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## Original article

# A study on ensuring the quality and safety of pharmaceuticals and medical devices derived from processing of autologous human induced pluripotent stem(-like) cells<sup>☆</sup>



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## ABSTRACT

As a series of endeavors to establish suitable measures for the sound development of regenerative medicine using human stem cell-based products, we studied scientific principles, concepts, and basic technical elements to ensure the quality and safety of therapeutic products derived from autologous human iPS cells or iPS cell-like cells, taking into consideration scientific and technological advances, ethics, regulatory rationale, and international trends in human stem cell-derived products. This led to the development of the Japanese official Notification No. 0907-4, "Guideline on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Autologous Human Induced Pluripotent Stem(-Like) Cells," issued by Pharmaceuticals and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, on September 7, 2012. The present paper addresses various aspects of products derived from autologous human iPS cells (or iPS cell-like cells), in addition to similar points to consider that are described previously for autologous human stem cell-based products. Major additional points include (1) possible existence of autologous human iPS cell-like cells that are different from iPS cells in terms of specific biological features; (2) the use of autologous human iPS(-like) cells as appropriate starting materials for regenerative medicine, where necessary and significant; (3) establishment of autologous human iPS(-like) cell lines and their characterization; (4) cell banking and/or possible establishment of intermediate cell lines derived from autologous human iPS(-like) cells at appropriate stage(s) of a manufacturing process, if necessary; and (5) concerns about the presence of undifferentiated cells in the final product; such cells may cause ectopic tissue formation and/or tumorigenesis. The ultimate goal of this guidance is to provide suitable medical opportunities as soon as possible to the patients with severe diseases that are difficult to treat with conventional modalities.

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<sup>☆</sup> Recently, this type of product has been defined as a distinct product from both conventional pharmaceuticals and medical devices according to the revised Pharmaceutical Affairs Law -renamed the Pharmaceuticals and Medical Devices, and Other Therapeutic Products Act. (Akinori Hara, Daisaku Sato, and Yasuyuki Sahara: New Governmental Regulatory System for Stem Cell-Based Therapies in Japan. *Therapeutic Innovation & Regulatory Science*. 2014; **48**(6): 681–688).

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## 1. Background (chronology and focus of the research)

The details of the series of the present studies have been described in the previous papers [1,2]. The present paper provides a summary of points that are closely related to those in the first paper.

Development of regenerative medicine using cell-based products derived from the processing of human cells and tissues is keenly anticipated in Japan because of difficulties in securing human organs and tissues in our country. With breakthroughs in technology and advances in research, more and more people are hopeful that this medical technology using novel cell-based products will result in the development of effective therapies.

In Japan, translational research on regenerative medicine is advancing rapidly. In particular, much work has been done on product development using human stem 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 on the evaluation of the products expected to be developed in the near future. Identifying the technical, medical, and ethical conditions necessary for utilizing various types of stem cells at an early stage of development is vital for their rapid application in clinical settings.

In the fiscal year 2008, the Japanese Ministry of Health, Labour and Welfare decided to form a panel of experts: the “Study Group on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from Processing of Human Stem Cells.” The panel was established as a scientific research project of the Ministry of Health, Labour and Welfare and has subsequently been chaired by Dr. Takao Hayakawa.

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, their ethical validity, the regulatory rationale, and international trends in human stem cell-derived products, and to establish and implement appropriate safety evaluation criteria.

As a result of the examination until 2009, in accordance with the Pharmaceutical Affairs Law, and with the goal of facilitating clinical application of products derived from human somatic stem cells, iPS cells, ES cells, and other cells, the study group concluded that to facilitate the conduct of efficient, effective, and rational research and development (R&D), the relevant guidelines should be tailored to specific cell sources and phenotypes (autologous human versus allogeneic human, and somatic stem cells vs. iPS cells vs. ES cells vs. other cells). Points to be considered include but are not limited to relevant technical details, the manufacturing process, characterization, quality control, and stability evaluation as well as the data required to determine the safety and efficacy of the products.

In 2009, 2 interim reports on draft guidelines on autologous human somatic stem cell-based products and autologous human iPS(-like) cell-based products were developed based on the existing Japanese Ministry of Health, Labour and Welfare Notification No. 0208003 and on the above considerations. Three other interim reports detailing draft guidelines were also developed for allogeneic human somatic stem cell-based products, allogeneic human iPS(-like) cell-based products, and human ES cell-based products according to Japanese Ministry of Health, Labour and Welfare Notification No. 0912006. These 5 sets of draft guidelines, still at the interim stage, were presented as the subjects of thorough discussions from a variety of viewpoints. They were widely circulated among the interested parties as articles published in a relevant scientific journal to elicit readers' comments (Hayakawa T., et al.: Regenerative Medicine [Journal of the Japanese Society for Regenerative Medicine], 9, 116–180 [2010], in Japanese). Thereafter, these articles were updated and published as a series of 8 articles (Journal

of the Japanese Society for Regenerative Medicine, 10, 86–152 [2011]), which would form the basis of the final draft guidelines. After extensive discussions with the study group and after public consultation, the Pharmaceutical and Food Safety Bureau of the Ministry of Health, Labour and Welfare of Japan issued 5 notifications on September 7, 2012, as described previously [1].

In this paper, in continuation of the previous papers [1,2], we introduce guidelines on the basic technological requirements for ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of autologous human iPS(-like) cells.

The generation of iPS cells by Yamanaka and colleagues demonstrated that differentiated cells can be reprogrammed artificially. This monumental work suggests that differentiation and dedifferentiation can be manipulated as needed. The technology raises great hopes of practical applications in basic biological research, medical research on pathogenesis, drug discovery through establishment of novel systems for efficacy and toxicity tests, and in regenerative medicine.

Needless to say, the ultimate goal of regenerative medicine is to treat patients. Therefore, we should always adopt a treatment (objective)-oriented approach and give priority to the consideration of potential target diseases and products for development. The paradigm shift brought by the discovery of iPS cells provides limitless possibilities for regenerative medicine. This, however, does not necessarily mean that all regenerative medicine should be practiced on the presupposition of a standardized degree of reprogramming or other properties of iPS cells. If iPS cells can be standardized and their state of pluripotency made precisely constant, iPS cells may serve as crucial and highly specific raw materials for the development of cell-based pharmaceuticals and medical devices for regenerative medicine. However, this does not necessarily mean that all products shall be produced using a specific iPS cell lineage. It is crucial to consider that when manufacturing an individual product from a certain type of cells, the cells chosen should be the “appropriate raw materials” for the product. In other words, the most important criterion for certain artificially induced pluripotent stem cells would be whether they have been confirmed to be a suitable raw material for the manufacture of a final product; this approach ensures quality, efficacy, and safety sufficient for a specific treatment (objective). The challenges for the researchers and developers would include (1) which types of pluripotent stem cells to use as a raw material: cell-of-origin, reprogramming method, and degree of reprogramming; and (2) how to obtain the final product from the pluripotent stem cells: the differentiation protocol and intermediate cell state.

Based on the concept mentioned above, these guidelines refer to both “human iPS cells” and “human iPS-like cells” and provisionally define these 2 types of cells as follows:

“Human induced pluripotent stem cells (iPS cells)”: Cells generated from somatic cells through artificial reprogramming by introducing genes or proteins, or, for instance, by chemical or drug treatment, or cells that are obtained from such cells through cell division, and which possess the ability to differentiate into the endoderm, mesoderm, and ectoderm, and furthermore, maintain the ability to self-renew or a similar ability.

“Human induced pluripotent stem-like cells (iPS-like cells)”: Cells generated from somatic cells through artificial dedifferentiation by introducing genes or proteins, or, for instance, by chemical or drug treatment, or cells that are obtained from such cells through cell division, and which at least possess the ability to differentiate into some type of endoderm, mesoderm, or ectoderm, and furthermore, to maintain the ability to self-renew or a similar ability.

Raw materials in biologics cannot be adequately characterized or quality controlled due to their indistinct origin and complexity; the same holds true for final products due to their limited quantity and complex quality attributes. To address these concerns, it is very important to ensure constancy and robustness of the manufacturing process in the production of all types of biologics. The core technical element required is to establish base camp(s), i.e., to prepare substrates for production of biologics at relevant stage(s) in the manufacturing process; these substrates can be extensively characterized and controlled and are of stable quality; constant processing of these substrates into the subsequent intermediate(s) and finally to a desired product is achievable.

The ideal base camp(s) in the sustainable manufacture of human iPS or iPS-like cell-based products are cells (banks) and/or intermediate cell products/lines that have been well characterized, are stable per se but can propagate under appropriate conditions, can be renewed, are ready for supply upon request, and can differentiate into target cells. For certain final products, it may be more feasible for the consistent, safe manufacture of the desired products to establish sustainable intermediate cell products/lines (as a form of a cell bank) at an intermediate stage of the manufacturing process, than to emphasize characterization, evaluation, or control of cells at the raw-material stage, which may be difficult to perform. It is, of course, essential to explain the advantages and appropriateness of such a procedure. When establishing cell lines at each stage of differentiation with different phenotypes, procedures for the process of cell generation such as differentiation, isolation, cultivation of target cells, generation of cell lines, the growth medium; culture conditions; culture period; and survival rate should be clearly documented and justified as much as possible. To maintain consistency and stability of intermediate cell products/lines, critical indicators such as purity, morphology, specific cell markers, karyotypes, proliferation, and differentiation should be selected, and acceptance criteria should be set accordingly. In addition, the passage number and/or population doublings of intermediate cell products/lines should be specified so that quality meets the acceptance criteria.

For products derived from human iPS cells or iPS-like cells (hereafter referred to as iPS(-like) cells), the presence of undifferentiated cells in final products is a major safety concern (e.g., ectopic tissue formation and tumorigenesis). However, because this concern is raised from one of iPS(-like) cells' strongest characteristics, it is quite difficult to avoid. Elimination of intrinsic characteristics of iPS(-like) cells is a trade-off at least in principle, and is thus considered very difficult. Accordingly, it is necessary to have a strategy and tactics to develop safer final products by improving manufacturing process and process control more effectively rather than discussing safety issues at iPS(-like) cell level. These draft guidelines, therefore, require demonstration of the absence of undifferentiated cell contamination at the level of an iPS(-like) cell-derived bank and/or intermediate and/or final cell products by thorough analysis, or an effort to develop efficient methods to eliminate or inactivate undifferentiated cells in the course of cell processing. Furthermore, selection of administration methods will help to minimize safety concerns. These guidelines also explain the importance of technical development to generate and characterize iPS(-like) cell-derived somatic stem cells, which may lead to safe, stable, characteristically well-defined, and appropriate raw materials. In addition, the need for R&D on examination techniques to predict the pluripotency and the differentiation potential of each iPS(-like) cell lineage and processing techniques to induce target cells efficiently and properly and to isolate differentiated cells from

undifferentiated cells during processing will provide novel business opportunities.

These draft guidelines include discussion of all of the above-stated aspects of iPS(-like) cells. The iPS(-like) cells possess pluripotency and self-replication abilities exceeding those of normal somatic stem cells and can therefore differentiate into a variety of cell types, depending on the processing technique used. Such iPS(-like) cell-based products may be clinically applied heterologously, i.e., in an environment that differs from the environment where the cells perform their natural endogenous function. Concerns about these points have been included in these human iPS(-like) cell guidelines in reference to Japanese Ministry of Health, Labour and Welfare Notifications No. 0208003 and No. 0912006, which serve as the basis for these guidelines.

When interpreting and implementing the present guidelines, the following arguments should be considered. The ultimate goal is to provide novel therapies to patients by means of regenerative medicine. The role of these guidelines is to present scientific principles, concepts, ideas, and technical elements that should help to achieve a specified goal in the most efficient and effective manner possible. Because a wide variety of products are anticipated, encompassing a variety of situations and circumstances, these guidelines describe comprehensive points of concern. It is necessary to determine the relevant testing parameters and evaluation methods by considering the characteristics of the cells in question, the specific clinical objective, and the method of application (among other parameters). Those items that are applicable should be justified and put into practice in an appropriate and flexible manner.

Several points should be kept in mind with regard to the development of products for regenerative medicine and the implementation of this guideline. The desired products are expected to show promise as a novel therapeutic method as a result of proof of concept (POC) and relevant data, indicating no critical concerns about product safety that might impede to the use of the products in humans for the first time. Thorough observance of the Declaration of Helsinki, including proper informed consent and the right of self-determination of the patient, is indispensable.

It should be emphasized again that our primary goal is to offer suitable medical opportunities as soon as possible to patients with severe diseases that are difficult to treat with conventional modalities. The present guidelines should serve this purpose. Therefore, it is important to interpret and employ these guidelines flexibly and meaningfully in this context. Stringent observance of these guidelines without primary consideration of the patient and his/her specific situation should be avoided.

Progress in the actual use of regenerative medicine is clearly desirable for maintaining and improving human health. The development of innovative and revolutionary medicinal products and therapeutic techniques should be beneficial to our country as well as the international community and is a way to make a peaceful international contribution that will be a legacy for all mankind. The role of the government here is to promote clinical research and industrialization; relevant regulations and guidelines are important measures undertaken to achieve this common goal in a scientific, rational, efficient, and effective way. All those involved, like players in the same arena with a common goal in mind, accumulating scientific data and concentrating wisdom, should continue to make efforts to deliver these revolutionary cell-based products and therapeutic techniques to patients as soon as possible.

**Guidelines on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from Processing of Autologous Human Induced Pluripotent Stem(-Like) Cells.** (Notification No. 0907-4, issued by Pharmaceuticals and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, on September 7, 2012).

## 2. Introduction

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

Autologous human iPS(-like) cell-based products are obtained by artificially inducing differentiation of various types of iPS(-like) cells generated artificially from autologous human somatic cells; they are used directly or after further processing. There are many types of manufacturing methods, intermediates, types and characteristics of the desired cell products, and methods for clinical application. In addition, the scientific progress in this field is incessant, while expertise and knowledge are constantly accumulating. Therefore, it is not always appropriate to consider the present guidelines all-inclusive and definitive. Consequently, when testing and evaluating each product, it is necessary to adopt, on a case-by-case basis, a flexible approach according to a rationale that reflects the scientific and technological advances at that point in time.

2. The main purpose of evaluating the quality and safety of the desired cell products before conducting investigational clinical trials (e.g., at the time of “clinical-trial consultation”) is to determine whether there are any quality and/or safety problems that would obviously hinder initiation of human clinical trials of the iPS(-like) cell-based products in question, whether certain quality attributes (QA) of the product are understood sufficiently to establish a relationship between the clinical findings and the QA, and whether consistency of the QA can be ensured within a definite range. Simultaneously, it is important to eliminate any known risk factors associated with the product quality and safety as much as possible, using up-to-date science and technology, and to describe the scientific appropriateness of the results of such an action. The remaining presumed risk factors should be weighed against the risks associated with not performing the trials on patients who suffer from diseases that are serious and life-threatening or that involve marked functional impairment or a marked decrease in quality of life (QOL) resulting from the loss of a certain degree of physical function or form, or for which existing therapies have limitations and do not result in a cure. Furthermore, it is also important to entrust the patient with the right to make a decision, after receiving all of the available information. When applying for approval of investigational clinical trials, applicants can submit a provisional nonclinical data package, which is prepared rationally by taking into account product aspects and patient aspects including a balance between the risk of the product vs. the risk facing the patient with/without treatment in question, for the decision to initiate investigational clinical trials, on the premise that the data package submitted at the time of marketing application/registration to ensure quality and safety will be enriched in line with the guidelines as the clinical trial progress. Finally, applicants are encouraged to discuss with the Pharmaceuticals and Medical Devices Agency (PMDA) the type and

amount of data that may be needed to initiate a particular clinical trial. Because of differences in product origin, target disease, target patients, application sites, application methods, and processing methods, there may be numerous variations among individual data packages; these differences cannot be definitively clarified in the present guidelines.

3. The items, test methods, criteria, and any other technical requirements described in the present guidelines are intended to be considered, selected, applied, and evaluated to serve each intended purpose; they do not necessarily require the most stringent level of interpretation and practice. Applicants are encouraged to explain and provide justification for any considerations regarding the background, selection, application, and the content as well as the extent of evaluation that are appropriate for their own purpose and are scientifically valid.

## 3. Chapter I. General principles

### 3.1. Objective

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

### 3.2. Definitions

The definitions of the technical terms used in these guidelines are as follows:

1. “Human induced pluripotent stem cells (iPS cells)”: Cells that are generated from somatic cells through artificial reprogramming by introducing genes or proteins, or via chemical or drug treatment, or cells that are obtained from such cells through cell division, and which possess the ability to differentiate into the endoderm, mesoderm, and ectoderm, and furthermore to maintain the ability to self-renew or a similar ability.
2. “Human induced pluripotent stem-like cells (iPS-like cells)”: Cells that are generated from somatic cells through artificial dedifferentiation by introducing genes or proteins, or via chemical or drug treatment, or cells that are obtained from such cells through cell division, and which at least possess the ability to differentiate into some type of endoderm, mesoderm, or ectoderm, and furthermore maintain the ability to self-renew or a similar ability.
3. “Processing of cells and tissues”: Any processing of a cell type 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, or manipulation using genetic engineering, with the aim of preparing desired cell products to treat a patient or to repair or regenerate tissues. Isolation of a tissue, homogenization of a 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 that are regarded as minimal manipulations, are not considered “processing.”
4. “Manufacture”: Actions undertaken before the final product (an autologous human iPS(-like) cell-based product) is released to

the market. This includes, in addition to processing of cells and tissues, minimal manipulations such as isolation of a tissue, homogenization of a 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.

5. "Phenotype": A morphological or physiological characteristic that is produced by a certain gene under constant environmental conditions.
6. "Donor": A person who donates his/her own somatic cells, which serve as a raw material for an autologous human iPS(-like) cell-based product. For such a product, a patient is definitely a donor. (Note: A patient is identified as a donor for actual treatment. It is also presumed that cells/tissues obtained from a donor other than a patient are used for the purpose of test production during R&D stages.)
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.
8. "Protein transductant": A construct that contains a target protein and elements such as reagents necessary for introducing the target protein into a target cell.

#### 4. Chapter II. Manufacturing methods

Describe all the important and relevant information concerning the manufacturing method, taking into account the items listed below. This information will contribute to ensuring the quality, safety, and efficacy of the final product, and is important for guaranteeing quality consistency from the manufacturing perspective. It should be noted that assurance of quality and safety and their consistency is achieved via mutual complementary measures throughout the manufacturing method as a whole, and it is very important that the measures be rational and serve the intended purpose. It is acceptable to omit a portion of the items listed below, if the appropriate scientific basis for ensuring the quality, safety, and constancy of the final products can be provided by means of suitably chosen quality tests or controls of the final product or intermediates, or control of the manufacturing process.

##### 4.1. Raw materials and materials used in manufacturing

###### 4.1.1. Human somatic cells that serve as raw materials

- (1) Features of biological structure and function, and selection criteria

Provide and explain the reasons for selecting the somatic cells used as raw materials based on the characteristics of their biological structure and function, such as morphological characteristics, growth characteristics, biochemical indicators, immunological indicators, specific substances produced, and other suitably chosen and appropriate genotype or phenotype indicators (or markers). It is acceptable to perform test production and tests using test specimens obtained from a donor other than a patient at the R&D stages, before beginning the clinical trials.

This should lead to the identification of the critical cell characteristics that are to be employed when preparing patient-derived somatic cells as raw materials. It is recognized that such a study can

only be performed with certain limitations because there are quantitative limits on samples as well as technological limitations.

##### (2) Donor considerations

To ensure the safety of patients, manufacturing personnel, and healthcare workers, establish test parameters for any infections that may be transmitted via the collected somatic cells, and provide justification for the parameters. Special consideration should be given to hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and human T-lymphotropic virus (HTLV).

Establish eligibility criteria that take into account the genetic characteristics, the medical history, and the health condition of the patient and explain the suitability of the donors. If donor genomic or gene analysis is undertaken, it shall be conducted in accordance with the "Ethical Guidelines for Analytical Research on Human Genome and Gene" published jointly on February 8, 2013, and revised on November 25, 2014, by the Japanese Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labour and Welfare; and the Ministry of Economy, Trade and Industry.

##### (3) Donor records

Complete donor records shall be retained so that any information required to ensure the safety of the somatic cells 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 specific information corresponding to the intended use of individual cells.

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

- (i) Eligibility of personnel and medical institutions collecting the samples

Describe the technical requirements for the 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 as well as the sampling method and clearly state why the selected sites are both scientifically and ethically appropriate. For the 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-up), and cross-contamination.

- (iii) Informed consent of donors

Describe the details of the informed consent obtained from the donor of the cells and/or tissues.

- (iv) Protection of donor privacy

Indicate the measures adopted to ensure protection of donor privacy.

- (v) Tests to ensure donor safety

If tests, such as those to confirm the state of the sampling site, must be performed to ensure donor safety at the time of cell or tissue sampling, describe the details of the tests as well as any interventions undertaken after test results indicate a problem.

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

If the somatic cells collected must be stored for a defined period of time, set the storage conditions and

storage period and explain their appropriateness (validity). Describe in detail the measures and procedures to be followed to prevent erroneous sampling (mix-up).

(vii) Transportation methods

If cells and/or tissues or iPS(-like) cells that were collected must be transported, define the containers to be used for transport and the transportation procedures (including temperature control), and provide the justification.

(viii) Preparation of records and storage procedures

Written records for items (i) through (vii) above shall be prepared, and proper record storage procedures shall be described in detail.

#### 4.1.2. Raw materials other than target cells and tissues as well as materials used in the manufacturing

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

When so-called Biological Products or Specific Biological Products (refer to Articles 2.9 and 2.10 of the Pharmaceutical Affairs Law) are used as raw materials, the amounts used should be kept to the minimum amount required and should strictly conform to the relevant laws and regulations, such as the “Standards for Biological Raw Materials” (Notification Number 210, Japanese Ministry of Health, Labour and Welfare, 2003; a partially revised version was issued on September 26, 2014). It is particularly important to adequately evaluate information related to the inactivation and elimination of viruses and to specify measures for encouraging retrospective survey 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, includes any relevant elements/concerns.

(1) Cell culture

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

(ii) Consider 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; quality should be controlled using standards equivalent to those for pharmaceuticals and pharmaceutical ingredients.

(b) 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 a single raw-material set.

(c) 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, to the extent possible, ways to avoid using these serum components. If the use of serum or other such materials is unavoidable, consider the following points, and investigate ways to prevent the contamination and transmission of bacteria, fungi, viruses, and prions from the serum and other related materials 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 components.

(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) Use these batches of serum only 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) 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, use combinations of heat treatment, filtration,  $\gamma$ -irradiation, and/or ultraviolet light treatment, if necessary.

(e) Preserve and store a portion of the serum used to enable monitoring of cultured cells for viral infections and onset of viral diseases among the patients 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 Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products” (Pharmaceutical Notification Number 873, July issued 14, 2000, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare), “Guidelines on Public Health Infection Issues Accompanying Xenotransplantations” (Notification 0709001, issued July 9, 2002, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labour and Welfare), 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, issued July 2, 2004, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labour and Welfare) in order to prevent contamination and transmission of bacteria, fungi, viruses, and prions from the feeder cells; indicate the methods used for

inactivation of the cell division potential; and state 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 is 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 much as possible. However, if antibiotics are deemed indispensable at the initial stages of processing, attempt to decrease their use at subsequent steps as much 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 determined that an antibiotic can be adequately eliminated from the final product, its use does not need to be restricted. However, if a patient has a history of allergy to the antibiotic used, this therapeutic method should not be used. If the 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 appropriate quality control methods using relevant parameters, for example purity and potency, for which established acceptance criteria and assay methods are employed, to guarantee the reproducibility of cell culture characteristics.
  - (vii) For media components and other components used in manipulation and those that may contaminate 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 cells of heterologous origin.
- (2) Combination of cells with noncellular components
- (i) Quality and safety of noncellular raw materials

If the final product consists of cells combined with noncellular components, such as a matrix, medical materials, scaffolds, support membranes, fibers, or beads, 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; the form and function in the final product; and evaluation of their quality, safety, and efficacy for the presumed clinical indication. When using materials that are absorbed by the body, perform the necessary tests for the safety of the 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. 0213001, issued February 13, 2003, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare), describe the test results, and provide justification for the use of such raw materials. Rational use of information from scientific literature is also encouraged.

- (ii) Interactions with target cells

Demonstrate the validity of the tests used and provide justification for the results obtained for the following 3 items with respect to the interactions between noncellular components and cells in the final product and in any intermediate products.

- (a) The noncellular components do not have any deleterious effects on the function, growth capacity, 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 cells and noncellular components, taking into consideration, for example, mutation, transformation, and/or dedifferentiation of the cells in the final or intermediate products.
  - (c) Demonstrate that there is no loss of the expected properties of the noncellular components for 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 components to isolate the desired cell products from the application site
- When using noncellular components with the objective of segregating the desired cell products from the application site, confirm their usefulness and safety by referring to points (a) through (d) below.
- (a) Membrane permeability kinetics and pharmacological effects of target physiologically active substances derived from the cells in the final product.
  - (b) Diffusion of nutritional components and excretory products.
  - (c) Effects of noncellular components on the area near the application site.
  - (d) 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 product and/or undifferentiated cells is the objective, confirm that the cells will not leak after, for example, degradation 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 (the specific gene encoding a desired protein or RNA): information related to its structure, origin, method by which it was obtained, cloning methods; for the cell bank of the target gene: methods of preparation of the cell bank, control, and renewal, and other relevant techniques.
- (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, their properties, and procedures (transgenic methods, and origin, properties, and method for obtaining the vector used for introduction of the transgene) required to produce the transgenic construct.
- (v) Structure and characteristics of the transgenic construct.
- (vi) Control and preparation methods for cell and virus banks that are 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” (hereafter referred to as “Gene Therapy Pharmaceutical Guidelines”), published as Notification No. 1062 by Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare on November 15, 1995. In addition, clearly state the appropriateness of the establishment of cell lines in accordance with the appendix of the same notification.

Be aware that, based on the law (Law No. 97, 2003) for ensuring the biodiversity of living organisms by regulating the use (and other aspects) of genetically modified organisms and related organisms, 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 organisms: “human cells” or “cells that have the ability to differentiate, or differentiated cells that 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 to simply describe how the quality and safety of the gene matches 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 protein in question into cells, provide information on its preparation, quality control, and renewal methods.
- (vi) Preparation of cell banks and cell bank control methods to produce the introduced proteins.

Regardless of the above, if a protein that is 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 to simply describe how the quality and safety of the protein corresponds to the intended use.

#### (5) Reprogramming or inducing dedifferentiation and/or differentiation of cells using drugs or any chemicals

When inducing reprogramming or dedifferentiation, and/or differentiation 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, for example, target drugs.

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

Describe the details of the methods used when inducing cell reprogramming or dedifferentiation and/or differentiation using physical methods in question.

#### (7) Cell reprogramming, 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 induce cell reprogramming, dedifferentiation, and/or differentiation.

#### 4.1.3. Establishment of autologous human iPS(-like) cell lines

Describe the methods used up until the establishment of autologous human iPS(-like) cell lines from the somatic cells that serve as the raw material, and indicate, as thoroughly as possible, the appropriateness of the methods. These include the methods for obtaining the human somatic cells, for separating and culturing of somatic cells, for inducing reprogramming or dedifferentiation of the somatic cells, for isolating and preparing cell lines of the reprogrammed or dedifferentiated cells as well as the media, culture conditions, culture period, yield, and other parameters at each step in the process until establishment of the autologous 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 population purity, morphological features, phenotype-specific markers, karyotype, cell growth properties, and pluripotency) and set acceptance criteria for them. In addition, demonstrate the number of passages or of cell divisions within which cells can proliferate while maintaining their quality in terms of the criteria specified.

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

For human iPS(-like) cell lines, perform appropriate stability tests based on the viability, potency, and other characteristics of the cells, establish the storage method and validity period, and clarify their suitability, considering storage duration, distribution, and the storage form. In particular, when freezing and thawing, confirm whether freezing and thawing affect the stability or any other characteristic of the cell line. Where necessary and possible, conduct stability studies on the cell line whose storage period exceeds normal periods in order to confirm, to the extent possible, the limits of stability. 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) shall be determined and their appropriateness clearly indicated.

#### 4.1.5. Preparation of records and storage procedures

Written records for items 4.1.2–4.1.4 above shall be prepared and proper record storage procedures shall be clearly described.

### 4.2. Manufacturing process

When manufacturing autologous human iPS(-like) cell-based products, describe in detail the manufacturing method and verify, as thoroughly as possible, the appropriateness of the method using the items listed below to maintain consistent product quality.

#### 4.2.1. Lot control

Indicate whether a lot control procedure is applied for final and intermediate products. If any lot control is adopted, establish standardized procedures for the makeup and control of the lot,

which may include the lot size, labeling/numbering, testing method and acceptance criteria.

#### 4.2.2. 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) to the establishment of autologous 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 or somatic 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 factors of target cells, cell viability, characterization of cells and tissues, and microbiological tests. At the stage of initiation of clinical trials, provide the actual measured values obtained on 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

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

##### (3) Tissue homogenization, cell separation, isolation of specific cells, and other techniques

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

##### (4) Establishment of autologous human iPS(-like) cell lines

Describe the methods used up until the establishment of iPS(-like) cells from somatic cells that serve as the raw material, and describe, as thoroughly as possible, the validity of the methods.

Identify critical quality attributes of the cells and set acceptance criteria for them. Demonstrate the number of passages or cell divisions within which cells can proliferate while maintaining their quality in terms of the criteria specified (refer to Chapter II-4-1-3).

##### (5) Establishment of an intermediate cell line derived from autologous 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 and for scientific validity of the 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 duration, the yield at each stage) until establishment of each respective cell line and explain their appropriateness to the extent possible.

To maintain the stability and consistency of the quality of the intermediate cell lines, identify critical quality attributes of the cells (for example, cell population purity, morphological features, phenotype-specific markers, karyotype, cell growth properties, and differentiation potency) and set acceptance criteria. Demonstrate the number of passages or of cell divisions within which cells can proliferate while maintaining their quality in terms of the criteria specified. Although comprehensive cell characterization is always desirable, it is recognized that quantitative limits on samples or technological limits may make it difficult to perform the study fully. If this is the case, it is acceptable to perform a limited study to the extent possible.

If establishing and utilizing a cell bank from an intermediate cell line in accordance with the above, refer to point (7).

##### (6) Preparation of cells that compose a principal component of the final product and serve 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, that are 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 as well as the media, culture conditions, culture duration, the yield of the desired cells, and other characteristics 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 autologous human iPS(-like) cell-based products, describe the details of the rationale for preparing the cell bank; the methods used to prepare the cell bank; characterization of the cell bank; and storage, maintenance, control, and renewal methods as well as any other processes and tests performed and provide justification for each. Refer to “Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products” (Pharmaceutical Notification No. 873, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, July 14, 2000) and other relevant documents. It is acceptable to omit a portion of the test items if the cells have been properly evaluated at an upstream point in the process (for a valid reason) or if the cells are of autologous origin.

##### (8) Measures to prevent erroneous sampling (mix-up) and cross-contamination during the manufacturing process

It is extremely important to prevent erroneous sampling and cross-contamination during the manufacturing process when manufacturing autologous human iPS(-like) cell-based products. Therefore, clearly describe preventive measures in the control process.

#### 4.2.3. Characterization of cells that comprise a principal component of a final product and serve as an active ingredient

Analyze various attributes of the cells, such as cell population purity, to control contamination with undifferentiated or nontarget 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 the cells that make up a principal component of the final product. Additionally, characterize the cells in relation to biological functions, where necessary. Furthermore, to evaluate the appropriateness of the culture duration and stability of the cells, use appropriate markers of cell characteristics to prove the absence of unintended changes in cells cultured beyond the proposed culture period. It is acceptable to perform these studies preliminarily, using test samples obtained from donors who are not patients in place of the products that will be prepared for a clinical trial. These results can be used to identify the critical cell characteristics that should be used when applying the real product to the treatment of a patient. Although comprehensive cell characterization is always desirable, quantitative limits on samples or technological limitations may prevent full characterization. In this case, it is acceptable to perform a limited 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.

#### 4.2.4. *The form and packaging of the final product*

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

#### 4.2.5. *Storage and transport of the final product*

If an 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) shall be stated and their appropriateness clearly indicated (refer to Chapter III).

#### 4.2.6. *Consistency of the manufacturing procedure*

To assess the consistency of the manufacturing process using each product (each lot) obtained from different production runs, determine whether they differ significantly with respect to the number of cells, cell viability, and cell characteristics (such as relevant markers of a phenotype and/or genotype, functional characteristics, and the percentage of desired cells), considering the application methods and intended use of the product. It is acceptable to use test samples obtained from donors who are not patients in place of the real products that will be prepared for a clinical trial. Evaluation using intermediate products may provide an accurate explanation of the suitability of the cells and tissues for use as raw materials and the validity of the manufacturing process until the point of production of the intermediate products as well as may serve 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 lengthy, perform sterilization tests and other relevant procedures at consistent intervals to confirm that sterility has been preserved.

#### 4.2.7. *Changes in the manufacturing process*

If the manufacturing process is altered at some point during development, and if test results that are 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.

### 4.3. *Quality control of the final product*

#### 4.3.1. *Introduction*

The overall quality control strategy for autologous human iPS(-like) cell-based products includes specifications (a set of acceptance criteria and analytical procedures) for final products, quality control of raw materials for each therapeutic application to each 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 the absence of contamination of the cells by undifferentiated cells other than the desired cells. Verification of the absence of contamination by nontarget undifferentiated cells is desirable, as much as possible, at the intermediate-product stage.

Specifications will differ among final products, depending on the type and properties of the desired cells and tissues, manufacturing methods, intended clinical use, the method of clinical application of each product, stability, and available test methods. These differences shall be taken into consideration when setting acceptance criteria and analytical procedures. In addition, specifications shall be set and justified from the standpoint of achieving the purpose of quality control as a whole, by taking into consideration the mutually complementary relationships among 1) verification of the suitability of the manufacturing process, 2) the method for 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 question is unlikely to pose significant quality/safety problems for use in investigational clinical trials. Therefore, it is possible to set provisional specifications, with allowance for some variation, based on values measured using a few test specimens, as long as one can be certain of the relationships between the results of clinical tests and such quality attributes after clinical trials. However, testing for sterility and the absence 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.

#### 4.3.2. *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 provide 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 lot is typically a unit subjected to quality control.

##### (1) Cell number and cell viability

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

##### (2) Tests of identity

Confirm that the cells are the intended target cells using markers for critical cell characteristic(s) selected from 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 the cell population in a final product, set the test parameters, test methods, and acceptance criteria for evaluating and controlling nontarget cells, such as undifferentiated cells, cells exhibiting abnormal growth, transformed cells as well as the presence of any other contaminating cells, considering the origin of the target cells and tissues, the culture conditions and other parameters of the manufacturing process, such as quality control of intermediate products. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on measured values obtained for a small number of test samples.

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

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

### (5) Tests for process-related impurities

For substances that may be present in the final product as, for instance, contaminants, residues, newly generated products or degradation products; that potentially originating from raw materials, noncellular 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 and antibiotics), it is necessary to 1) 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 2) establish appropriate tests to control the amount of the substance in the final product within permissible levels. When selecting substances to be tested and setting their acceptance criteria, their suitability should be explained and justified.

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

### (6) Sterility tests and tests for the absence of mycoplasma

The sterility of the final product should be adequately assessed to ensure sterility throughout the entire manufacturing process, using test samples. The sterility (negative results of tests 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 be obtained only after administration to the patient, the proper measures for dealing with the potential lack of sterility should be established beforehand. In such cases, the intermediate products must be demonstrated to be sterile, and sterility should be strictly maintained 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 ensured, tests using only representative samples are sufficient. When tests must be conducted for each clinical application, and if the results of sterility and other tests can be obtained only after

administration to the patient, the decision on whether the clinical application should proceed will be determined based on the most recent data. However, even in this case, sterility tests and other tests shall be performed on the final product.

It is desirable that every effort be made to avoid the 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 test

Perform an endotoxin test, considering the impact of a 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 based on a single dose of the final product. Endotoxin testing can be established as an in-process control test; however, in such cases, establish criteria, including validation results, and provide the justification.

### (8) Virus tests

If the absence of HBV, HCV, HIV, and HTLV cannot be proven at the patient level, and if these viruses may proliferate in the cells, conduct virus titer tests and confirm that administration of the iPS(-like) cell-based products will not lead to any adverse effects on the patient. This does not apply if tests proving the absence of viruses are performed on intermediate products or at the cell bank. If components of biological origin are used in the manufacturing process, it may be necessary to conduct tests on the final product for viruses originating from those components. However, whenever possible, it is preferable to verify the absence of contamination by testing or via process evaluation at the upstream stage, including tests on the original components.

### (9) Specific biological tests

In some instances, it will be necessary to consider specific (quantitative or qualitative) biological testing that takes into consideration 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 based on measured values obtained for a small number of test samples.

### (10) Potency assay

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 autologous human iPS(-like) cell-based product during its intended clinical use, establish test parameters and/or acceptance criteria related to this substance in order to demonstrate the intended effect. Set acceptance criteria for potency or quantitation for a gene expression product secreted from the cells when a transgene was introduced. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on measured values obtained for a small number of test samples.

### (11) Mechanical compatibility tests

For products that require a certain degree of dynamic 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 obtained for a small number of test samples.

### 5. Chapter III. Stability of autologous human iPS(-like) cell-based products

Taking into full consideration the storage and distribution periods and the storage form, perform suitable stability testing on autologous human iPS(-like) cell-based products and/or critical intermediate products based on cell viability, potency, and other characteristics to establish storage methods and expiration date, and provide justification for their suitability. 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 criteria of the product. Where necessary and possible, it is recommended to conduct stability studies on products whose manufacturing or storage period exceeds the normal period, in order to confirm, as much as 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) shall be set and their appropriateness justified.

### 6. Chapter IV. Preclinical safety testing of autologous human iPS(-like) cell-based products

Relevant animal tests and/or *in vitro* tests may be performed to elucidate concerns about the safety of an autologous human iPS(-like) cell-based product when it is scientifically reasonable and technically possible. Safety concerns about noncellular constituents and process-related impurities should be resolved, as much 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 are 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 at the intermediate-product stage, or 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 risks.

Animal testing of products of human origin does not always yield meaningful results. Thus, there may be a scientific rationale for preparing product models of animal origin and testing on appropriate experimental animals if more useful information may be obtained. In such a case, consider conducting tests on suitable animal models for each target disease. (Note: For example, monkeys may be suitable for neurological diseases, while pigs and/or dogs may be suitable for cardiovascular diseases.) However, because the use of identical procedures in nonhuman animals will not necessarily yield cell groups that possess characteristics identical to those of cells that constitute an autologous human iPS(-like) cell-based product, and because a product of animal cell origin that was manufactured using identical processing, including culture conditions, 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 nonhuman animal species, explain the suitability of the 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 that tests be conducted without a rational basis. Conduct necessary and appropriate tests, taking into account the characteristics of the product, intended clinical use, and other parameters, and evaluate and discuss the results in a comprehensive manner.

1. For cells expanded beyond the defined limit for cultivation (duration of culture, the population doubling level, or the passage number of the cells) for routine production, clearly demonstrate that undesirable alterations other than the intended transformation and abnormal proliferation of nontarget cells have not occurred.
2. It may be necessary to conduct quantitative assays of particular physiologically active substances produced by the cells and tissues and to 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 of the product on the healthy 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, appropriateness of the test system, and other characteristics.
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 an intermediate product. These studies should be performed suitably by taking into account the type and characteristics of the product, number of cells, route of administration, mode of application (e.g., cell sheet or cell suspension), cell engraftment site, target diseases, appropriateness of the tests systems, and other characteristics. If there is a possibility of tumorigenicity or malignant transformation, provide justification for 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 impossibility to obtain a sufficient number of cells. Furthermore, in tumorigenicity tests using animal models, various conditions, such as cell dispersion and cell adhesion to scaffolding, cell density, and administration site, are not always 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 as a result of treating the disease.)

7. If an exogenous gene is introduced into cells during the manufacturing process, and if it may function or remain as a residue in the final product, conduct tests in accordance with the “Gene Therapy Pharmaceutical Guidelines.” In particular, if viral vectors are used, determine quantitatively the potential presence of any replication-competent viruses and provide justification for the test 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 the risk of tumor formation, including benign tumors and malignant transformation. Whenever a vector, which may get inserted into a chromosome, is used, consider the necessity of evaluating possible occurrence of abnormal proliferative characteristics and/or tumorigenicity due to an insertion mutation in the cells, and the necessity of implementing long-term follow-up for clinical applications.
8. Consider conducting rationally designed general toxicological tests, if the product, including an animal-derived model of product, is easy to obtain, and if doing so will generate useful information regarding its clinical application. When conducting general toxicological 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, issued September 11, 1988, New Drug Division/Evaluation and Licensing Division, Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare).

#### **7. Chapter V. Studies supporting the potency or efficacy of autologous 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 an effect, and/or anticipated clinical efficacy (proof of concept) of an autologous human iPS(-like) cell-based product to the scientifically reasonable and technically possible extent.
2. For transgenic cells, demonstrate the expression efficiency, sustainability of expression, and biological activity of desired products derived from the (trans)gene. Discuss the rationale of the transgene expression products as active ingredients for-anticipated clinical efficacy (proof of concept) of the autologous human iPS(-like) cell-based product in question.
3. Where appropriate models of products derived from processing of animal iPS(-like) cells and/or animal models of a disease 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.

#### **8. Chapter VI. Pharmacokinetics of autologous human iPS(-like) cell-based products**

1. Pharmacokinetic studies of the internal behavior of cells/tissues that constitute the final products or expression products of transgenes (these studies may include assessment of the absorption and distribution in experimental animals), should be

performed to the technically possible and scientifically reasonable extent. Therefore, these studies are expected to estimate the survival of cells/tissues administered to patients and the duration of their effect and to determine whether the intended efficacy is successfully achieved. (Note: Testing methods may include histological studies, human Alu sequences amplification by polymerase chain reaction (Alu-PCR), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT) and bioimaging).

2. Clarify, using animal studies, the rationale for the administration method for the autologous 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 with each administration route, it is assumed that local administration is preferable to systemic administration. However, if the benefits to patients can be explained in a rational manner, it is acceptable to use systemic administration. In any case, an administration method that minimizes distribution of an iPS(-like) cell-based product to organs other than the target organ is preferred. Even if the cells localize to a site other than the intended transplantation site, the administration method is acceptable if patients experience no adverse effects. Arrhythmia caused by osteogenesis of some types of cells that ectopically locate to the heart is an example of an adverse effect that can result from ectopic differentiation.)
3. When the cells or tissues are directly applied or alternatively targeted to a specified site (e.g., tissue) 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.

#### **9. Chapter VII. Preliminary analysis of clinical trials**

The main purpose of the present guidelines is to address points to consider for evaluating the quality and safety of autologous human iPS(-like) cell-based products at the time of application for marketing authorization and at the beginning of investigational clinical trials. In the latter case, it is necessary to determine whether any quality or safety problems exist that might pose an obstacle to initiation of human clinical trials, taking into consideration the product's clinical usefulness. Thus, quality and nonclinical safety evaluation for the decision to initiate the investigational clinical trials of the product in question should be conducted with reference to the points outlined below. Any 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 on patients that suffer from diseases that are serious and life-threatening, that involve marked functional impairment, or a marked decrease of quality of life resulting from the loss of a certain degree of a physical function or form, and for which existing therapies have limitations and do not result in a cure. Furthermore, it is also important to entrust the patient with the right to make a decision after receiving all of the available information, including all information on identified/presumed 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 on the subjects, including the application of autologous human iPS(-like) cell-based products and drugs used concomitantly. (Note: If it is anticipated that drugs will be coadministered in order to maintain, enhance, and/or induce the function of the 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.

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

#### Disclosures

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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