C.1.5 ヒト（自己）iPS（様）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 Quality and Safety of Pharmaceuticals and Medical Devices Derived from Processing of Autologous Human Induced Pluripotent Stem Cell (-Like Cells)

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Background (Chronology and Focus of the Research)

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

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 in regenerative medicine is advancing rapidly. In particular, much work has been conducted 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. It is therefore urgent to prepare relevant guidelines for the evaluation of 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, Labor, and Welfare (MHLW) decided to form a panel of experts entitled 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 MHLW scientific research project and has subsequently been chaired by Dr. Takao Hayakawa.

The objective of the study group is to promote the development of products derived from human stem cells

through investigation and research into scientific and technological advances, their ethical validity, 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 a 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 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 *vs.* allogenic 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, 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, two 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 MHLW Notification No. 0208003 and on the above considerations. Three other interim reports detailing draft guidelines were also developed for allogenic human somatic stem cell-based products, allogenic human iPS(-like) cell-based products, and human ES cell-based products based on MHLW Notification No. 0912006. These five sets of draft guidelines, still in 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, in Japanese]. Thereafter, these articles were updated and published as a series of eight 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 implementation of public consultation, the Pharmaceutical and Food Safety Bureau of MHLW issued five Notifications on September 7, 2012, as described previously [1].

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

The generation of iPS cells by Yamanaka and colleagues has demonstrated that differentiated cells can be reprogrammed artificially. This monumental work suggests that differentiation and dedifferentiation can be manipulated as desired. The technology raises great hope for application in basic biological

research, medical research on pathogenesis, drug discovery through establishment of novel systems for efficacy and toxicity tests, and regenerative medicine.

Needless to say, the ultimate goal of regenerative medicine is to treat patients. Therefore, we should always take 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 could provide 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 with a specific iPS cell. 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 determined to be a suitable raw material for the manufacture of a final product, which achieves 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: 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 two 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 by chemical or drug treatment etc., or cells that are obtained from such cells through cell division, and that possess the ability to differentiate into endoderm, mesoderm, and ectoderm, and furthermore, maintain the ability to self-renew or have 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 by chemical or drug treatment etc., or cells that are obtained from such cells through cell division, and they at least possess the ability to differentiate into some type of endoderm, mesoderm, or ectoderm, and furthermore, maintain the ability to self-renew or have a similar ability.

Raw materials in biologics cannot be sufficiently 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 minimize these concerns, it is most 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 biologics production substrates at relevant stage(s) in the manufacturing process, which can be extensively characterized and controlled, which are of stable quality, and from which constant processing to 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 cell bank) at an intermediate stage of the manufacturing process, than to emphasize characterization, evaluation, or control of cells at the raw materials 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 and cultivation of target cells, generation of cell lines, growth medium, culture conditions, culture period, and survival rate, should be clearly documented and justified as much as possible. To maintain the 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, passage number and/or population doublings of intermediate cell products/lines should be set 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, i.e., ectopic tissue formation and tumorigenesis. However, because this is 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 significant to have strategy and tactics to develop safer final products by improving manufacturing process and process control more properly rather than discussing safety issues at iPS(-like) cell level.These draft guidelines, therefore, require the demonstration of potential free of undifferentiated cell contamination at the level of an iPS(-like) cell-derived bank and/or intermediate 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 describe 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 differentiation potential of each iPS(-like) cell, 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. 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 related to these points have been included in these human iPS(-like) cell guidelines in comparison with MHLW Notification No. 0208003 and MHLW Notification No. 0912006, which serve as the basis for these guidelines.

When interpreting and implementing the present guidelines, the following should be considered. The ultimate goal is to provide novel therapies to patients from regenerative medicine. The role of these guidelines is to present scientific principles, concepts, ideas, and technical elements that should serve 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 critical to determine the relevant testing parameters and evaluation methods by considering the characteristics of the cells in question, the specific clinical objective, the method of application, etc. Those that are applicable items should be justified and put into practice in an appropriate and flexible manner.

Several points should be kept in mind with regard to thedevelopment of products for regenerative medicine and the employment of this guideline. The desired products are expected to show a potential as a novel therapeutic method through proof of concept (POC) and relevant data, indicating no critical concerns for product safety that might impede to the use of the products in humans for the first time. Thorough the observance of the Declaration of Helsinki, including proper informed consent and 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 rapidly as possible to patients suffering from severe diseases that are difficult to treat using conventional medicine. The present guidelines should further 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 peoples' 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, and regulations and guidelines are important measures undertaken to advance towards 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 great efforts to deliver these revolutionary cell-based products and therapeutic techniques to patients as rapidly 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
(September 7, 2012)

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 hereinafter referred to as autologous human iPS(-like) cell-based products or merely as the "desired cell products." Autologous human iPS(-like) cell-based products are obtained by artificially inducing the 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 different types of manufacturing methods, intermediates, types and characteristics of desired cell products, and methods of clinical application. In addition, the scientific progress in this field is constantly advancing and experience 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 individual product, it is necessary to take, on a case-by-case basis, a flexible approach based on a rationale that

reflects the scientific and technological advancesat 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 to initiating 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 insofar as possible, using up-to-date science and technology, and to describe the scientific appropriateness of the results of such action. The remaining unidentified risk factors should be weighed against the risks associated with not performing the trials in patients who suffer from diseases that are serious and life-threatening, that involve marked functional impairment or a marked decrease in quality of life (QOL) resulting from loss of a certain degree of physical function or form, or for which existing therapies have limitations and do not provide cures. Furthermore, it is also important to entrust to the patient the right to make a decision, after providing all of the information available. When applying for investigational clinical trials, applicants can submit a reasonably prepared provisional non-clinical data package, which is prepared reasonably by taking into account product aspects and patient aspects including a balance between risk of product *vs* risk of patient with/without treatment in question, ; for determining 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 an individual clinical trial. Because of differences in product origin, target disease, target patients, application sites, application methods, and processing methods, there may be numerous variations between individual data packages that 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 justify any considerations of background, selection, application, and the content and extent of evaluation that are appropriate to their own purpose and are scientifically rational.

Chapter I General Principles

I. Objective

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

II. 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 by chemical or drug treatment, or cells that are obtained from such cells through cell division, and they possess the ability to differentiate into endoderm, mesoderm, and ectoderm, and furthermore maintain the ability to self-renew or have 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 by chemical or drug treatment, or cells that are obtained from such cells through cell division, and they at least possess the ability to differentiate into some type of endoderm, mesoderm, or ectoderm, and furthermore maintain the ability to self-renew or have a similar ability.

3. "Processing of cells and tissues:" Any processing of a cell or tissue, such as propagation and/or differentiation, production of a cell line, activation of a cell by pharmaceutical or chemical treatment, alteration of a biological characteristic, combination with a noncellular component, or manipulation by genetic engineering, with the aim of preparing desired cell products to treat a patient, or repair or regenerate tissues.
Isolation of tissue, disintegration of tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by gamma irradiation or other methods, freezing, thawing, and other such procedures regarded as minimal manipulations, are not considered to be processing.

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

5. "Phenotype:" A morphological or physiological characteristic that is expressed by a certain gene under

constant environmental conditions.

6. "Donor:" persons who donate their own somatic cells, which serve as the raw material for an autologous human iPS(-like) cell-based product. For an autologous human iPS(-like) cell-based 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 research and development 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.

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 a manufacturing perspective. It should be noted that assurance of quality and safety, and their consistency, is achieved by mutual complementary measures throughout the manufacturing method as a whole, and it is most important that the measures are rational and serve the intended purpose. It may be 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.

I. Raw Materials and Materials Used in Manufacturing

1. Human somatic cells that serve as raw materials

(1) Features of biological structure and function, and selection criteria.
Explain and justify the reasons for selecting the somatic cells used as raw materials based on the characteristics of their biological structure and function, such as for example, 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 research and development stages, before beginning the clinical trials.
This should lead to the identification of the primary cell characteristic indicators to be employed when applying the cells to the patient. It is recognized that such study can only be performed within reason, because

there are quantitative limits to samples as well as technological limits.

(2) Donor considerations

To ensure the safety of patients, manufacturing personnel, and health care workers, establish test parameters for any infections that may be transmitted via the collected somatic cells, and justify the appropriateness of the parameters. Particular consideration shall 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, history, and health condition of the patient, and justify the appropriateness of the donors. If donor genome or gene analysis is undertaken, it shall be conducted in accordance with the "Ethical Guidelines for Human Genome and Gene Analysis Research" published jointly on December 28, 2004 and revised on December 1, 2008 by the Japanese Ministry of Education, Culture, Sports, Science, and Technology, the Ministry of Health, Labor 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 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 how 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-ups), and cross-contamination.

(iii) Informed consent from 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 following test results indicating a problem.

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

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

period, and justify their appropriateness (validity). Describe in detail the measures and procedures to be followed to prevent erroneous sampling (mix-ups).

(vii) Transportation methods

If cells and/or tissues or iPS(-like) cells collected must be transported, define the containers to be used for transport and the transportation procedures (including temperature control, etc.), and justify their appropriateness.

(viii) Record preparation and storage procedures

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

2. Raw materials other than target cells and tissues, as well as materials used in the manufacturing

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

When so-called 'Biological Products' or 'Specific Biological Products' (refer to Article 2.9 and 2.10 of the Pharmaceutical Affairs Law) are used as raw materials, the amounts used should be kept to the minimum amount required and should strictly obey the relevant laws and notifications, such as the "Standards for Biological Raw Materials" (Notification Number 210, Japanese Ministry of Health, Labor, and Welfare, 2003; note that a new version will soon be issued). It is particularly important to sufficiently evaluate information related to the inactivation and elimination of viruses, and to indicate measures for ensuring retrospective and other studies.

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, additives (serum, growth factors, antibiotics, etc.) and reagents, etc. used in the treatment of cells, and set specifications if necessary. Consider 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

(a) 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.

(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 individual raw materials.

(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 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 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 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 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 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 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 detail the quality and safety of the noncellular components.

Provide any relevant information concerning the noncellular raw materials, taking into consideration their type and characteristics, form and function in the final product, and 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, 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.

(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 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 (a) through (d) below.

(a) Membrane permeability kinetics and pharmacological effects of target physiologically active substances derived from 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 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; for the cell bank of the target gene, methods of preparation of its cell bank, 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 methods, 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 of living organisms 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 organisms: "human cells, etc." or "cells that have the ability to differentiate, or differentiated cells that are not viable when alone, under natural conditions."

Regardless of the 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 conforms 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 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 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 conforms to the intended use.

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

When reprogramming or dedifferentiating 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 lines

Describe the methods used up until the establishment of human 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 preparing 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, phenotype-specific markers, karyotype, 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 storage 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 2–4 above shall be prepared, and proper record storage procedures shall be clearly described.

II. Manufacturing Process

When manufacturing pharmaceuticals and medical devices derived from the processing of autologous human iPS cells or autologous 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.

1. 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 makeup and control of the lot.

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 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 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 factor of target cells, cell viability, characterization of cells and tissues, microbiological tests, and so on. At the stage of initiation of clinical trials, provide the actual measured values obtained using 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 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 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 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 lines 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, 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 lines, 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 the 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 bank, the methods used to prepare the cell bank, characterization of the cell banks, and storage, maintenance, control, 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 the cells have been properly evaluated at an upstream point in the process for a good reason, or if the cells are of autologous origin.

(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-based 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 beyond the proposed culture period. It may be acceptable to perform these studies using test samples obtained from donors who are not patients in place of the 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 product

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 autologous 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 of desired cells), considering the application methods and intended use of the product. It may be acceptable to use test samples obtained from donors who are not patients 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.

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 includes specifications (a set of acceptance criteria and analytical procedures) for final products, quality