins in question into cells, provide information on its preparation, quality control, and renewal methods.

(vi) Preparation of cell banks and cell bank control methods i to produce the introduced proteins.

Regardless of the above, if a protein introduced into cells is used as a reagent in the manufacturing process but is neither chemically nor functionally present in the final product, it is acceptable simply to describe how the quality and safety of the protein conform to the intended use.

(5) Reprogramming or dedifferentiating, and/or differentiating cells using drugs or any chemicals

When reprogramming or dedifferentiating cells, and/or differentiating using drugs or any other chemicals, provide the details on the following items.

(i) Origin, and quality attributes including structure biological activity, (if any) and physicochemical properties of the drugs or chemicals in question.

(ii) Information concerning the procurement, manufacturing, quality control, and renewal methods for the target drugs or chemicals.

(iii) Cell treatment methods using target drugs, etc.

(6) Cell reprogramming or dedifferentiation, and/or differentiation using physical methods

Describe the details of the methods

used when performing cell reprogramming or dedifferentiation, and/or differentiation using physical methods in question.

(7) Cell reprogramming or dedifferentiation, and/or differentiation using a combination of methods

Describe the details of the methods when using any combination of genetic modification, introduction of a protein, drug/chemical treatment, or physical methods to conduct cell reprogramming or dedifferentiation, and/or differentiation.

3. Establishment of human iPS(-like) cell line

Establish human iPS(-like) cell line after having determined to the greatest extent possible the genetic background of the donor. Describe the methods used up until the establishment of iPS(-like) cells from the somatic cells that serve as the raw material, and indicate, to the greatest extent possible, the appropriateness of the methods. These include the methods for obtaining the human somatic cells, for separating and culturing of somatic cells, for reprogramming or dedifferentiation of the somatic cells, for isolating and preparating cell lines of the reprogrammed or dedifferentiated cells, as well as the media, culture conditions, culture period, yield, and so on at each step in the process until establishment of the human iPS(-like) cell line.

In order to maintain stability and consistency of the quality of the human iPS(-like) cell lines, identify critical quality attributes of the cells (for example: cell purity, morphological features, HLA typing, phenotype specific markers, karyotype, DNA fingerprinting, cell growth properties, pluripotency, etc.) and set acceptance criteria for them. In addition, demonstrate the potent number of passages or of cell divisions within which cells can be proliferated while maintaining their quality in terms of the criteria specified.

4. Storage and transport of human iPS(-like) cell lines

For human iPS(-like) cell lines, perform appropriate stability tests based on the viability, potency, etc., of the cells, establish storage method and validity period, and clarify their appropriateness, considering strage duration, distribution, and storage form. In particular, when freezing and thawing, confirm whether freezing and thawing affect the stability or any criterion of the cell line. Evaluate storage over a standard storage period, and confirm the margin of stability to the extent possible. However, this does not apply if the cells will be used immediately after being established.

When transporting human iPS(-like) cells, the containers used for transport and the transportation procedures (including temperature control, etc.) shall be determined and their appropriateness clearly indicated.

5. Preparation of records and storage procedures

Written records for items 2above shall be prepared and proper record storage procedures f shall be clearly described.

II. Manufacturing Process

When manufacturing pharmaceuticals and medical devices derived from the processing of allogenic human iPS cells or allogenic human iPS-like cells (i.e., human iPS(-like) cell-based products), describe in detail the manufacturing method and verify, to the greatest extent possible, the appropriateness of the method using the items listed below to maintain consistent product quality.

a. Lot composition and control

Indicate whether or not a lot comprises both final and intermediate products. If a lot is composed of both final and intermediate products, establish standardized procedures for make up and control of the lot.

b. Manufacturing method

Provide an outline of the manufacturing method from the time of receipt of the cells and tissues or somatic cells that serve as the raw materials through to the establishment of human iPS(-like) cells and cells that have progressed to the differentiation stage and then to the final product. Describe the technical details of the process and the required process and product quality control.

(1) Tests upon receipt

Establish a battery of tests and acceptance criteria to assess appropriateness of the cells and tissues, somatic cells or human iPS(-like) cells, 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, characterization of cells and tissues, microbiological tests, and so on. At the stage of initiation ofclinical trials, provide the actual measured values obtained using test samples, and propose a provisional a set of acceptance criteria based on these values.

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

For cells and tissues, human somatic cells or human iPS(-like) cells that serve as raw materials, inactivate and eliminate bacteria, fungi, viruses, and other microorganisms if needed and whenever possible, to such an extent that the procedures do not have any effect on the cell viability, phenotype, genetic traits, specific functions, or other characteristics and quality of the cells and tissues serving as raw materials. State the appropriateness of measures, procedures and evaluation methods employed, if any.

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

Describe the methods for the disintegration of tissue, separation of somatic cells, isolation of specific somatic cells, and methods for washing, etc. of these cells and tissues in order to generate the iPS(-like) cells, that are performed in the early stages of manufacture of the iPS(-like) cell-based products from collected cells and tissues. Upon isolating specific somatic cells, establish identification methods for the cells.

(4) Establishment of allogenic human iPS(-like) cell lines

Establish human iPS(-like) cell line after having determined to the

greatest extent possible the genetic background of the donor. Describe the methods used up until the establishment of iPS(-like) cells from somatic cells that serve as the raw material, and indicate, to the greatest extent possible, the appropriateness of the methods.
Identify critical quality attributes of the cells and set acceptance criteria for them. Demonstrate the potent number of passages or cell divisions within which cells can be proliferated while maintaining their quality in terms of the criteria specified. (refer to Chapter II-I.-3).

(5) Establishment of intermediate cell line derived from allogenic human iPS(-like) cells
It should be noted that in some cases the establishment of a cell line (intermediate cell line) as an intermediate product may be important for the stable manufacture of a safe final product, as well as a scientifically rational procedure. When such a measure is chosen, explain its advantages and appropriateness. If a cell line that exhibits a different phenotype is established in stages, describe the methods (for example, methods for induction of differentiation isolation, culturing, and cell line establishment of the target cells, as well as the media, culture conditions, culture period, yield, and so on at each stage) until establishment of each respective cell line, and justify their appropriateness to the extent possible.
To maintain the stability and consistency of the quality of the intermediate cell line, identify critical quality attributes of the cells (for example; cell purity, morphological features, phenotype specific markers, karyotype, cell growth properties, pluripotency, etc.) and set acceptance criteria. Demonstrate the potent number of passages or of cell divisions within which cells can be proliferated while maintaining their quality in terms of the criteria specified. Although comprehensive cell characterization is always desirable, it is recognized that quantitative limits to samples or technological limits may make it difficult to perform the study fully. If this is the case, it is acceptable to perform the study to some extent possible.
If establishing and utilizing a cell bank from an intermediate cell line in accordance with the above refer to (7).

(6) Preparation of cells that compose a principal component of the final product as an active ingredient
Describe the methods, either directly from a human iPS(-like) cell line or from an intermediate cell line derived from human iPS(-like) cells, used to prepare the cells that serve as the active ingredient in the final product. Describe the induction of differentiation, isolation, and culturing of the desired cells, and the media, culture conditions, culture period, yields of the desired cells, and so on used at each step. Describe the appropriateness of each method, to the extent possible.

(7) Establishment of cell banks
When a cell bank is established at any stage during the process of manufacturing human iPS(-like) cell products, describe the details of the rationale for preparing the cell banks,

the methods used to prepare the cell banks, characterization of the cell banks, and storage, maintenance, control methods, and renewal methods, as well as any other processes and tests performed, and justify the appropriateness of each. Refer to "Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products" (Pharmaceutical Notification Number 873, Ministry of Health, Labor, and Welfare, July 14, 2000) and other relevant documents. It is acceptable to omit a portion of the test items, if t the cells have been properly evaluated at an upstream point in the process for a good reason.

(8) 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 human iPS(-like) cell-baced products. Therefore, clearly describe preventative measures in the control process.

3. Characterization of cells that comprise a principal component of a final product as an active ingredient
Analyze various attributes of the cells, such as cell purity to control contamination by undifferentiated or non-target cells, cell viability, morphological characteristics, growth characteristics, biochemical markers, immunological markers, distinctive substances produced by cells, karyotype, differentiation potency, and other appropriate genotypic and phenotypic markers of cells that make up a principal component of the final product. Additionally, characterize cells with respect to biological functions, where necessary. Furthermore, to evaluate the appropriateness of the culture period and stability of the cells, use appropriate cell characteristic markers to prove the absence of unintended changes in cells cultured f beyond 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. These results can be used, to identify the critical cell characteristics that should be used when applying the real product to a patient. Although comprehensive cell characterization is always desirable, quantitative limits to samples or technological limits may prevent its full performance. In this case, it is acceptable to perform the study to the extent possible. When cell processing such as growth within the body is anticipated after clinical application, clearly demonstrate the functions expected using the passage number or number of cell divisions based on the specified criteria.

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 final product
If intermediate or final product must 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 iPS(-like) cell-based products, assess whether or not any significant differences were present during the manufacturing process for each individual product between production runs (each lot), with respect to the number of cells, cell viability, and cell characteristics (such as relevant markers of phenotype and/or genotype, functional characteristics, and the percentage content of desired cells), considering the application methods and intended use of the product. It is acceptable to use test samples in place of the real products that will be prepared for clinical trial. Evaluation using intermediate products may provide an accurate reflection of the appropriateness of the cells and tissues used as raw materials and the validity of the manufacturing process until the point of production of the intermediate products, as well as serving as an appropriate guidepost leading up to the final product. Therefore, it may be reasonable to adopt such an approach, where necessary and appropriate.

When the duration of the cryopreservation or cell cultivation portion of the manufacturing process is long, perform sterilization tests and so on at consistent intervals to confirm sterility has been ensured.

7. Changes in manufacturing process

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

III. Quality Control of Final Product

1. Introduction

The overall quality control strategy for human iPS(-like) cell-based products include specifications (a set of acceptance criteria and analytical procedures) for final products, quality control of raw materials for each different application to each individual patient, verification of the appropriateness of the manufacturing process and maintenance of its consistency, as well as proper quality control of intermediate products, if any. One of the most critical issues in case of iPS(-like) cell-based products is a measure to ensure absence of contamination of the cells by undifferentiated cells other than the desired cells. Verification of the absence of contamination by non-target undifferentiated cells is desirable, as much as possible, at the intermediate product stage.

Because specifications for the final product differ depending upon the type and properties of the desired cells and tissues, manufacturing methods, intended clinical use and method of application for each product, stability, and available test

methods differences that depend on cell or tissue handling shall be taken into sufficient consideration when setting 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 for initiating clinical trials is to confirm that the product in queston can be deemed to have no significant quality/safety problems for use in investigational clinical trials. Therefore, it may be possible to set provisional specifications with allowances for some variation, based on values measured using a few test specimens, as long as one can argue the relationships between the results of clinical tests and such quality attributes after clinical trials. However, testing for sterility and presence of mycoplasma is essential. It should be noted that the quality control strategy including specifications, shall be enriched and developed in tandem with the progress of clinical trials.

2. Quality control of the final product
Refer to the general quality control parameters and tests described below set 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 comprise a lot as well as for individual products that do comprise a lot, because each individual lot is typically the unit subjected to quality control.

(1) Cell number and cell viability
The number and viability of cells that are active ingredient in the final product or, in an appropriate intermediate product, if required, i should be determined. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on actual measured values from a small number of test samples.

(2) Tests of Identity
Confirm that the cells are the intended target cells using markers for important cell characteristic selected from among the morphological characteristics, biochemical markers, immunological markers, characteristic products, and other appropriate genotypes or phenotypes of the intended target cells and tissues.

(3) Tests of Purity
To test the purity of cells in a final product, set the test parameters, test methods, and acceptance criteria for evaluating and controlling non-target cells, such as undifferentiated cells, cells exhibiting abnormal growth, transformed cells, and the presence of any contaminating cells, considering the origin of the target cells and tissues, the culture conditions and other parameters of the manufacturing process, quality control of intermediate products, and so on. At the beginning of the clinical trial, it is acceptable to set provisional

acceptance critetia based on actual measured values from a small number of test samples.

(4) Tests for cell-derived undesirable physiologically active substances
Specify the appropriate and permissible dose-limiting tests for any potential undesirable physiologically active substances derived from target cells, the significant presence of which in the product is presumed clearly to impact the safety of the patient. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on actual measured values from 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 as newly generated products or degradation products, etc., potentially originating from raw materials, non-cellular components, media ingredients (including feeder cells), chemical reagents, or any other process-related materials, and that may have deleterious effect on the quality and safety (for example, albumin derived from fetal calf serum, antibiotics, etc.), it is necessary either to prove that the substance is not present in the final product using the results of process evaluation for the elimination of the substance or the results of in-process control of the substance, or to establish appropriate tests to control the amount of the substance in the final product within permissible levels for 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 based on actual measured values from a small number of test samples.

(6) Sterility tests and tests for the presence of mycoplasma
The sterility of the final product should be sufficiently assessed to ensure sterility throughout the entire manufacturing process using test samples. The sterility (negative for common bacteria and fungi) of the final product should be demonstrated in tests before use in a patient. Appropriate tests confirming the absence of mycoplasma should also be carried out. A validated nucleic acid amplification test can be used. If the results of the sterility and other tests of the final product can only be obtained after administration to the patient, the proper measures for dealing with potential non-sterility should be established beforehand. In such an instance, the intermediate products must be demonstrated to be sterile, and sterility should be strictly controlled in all processes leading up 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 it in all patients. If complete closure (hermetic seal) of an individual lot of the product has been assured, tests using only representative samples are sufficient. When tests must be conducted for each different application and if the results of sterility and other tests can only be obtained after administration to the patient, the determination of whether

or not application should proceed will be determined based on the most recent data. However, even in this instance, sterility tests and other tests on the final product shall be conducted.
It is desirable that every possible effort be made to avoid use of antibiotics in cell culture systems; however, if they are used, adopt measures to ensure that the antibiotics do not influence the sterility tests.

(7) Endotoxin tests
Perform anendotoxin test, considering the impact of potential contaminant in the samples. The acceptance criteria do not necessarily depend on the actual measured values. It is recommended to set acceptance criteria considering the safety ranges given in the Japanese Pharmacopoeia and/or any other relevant compendia t 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 criteria, including validation results, and justify their appropriateness.

(8) Virus tests
Use titer test to detect viruses in the intermediate and final product and confirm administration of the iPS(-like) cell-based products do not leads any disbenefit to the patient, when using cells which are not banked in neither raw materials nor manufacturing processes, and are from donors not proved in the window period of infection, and in which HBV, HCV, HIV or HTLV can propagate. If components of a biological origin are used in the manufacturing process, it may be necessary to consider conducting tests on the final product for viruses originating from those components. However, whenever possible, it is preferable to verify there is no contamination by testing or process evaluation at the upstream stage including that of the original component.

(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 the secretion of a specific physiologically-active substance from the cells or tissues is responsible for the efficacy or the essential effect of an iPS(-like) cell-based product in its intended clinical use, establish test parameters and/or acceptance criteria related to the substance in order to demonstrate the intended effect. Set acceptance criteria for potency, amount produced, etc. for phenotype products from 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 based on actual measured values from a small number of test samples.

(11) Mechanical compatibility tests

For products that require a certain

degree of mechanical strength, set acceptance criteria to confirm 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 based on actual measured values from a small number of test samples.

Chapter III Stability of Allogenic Human iPS(-like) Cell-based Products

Taking into full consideration the storage and distribution periods and the storage form, perform suitable stability testing on allogenic human iPS(-like) cell-based products and/or critical intermediate products based on cell viability, potency, etc. to establish storage methods and expiration date, and justify their appropriateness. In particular, when freezing and thawing are involved in the storage and use of the products, confirm that the freezing and thawing processes do not affect the stability or acceptance critetia of the product. Where necessary and possible, it is recommended to conduct stability studies the products whose manufacturing or storage periods exceeds the normal periods, in order to confirm, to the greatest extent possible, the limits of stability. This does not apply if a product will be used immediately after production.
If a human iPS(-like) 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 (iPS)-like Cell-based Products

Relevant animal tests and/or in vitro tests may be performed to elucidate concerns an regarding the safety of allogenic human iPS(-like) cell-based product when it is scientifically reasonable and technically possible.
Safety concerns regarding non-cellular constituents and process-related impurities should be resolved, as far as possible, using physicochemical analyses and not animal testing. In addition, the presence of undifferentiated cells in the final product and their potential to cause ectopic tissue formation, tumorigenicity, or malignant transformation is a safety concern. Therefore, it is necessary to reduce the risk of contamination with such cells as much as possible via thorough analysis at the cell bank and/or intermediate product stage, or alternatively by developing and utilizing methods that effectively separate, remove, and/or inactivate these contaminating undifferentiated cells from the target cells during the manufacturing process. Furthermore, the administration route for the target cells may be selected to aid in the minimization of the safety concerns.

Animal testing of products of human origin do not always yield meaningful results. Thus, there may be a scientific rationale for preparing product models of animal origin and testing with appropriate experimental animals, if more useful information may be obtained. In such a case, consider conducting tests using suitable animal models for each target diseases. (Note: For example,

monkeys may be suitable for nervous system diseases, while pigs and/or dogs may be suitable for cardiovascular diseases). However, because use of identical procedures in non-human animals will not necessary yield cell groups that possess identical characteristics to cells that constitute an allogenic human iPS(-like) cell-based product, and aproduct of animal cell origin manufactured using identical processing, including culture conditions, etc. It will not necessarily be comparable to a human cell product, careful feasibility studies are required beforehand when adopting, conducting, and evaluating such studies. When conducting animal experiments using iPS(-like) cell-based products obtained from non-human animal species, explain the suitability of extrapolation. Depending on the case, consider test systems that employ cells, and clearly explain the appropriateness of the test system when conducting tests using this kind of approach.

The examples below present points to consider when confirming the preclinical safety of a product. These are merely examples for illustration and are not meant to suggest conducting tests with no rational basis. Conduct necessary and appropriate tests, taking into account the characteristics of the product, intended clinical use, and evaluate and discuss the results in a comprehensive manner.

1. For cells expanded beyond the defined limit for cultivation (by a period of time, population doubling level of the cells, or passage level of the cells) for routine production, clearly demonstrate that transformations other than the intended transformation and abnormal proliferation of non-target cells have not occurred.
2. It may be necessary to conduct quantitative assays for particular physiologically-active substances produced by the cells and tissues and discuss their effects when given 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. Examine and discuss the potential effects and safety consequences f of the product on the normal cells and tissues of a patient,.
4. Investigate and discuss the possibility and potential safety consequences of the formation of ectopic tissue by cells in the product and/or contaminating undifferentiated cells when the product is given to the patient. Discuss in a comprehensive manner, taking into account the type and characteristics of the product, the route of administration, target diseases and appropriateness of the test system, etc.
5. Investigate and discuss the possibility and safety of undesirable immunological reactions to the product and/or expression product of a transgene, and the relevant safety concerns..
6. Using an appropriate animal model or other system,

investigate and discuss the possibility of tumor formation including benign tumors and/or malignant transformation of cells in the final product or in an intermediate product. Upon testing, take into account 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 there is a possibility of tumorigenicity or malignant transformation, justify the appropriateness of the use of the product in question and its rationale, considering the relationship with the anticipated efficacy. (Note: The most important aspect of a tumorigenicity test is to accurately assess the tumorigenicity of a final product that will be used in patients. However, it is conceivable that tumorigenicity will need to be evaluated using cells from an intermediate product because the cells comprising the final product cannot be used for various reasons, such as the inability to obtain a sufficient number of cells. Furthermore, in tumorigenicity tests using animal models various conditions such as cell dispersion and cell adhesion to the scaffolding, cell density, and administration site, are not necessarily identical to those for the final product. Sensitivity may differ depending on the species, strain, and immunological state of the animal. The tumorigenicity of the final product should be evaluated with comprehensive consideration of these circumstances. The risks to the patient arising from tumorigenicity of the final product should be rationally evaluated based on the balance between any risks and the benefits to the patient by treating the disease.).

7. If an exogenous gene is introduced into cells during the manufacturing process, and if it may functions or remain as a residue in the final product, conduct tests in accordance with the "Gene Therapy Pharmaceutical Guidelines", published as Notification 1062 by the Ministry of Health and Welfare on November 15, 1995. In particular, if virus vectors are used, test quantitatively to determine the potential presence of any replication-competent retroviruses 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 or, tumor formation including benign tumor and malignant transformation. Whenever a vector, which may be inserted into a chromosome is used, consider the necessity of evaluating possible occurrences of abnormal proliferative characteristic and/or tumorigenicity due to insertion mutation in the cells and of implementing long-term

follow-up for clinical applications.

8. Consider conducting rationally designed general toxicology tests if the product, including an animal-derived model product is easy to obtain, and if doing so will generate useful information regarding its clinical application. When conducting general toxicology tests, refer to the "Guidelines for Toxicology Studies on Pharmaceuticals", which is an appendix to 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 iPS(-like) Cell–based Products

1. A well designed study using experimental animals and/or cells should be performed in order to demonstrate the functional expression, sustainability of effect, and/or anticipated clinical efficacy (Proof-of-Concept) of an allogenic human iPS(-like) cell-based product to the scientifically reasonable and technically possible extent.

2. For transgenic cells, demonstrate expression efficiency, sustainability of expression and biological activity of desired products from the transgene, and discuss the feasibility of the anticipated clinical efficacy (Proof-of-Concept) of the human iPS(-like) cell-based product in question..

3. Where appropriate models of products derived from processing of animal iPS(-like) 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 the potency or efficacy of the therapy employing the product in question is expected to be markedly superior to other therapeutic methods, and if this can be justified by means of scientific literature and/or other available information.

ChapterVI Pharmacokinetics of Allogenic Human iPS（-like）Cell-based Products

1. Pharmacokinetic tudies of the internal behavior of cells/tissues that constitute the final products or expression products of transgenes, which may include absorption and distribution in experimental animals, should be performed to the extent 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. Clarify, using animal studies, the

rationale for the administration method for the allogenic human iPS(-like) cell-based products. 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 the cells adhere for each administration route, it is assumed that local administration may be preferable to systemic administration. However, if the benefits to patients can be explained in a rational manner, it may be acceptable to use systemic administration. In any case, an administration method that minimizes distribution of iPS(-like) cell-based product to organs other than target organ is preferred. Even if the cells localize to a site other than the intended transplantation site, an administration method may be acceptable if patients experience no adverse effects. For example, a disadvantage caused by ectopic differentiation may be, for example, arrhythmia caused by osteogenesis of some types of cells that ectopically locate to the heart.).

3. When the cells or tissues are directly applied or alternatively targeted to a specified site (tissue, etc.) where they can be expected to perform their actions, 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 address points to consider for evaluating the quality and safety of allogenic human iPS (-like) cell-based products at the time of application for marketing authorization as well as at the beginning of investigational clinical trials. In the latter case, it is necessary to evaluate, whether any quality or safety problems exist that might pose an obstacle to initiating human clinical trials, taking into consideration the product's clinical usefulness. 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. evaluation referring to the points outlined belowis necessary. F Any presumed known risk factors associated with the product's quality and safety should be eliminated as much as possible, using up-to-date science and technology, 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 of QOL resulting from the loss of a certain degree of physical function or form, and for which existing therapies have limitations and do not provide cures. Furthermore, it is also 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 subjects
3. Details of the therapy to be performed on the subjects, including the application of human iPS(-like) 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 existing therapeutic methods.
5. Plan for explaining the clinical trial to the patients, including the currently known risks and benefits of the product.

Clinical trials should have an appropriate study design and specified endpoints. They should be designed based on the desired cells/tissues, target disease, and method of application.

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C. 1. 7 ヒトES細胞加工医薬品等の品質及び安全性の確保に関する研究の経緯と視点及びガイドライン英文版

Study on Ensuring the Safety and Quality of Pharmaceuticals and Medical Devices Derived from the Processing of Human Embryonic Stem Cells

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Background (Chronology and Focus of the Research)

The details of the present study were described in a previous paper[1-4]. The present paper summarizes points that are closely related to those presented in the earlier paper.

Regenerative medicine using cell-based products derived from the processing of human cells and tissues is keenly anticipated in Japan because of difficulties with securing human organs and tissues in our country. With technology breakthroughs and research advances, people are increasingly hopeful that medical technology using novel cell-based products will develop into therapies.

In Japan, translational research into regenerative medicine is advancing rapidly. In particular, considerable work has been done to develop products that make use of human pluripotent cells, i.e., somatic stem cells such as mesenchymal stem cells, embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells. Thus, there is an urgent need to prepare relevant guidelines for the evaluation of products expected in the near future. Identifying at an early stage of development the technical, medical, and ethical conditions necessary for the utilization of various types of stem cells is vital for their rapid application in patients.

In fiscal year 2008, the Japanese Ministry of Health, Labor, and Welfare (MHLW) convened a panel of experts entitled "Study Group on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Human Stem Cells." The panel was established as an MHLW scientific research project and has been chaired by Dr. Takao Hayakawa since its conception.

The objective of the study group is to promote the sound development of products derived from human stem cells by investigating scientific and technological advances, ethics, regulatory rationales, and international trends regarding human stem cell-derived products and to establish and implement appropriate safety evaluation criteria.

As a result of analyses conducted up to 2009, in accordance with the Pharmaceutical Affairs Law, and with clinical application of products derived from human somatic stem cells, iPS cells, ES cells, and other