cells as the goal, the study group concluded that relevant guidelines should be tailored to specific cell sources and phenotypes (human autologous *vs*. human allogenic; somatic stem cells *vs*. iPS cells *vs*. ES cells *vs*. other cells) to facilitate efficient, effective, and rational R&D. Points to be considered include but are not limited to: technical details, the manufacturing process, characterization, quality control, stability evaluation, and the data necessary to guarantee the safety and efficacy of the products.

With this perspective in mind and with a desire for consistency in scientific principles and concepts, two interim reports on draft guidelines for autologous human somatic stem cell-based products and autologous human iPS cell-based products were prepared in 2009 on the basis of MHLW Notification No. 0208003. Three other interim reports of draft guidelines for allogenic human somatic stem cell-based products, allogenic human iPS cell-based products, and human ES cell-based products were also prepared on the basis of MHLW Notification No. 0912006. These five sets of draft guidelines were thoroughly discussed from a variety of viewpoints. They were then widely circulated among interested parties as articles in a relevant scientific journal to allow readers to comment (Hayakawa T., et al.: Regenerative Medicine (Journal of the Japanese Society for Regenerative Medicine), 9, 116–180, in Japanese (2010)). Thereafter, these articles were updated and published as eight articles (Journal of the Japanese Society for Regenerative Medicine, 10, 86–152 (2011)) that served as the basis for the final draft guidelines. After extensive discussions with the study group and public consultation, the Pharmaceutical and Food Safety Bureau of MHLW issued five notifications on September 17, 2012, as described in the previous paper[1].

In this paper, a continuation of the previous paper, we introduce the basic technological requirements for ensuring the quality and safety of pharmaceuticals and medical devices derived from human ES cells.

Human ES cells can provide raw material for the production of a variety of cell types because of their pluripotency, which is greater than that of somatic stem cells, whose ability to differentiate and self-replicate is limited. Once effective and efficient differentiation protocols for the generation of a target cell are established, human ES cells are expected to stably supply large amounts of cell substrates for use in cell-based therapies.

Human ES cell-based products have recently been evaluated in clinical trials in the USA. However, human ES cells are generated by the disruption of human embryos. Because this raises ethical issues, the generation and usage of human ES cells requires careful consideration. To ensure human dignity, the derivation, distribution, and utilization of human ES cells should adhere to the Guidelines for the Derivation and Distribution of Human Embryonic Stem Cells (Notification of the Ministry of Education, Culture, Sports, and Technology (MEXT), No. 156 of August 21, 2009 and No. 86 of May 20, 2010) and the Guidelines for the Utilization of Human Embryonic Stem Cells (Notification of MEXT, No. 157 of August 21, 2009 and No. 87 of May 20, 2010). In these two guidelines, basic issues concerning the protection of personal information and protocols for the derivation and use of human ES cells are defined from the viewpoint of bioethics. According to the guidelines, the derivation and use of human ES cells with human fertilized embryos is permitted only for basic research that

serves to elucidate human development/differentiation and tissue regeneration or to develop new diagnostic methods, approaches to prevention, treatments, or products intended for medical use.

The goal of basic research that contributes to the development of "new diagnostic methods, approaches to prevention, treatments, or products intended for medical use" can be interpreted to mean the development of novel treatments, pharmaceuticals, and medical devices.

We have developed a draft guideline of points to consider for ensuring the safety and quality of cellular products throughout the process, beginning with the establishment of human ES cells. The draft guideline by this research team can be practically applied at present to pharmaceuticals and medical devices manufactured by producing differentiated cells from pre-existing ES cells. However, the guideline needs to be also prepared for pharmaceuticals and medical devices derived from the processing of newly established human ES cells in the future.

When human ES cells are used a source of cell substrates for the manufacture of cell- and tissue-based products, data and information on their characteristics and competence as raw materials should be comparable to those described in the "Guidelines on Ensuring the Safety and Quality of Pharmaceuticals and Medical Devices Derived from the Processing of Human (Allogenic) Cells" (Notification from the Director of the Pharmaceutical and Food Safety Bureau, MHLW, No. 0912006) in order to ensure the safety and quality of the final products. This is because the clinical application of products made from both cell types is allogenic in nature. Thus, the extent and depth of evaluation, particularly in terms of adventitious agents and immunogenicity, should be equal for both cell types.

At present, however, the notifications of MEXT (Nos. 156 and 157) and MHLW (No. 0912006) have different standpoints, and their recommendations may therefore conflict. The two MEXT notifications focus on the protection of donors' personal information; the MHLW notification prioritizes the safety of the raw materials. It is impractical to require infertility clinics and basic research laboratories that generate ES cells from human embryos to maintain quality control at the level required for pharmaceutical manufacturing. Thus, the draft guideline does not resolve all of these problematic issues.

Nevertheless, we thought it important to prepare a draft guideline that outlines points to consider when developing pharmaceutical and medical device derived from newly established human ES cells. Upon obtaining informed consent from donors to use human ES cells as a raw material for cell- and tissue-based products, the requirements in "Chapter II. Manufacturing Methods, I. Raw Materials and Materials Used in Manufacturing, 1. In vitro fertilized embryos" should be provided, and unlinkable anonymization should be used. Appropriate measures should be taken and then justified according to "Chapter II. I. 3. Establishment of human ES cell lines and human ES cell-derived differentiated cell lines." Human ES cells may be accepted as raw materials for pharmaceuticals or medical devices if a basic study has taken into consideration both the information required for pharmaceutical manufacturing and the two MEXT notifications. This is indeed a measure to realize "bench to bed" in ES cell-based therapy. We exclude the use of cloned ES cells from the draft guideline because many ethical arguments arise.

Characterization and quality control of raw materials in biologics cannot be sufficiently performed because of the material's indistinct origin and complexity. Likewise, characterization and quality control of final products cannot be sufficiently performed because of the product's limited quantity and complex attributes. To minimize these concerns as much as possible, it is most important to ensure constancy and robustness in the manufacturing process for all types of biologics. A key technical element for the proper and consistent production of biologics is the establishment of a base camp(s), i.e., the preparation at relevant stage(s) in the manufacturing process of biologic production substrates that can be extensively characterized and controlled, whose quality is stable, and from which subsequent intermediate(s) and the desired product can be constantly produced.

The ideal base camp(s) in the sustainable manufacture of human ES 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 readily available upon request; and are able to differentiate properly into target cells. With regard to the consistent manufacture of safe products, for certain final products, the proper establishment of sustainable intermediate cell products/lines (as a sort of cell bank) at an intermediate stage of the manufacturing process may be more important than emphasizing the characterization, evaluation, or control of cells at the raw material stage, which may be difficult. It is, of course, essential to explain the advantages and appropriateness of such an approach. When establishing cell lines with different phenotypes at each stage of differentiation, details of the cell generation process, such as growth medium, culture conditions, culture period, survival rate, the generation of cell lines, and methods for the differentiation, isolation, and cultivation of target cells, should be clearly documented and justified, to the extent possible. To maintain the consistency and stability of intermediate cell products/lines, critical indicators should be selected that describe cell attributes such as purity, morphology, specific cell markers, karyotypes, proliferation, and differentiation; acceptance criteria should be set accordingly. In addition, for the intermediate cell products/lines, indicate the passage number and/or population doublings limit for meeting the acceptance criteria.

This draft guideline lists points to consider when manufacturing ES cell-based products from in vitro fertilized embryos and requires documentation of necessary information. These requirements are important to guarantee consistency in quality and to ensure the quality and safety of the final products. It should be emphasized that quality, safety, and constancy can be ensured by complementary approaches throughout the manufacturing process, and such measures should be undertaken in a logical and appropriate way in order to serve the intended purpose. If the quality, safety, and constancy of the final product is ensured and scientifically verified, it might be possible to omit certain quality tests and controls over cell banks, intermediate cell products, final products, and the manufacturing process listed in the draft guideline. In this context, if the characterization and control of ES cell-derived differentiated cells or other intermediate cell products (intermediate cell lines, etc.) as a base camp and the robustness and consistency of the manufacturing process are guaranteed, it is not

always necessary to follow the guideline, particularly with regard to upstream processes.

As for ES cell-based products, the presence of undifferentiated cells in the final product is a major concern from a safety point of view, i.e., the potential for ectopic tissue formation and tumorigenesis. This is one of the characteristics of ES cells, and it is thus quite difficult to avoid and counteract. Elimination of the intrinsic characteristics of ES cells is a trade-off, at least in principle, and thus considered quite difficult. Accordingly, it will be important to have a strategy for the development of safer final products in the future that involves improved manufacturing processes and process controls, rather than addressing safety issues at the ES cell level. Therefore, this draft guideline requires the elimination of undifferentiated cell contamination at the cell bank and intermediate cell product level by thorough analysis or efforts to minimize potential contamination by developing efficient methods to eliminate or inactivate undifferentiated cells during cell processing. Furthermore, selection of the appropriate administration method can minimize safety concerns. The guideline also describes the importance of technical developments for the generation and characterization of ES cell-derived somatic stem cells; these developments may lead to safe, stable, characteristically well-defined, and appropriate raw material. R&D of examination techniques to predict the pluripotency and differentiation potential of each ES cell and processing techniques to induce target cells efficiently and properly and to isolate differentiated cells from undifferentiated cells during cell processing will produce novel opportunities for business.

We have included these aspects of ES cells in this draft guideline. The natural pluripotency and self-renewal ability of ES cells exceeds those of normal somatic stem cells. Thus, ES cells can differentiate into a variety of cell types depending on the processing technique. In clinical applications, the use of ES cell-based products will involve heterologous transplantation, i.e., administration into a cell environment that is essentially different from the environment where the cells exert their natural endogenous function. Points to consider with respect to these issues are included in this guideline and were based on MHLW Notification No. 0912006.

Before interpreting and implementing the present guideline, the following should be considered. The ultimate goal is to provide patients with new therapies that utilize regenerative medicine. The role of the guideline is to present the scientific principles, concepts, ideas, and technical elements that will achieve the specified goal in the most efficient and effective manner possible. Because situations, circumstances, and products will vary, the guideline addresses points of concern in a comprehensive manner. Therefore, it is critical to identify the relevant testing parameters and evaluation methods by taking into consideration the characteristics of the cells in question, the specific clinical objective, the method of application, etc. Those that are applicable should be justified and implemented in an appropriate and flexible manner.

Several points should be kept in mind with regard to the development of medicinal 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 relevant proof of concept (POC). Relevant data and/or information should establish that there are no critical concerns for product safety

that might impede the use of the product in humans for the first time. Thorough observance of the Declaration of Helsinki, including proper informed consent and right of self-determination on the part of the patient, is indispensable.

It should be emphasized again that the primary goal of our endeavor is to offer suitable treatment options as fast as possible to patients suffering from severe diseases that are difficult to treat with conventional medicine. The present guideline should be useful for this purpose. Therefore, it is important to interpret and employ the guideline in a flexible and meaningful way. Stringent observance of the guideline without taking into account the patients and their specific situation, which is like putting the cart before the horse, should be avoided.

It is evident that progress in the application of regenerative medicine is desirable for maintaining and improving peoples' health. The development of innovative and revolutionary medicinal products and therapeutic techniques should benefit our country as well as the international community. Regenerative medicine is a great way to make a peaceful international contribution that will be a legacy to mankind. In this context, the role of government is to promote clinical research and industrialization; regulations and guidelines are adopted such that we advance towards this common goal in a scientific, rational, efficient, and effective manner. All those involved, like players with a common goal in the same arena, should continue to make great efforts to deliver to patients as fast as possible revolutionary, cell-based products and therapeutic techniques.

Guidelines on Ensuring the Safety and Quality of Pharmaceuticals and Medical Devices Derived from the Processing of Human Embryonic Stem 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 human embryonic stem (ES) cells. These products are hereafter referred to as human ES cell-based products or merely as the "desired cell products."

At the present time, it is assumed these guidelines will apply to pharmaceuticals and medical devices manufactured from already existing ES cell-derived differentiated cells. In the future, when the intention is to manufacture desired cell products using newly established human ES cells, donors must be thoroughly briefed about the purpose of establishing the cells, and their consent must be obtained. Provide the donors with as much of the information outlined below in "1. In vitro fertilized embryos" (see Chapter II. Manufacturing Methods, I. Raw Materials and Materials Used in Manufacturing) as possible and ensure the anonymity of the donor. Following this, devise appropriate measures in accordance with "Chapter II, I. 3. Human ES cell strains and human ES cell-derived differentiated cell strains" and clearly explain their appropriateness. There are many different types of human ES cell-based products, and methods of clinical application. In addition,

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 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 initiating human clinical trials of the human ES 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 the consistency of the QA can be ensured within a definite range. Simultaneously, it is important to eliminate as much as possible any presumed known risk factors associated with product quality and safety 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 the loss of physical function or form, or for which existing therapies have limitations and do not provide cures. Furthermore, it is 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 submit a 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 and developed in line with the guidelines as the clinical trial progresses.

Finally, applicants are encouraged to discuss with the Pharmaceuticals and Medical Devices Agency (PMDA) the type and extent 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 guideline 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. In accordance with the purpose of the present guideline, applicants are encouraged to explain and justify how the background, selection, application, and the content and extent of evaluation are appropriate and 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 the processing of human embryonic stem (ES) cells. These products are hereafter referred to as human ES cell-based products or merely as "desired cell products."

II. Definitions

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

1. "Human embryonic stem cells (ES cells)": Cells that are collected from a human embryo or cells that are obtained from such cells through cell division and for cells that are not embryo itself; that possess the ability to differentiate into endoderm, mesoderm, and ectoderm; and that maintain the ability to self-renew or a similar ability.

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

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

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

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

6. "Donor": Persons who donate their own cells, which serve as the raw material of a human ES cell-based

product. Persons who provide sperm and unfertilized eggs.

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

Chapter II Manufacturing Methods

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

I. Raw Materials and Materials Used in Manufacturing

1. In vitro fertilized embryos

(1) Source and origin, justification of their selection
Explain the source and origin of the in vitro fertilized embryos used to establish the human ES cell line and justify the reasons for selecting these embryos.

(2) Characteristics and eligibility of in vitro embryos used as raw materials

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

Explain and justify the reasons for selecting the in vitro embryos used as raw materials with reference to characteristics of their biological structure and function, such as morphological characteristics, growth characteristics, and other suitably chosen and appropriate indicators.

(ii) Ethical propriety of donor selection

If a new human ES cell line intended for clinical use is established after publication of the present guidelines, indicate that the donor was selected in an appropriate and ethical manner and that the proper procedure was followed by providing a record of the review process conducted by the ethical review committee of the medical facility providing the in vitro fertilized eggs. For ES cell strains established before publication of this guideline, it is the responsibility of the manufacturer of the human ES cell-based product to demonstrate that selection of the donor was carried out in an appropriate and ethical manner and that the proper procedure was followed.

(iii) Donor selection criteria and

eligibility

Establish selection criteria and eligibility criteria that take into consideration age, sex, genetic characteristics, disease history, health conditions, test parameters related to any type of infection that may occur via sampled gametes, immunological compatibility, etc., and justify their appropriateness. If donor genome or gene analysis is undertaken, they shall be performed in accordance with "Ethical Guidelines for Human Genome/Gene Analysis Research," issued jointly on December 28, 2004 (partially revised on December 1, 2008) by the Japanese Ministry of Education, Culture, Sports, Science, and Technology, Ministry of Health, Labor, and Welfare, and Ministry of Economy, Trade, and Industry.

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

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

- Bacterial infections, such as syphilis (*Treponema pallidum*), chlamydia, gonorrhea, and tubercle bacillus
- Sepsis or suspected sepsis
- Malignant neoplasm
- Serious metabolic or endocrine diseases
- Collagen and blood diseases
- Hepatic diseases
- Confirmed or suspected transmissible spongiform encephalopathy (TSE) or other cognitive disorders
- Specific genetic disease or a family history of a specific genetic disease

If no link can be made between the name of the donor and the in vitro embryo, collect as much information about the donor as possible as described above in "(iii) Donor selection criteria and eligibility." It may be acceptable to perform some parts of the aforementioned studies at the stage of ES cell-derived differentiated cells or at a stage further downstream in the investigation, after having justified their appropriateness.

If differentiated cells derived from ES cells are used as raw materials, collect as much information related to the above as possible. Alternatively, at a stage (intermediate product) where differentiation has further progressed, it may be acceptable to perform the aforementioned studies and justify their appropriateness.

In conclusion, it is important to perform this analysis to the extent possible at a proper stage, either raw

materials or intermediate products, and to justify their appropriateness.

(3) Records related to the donor

Retain complete records related to the donor in order that any information necessary to ensure the safety of an in vitro embryo can be verified. Concrete measures shall be described to the extent possible.

(4) Collection of gametes and the preparation, storage, and transport of in vitro embryos

The collection of gametes used to establish a human ES cell line, preparation of in vitro embryos, and their storage and transport should be carried out in accordance with (i) to (viii) below. The establishment and distribution of a human ES cell line should be conducted in accordance with "Guideline on the Establishment and Distribution of Human ES Cells" (Notification No. 156, Japanese Ministry of Education Culture, Sports, and Science, published on August 21, 2009). Human ES cells are established (primary establishment) using human in vitro embryos. Do not use ES cells established by preparing a human cloned embryo and then using this cloned embryo to establish the ES cells (secondary establishment). In addition, do not use entosomatic fertilized embryos.

(i) Eligibility of personnel and medical institutions collecting samples

When preparing and using human in vitro embryos, describe the technical requirements for personnel and medical institutions that collect the male and female gametes.

(ii) Suitability of the embryo collection method

Describe the method used to prepare the in vitro embryos. Describe steps taken to select embryos in a scientifically and ethically appropriate manner. Establish that appropriate procedures were followed. For gamete collection methods and in vitro fertilization methods, indicate the suitability of the equipment and reagents, including the drugs used, and the measures adopted to prevent microbial contamination, erroneous sampling (mix-ups), and cross contamination.

(iii) Informed consent from donors

Describe the details of the informed consent, including the clinical application, provided by the donors of the gametes.

(iv) Protection of donor privacy

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

(v) Tests to ensure donor safety

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

(vi) Storage method and measures to

prevent erroneous sampling (mix-ups)

If the gametes or in vitro embryos need to be stored for a definite period of time, set the storage conditions and storage period and justify their appropriateness. Describe in detail the measures and procedures to be taken to prevent erroneous sampling (mix-ups), referring to "Safety Control in Infertility Treatments" (dated February 20, 2009, Notification No. 0220001, Equal Employment, Children and Families Bureau, Japanese Ministry of Health, Labor, and Welfare).

(vii) Transportation methods

If gametes or in vitro embryos need to be transported, set the containers used for transport and the transportation procedure (including temperature control, etc.) and justify their appropriateness.

(viii) Preparation of records and record-keeping procedures

Written records for (i) through (vii) above shall be prepared, and record-keeping procedures shall be described in detail. If differentiated cells derived from ES cells are used as raw materials, collect as much information related to the above as possible.

2. Raw materials other than in vitro embryos, existing ES cells, and ES cell-derived differentiated cells; materials used in manufacturing

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

When so-called "Biological Products" or "Specific Biological Products" (refer to Article 2.9 and 2.10 of the Pharmaceutical Affairs Law) serve as raw materials, use the minimum amount required and strictly obey the relevant laws and notifications, such as "Standards for Biological Raw Materials" (Notification No. 210, Japanese Ministry of Health, Labor, and Welfare, 2003; note that a new version will soon be issued). It is particularly important to sufficiently evaluate information related to the inactivation and elimination of viruses and to indicate measures for ensuring retrospective and other studies. The technical requirements described in this paragraph should be taken into consideration when ES cells are prepared from in vitro embryos derived from raw materials (gametes) and when ES cell-derived differentiated cells and the final products are prepared by directed differentiation from ES cells.

(1) When culturing cells

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

specifications concerning the appropriateness of each component.

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

(a) The ingredients and water used in media should be of high quality and high biological purity, and their quality should be controlled at standards equivalent to those used with pharmaceuticals and pharmaceutical raw materials.
(b) Provide information on all components of the media, as well as the rationale for their selection and, if necessary, the quality control and other procedures. However, widely known and commercially available media products such as DMEM, MCDB, HAM, and RPMI are regarded as a single raw material set.
(c) Conduct sterility tests and performance tests on media that contain all components in order to determine whether they are suitable as target media. Set specifications for any other relevant parameters thought to be controlled in the process and perform proper quality control.

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

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

(iv) When using feeder cells, conduct a quality evaluation while referring to

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

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

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

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

(2) When combining with noncellular components

(i) Quality and safety of noncellular raw materials

If the final product consists of cells and noncellular components, such as matrix, medical materials, scaffolds, support membranes, fibers, and beads,

describe in detail the quality and safety of the noncellular components.

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

With respect to the tests that should be carried out, refer to "Basic Views on Biological Tests Necessary for Regulatory Approval for Manufactured or Imported Medical Devices" (Notification No. 02013001, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labor, and Welfare, issued February 13, 2003), describe the test results, and justify the use of such raw materials. The use of information obtained from the literature in encouraged.

(ii) Interactions with target cells

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

(a) The noncellular components do not have any deleterious effects on the function, growth capability, activity, or stability of the cells in the final product required for the presumed clinical indication or the cells in any intermediate products.
(b) Evaluate to the extent possible any potential interactions between the cells and noncellular components, taking into consideration, for example, the mutation, transformation, and/or dedifferentiation of cells in the final product or cells in intermediate products.
(c) Show there is no loss of the expected properties of the noncellular components in the presumed clinical indication as a result of any interactions between the noncellular components and the cells in the final and intermediate products.

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

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

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

objective is segregation of the application site and the desired cell product and/or undifferentiated cells, confirm that the cells do not leak out, which might result from the degradation, etc. of noncellular components.

(3) When cells undergo genetic modification

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

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

(ii) Nature of the transgene.

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

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

(v) Structure and characteristics of the transgene construct.

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

For manufacturing methods for transgenic cells, refer to Chapter 2 and other sections of "Guidelines for Ensuring the Quality and Safety of Gene Therapy Pharmaceuticals," which is an appendix in "Concerning Guidelines for Ensuring the Quality and Safety of Gene Therapy Pharmaceuticals" (hereafter referred to as "Gene Therapy Pharmaceutical Guidelines"), published as Notification No. 1062 by the Ministry of Health and Welfare on November 15, 1995. In addition, state clearly the appropriateness of the establishment in accordance with the appendix of the same notification.

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

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

3. Establishment of human ES cell lines and human ES cell-derived differentiated cell lines

(1) Establishment of human ES cell lines

Establish human ES cell lines after having determined, to the extent possible, the genetic background of the donors of the male and female gametes used to produce the in vitro fertilized embryo. Describe the methods used to establish the ES cells from the in vitro fertilized embryo stage and indicate, to the extent possible, the appropriateness of the methods. These include the method for obtaining the human blastocysts, methods for the separation and cultivation of the inner cell mass (ICM) from blastocysts, and methods for the separation and establishment of undifferentiated cells, as well as the media, culture conditions, cultivation period, etc. at each step in the process used to establish the human ES cell line.

To ensure that the quality of the human ES cell line remains stable and consistent, identify the critical quality attributes of the cells by describing cell characteristics such as cell purity, morphological features, HLA typing, phenotype specific markers, karyotype, DNA fingerprinting, cell growth properties, and pluripotency, and set acceptance criteria for them. In addition, demonstrate the potent number of passages or cell divisions through which the cells maintain their quality in terms of the criteria specified. Although comprehensive cell characterization is always desirable, it is recognized that full characterization may be difficult because there are quantitative limits and technological limits to sample analysis. Thus, it is considered acceptable to perform the studies to the extent possible.

If information related to infections in donors cannot be obtained because of donor anonymity or other reasons, rule out the presence of HBV, HCV, HIV, adult HTLV, and parvovirus B19 in the established human ES cell line by relevant testing. In addition, rule out infection with cytomegalovirus, EB virus, and West Nile virus, if necessary, by testing. If the genetic traits of a donor cannot be obtained, analyze the genetic information of the ES cell line itself to determine if any factors related to genetic diseases are present. Although it is acceptable to perform these tests at the stage when an ES-derived differentiated cell line has been established as a cell substrate with which to manufacture ES cell-based products, it is preferable to perform the tests in the ES cell line.

(2) Establishment of a human ES cell-derived differentiated cell line by an institution that uses human ES cells

In some cases, the establishment of a differentiated cell line derived from human ES cells may be important for the constant manufacture of a safe, final product. In other words, there may be some cases where such an approach is encouraged as a scientifically rational procedure. When such a measure is chosen, describe the intended use at the facility and explain the advantages and appropriateness with respect to the manufacture of the human ES cell-based product. If a cell line that

exhibits a different phenotype is established in stages, describe the procedure used to establish each respective cell line, including, for example, differentiation induction methods and methods for the isolation, culture, and establishment of the target cell line, as well as media, culture conditions, culture period, yield, etc., and justify the appropriateness for the manufacture of a human ES cell-based product.

In order to ensure the quality of the differentiated cell line remains stable and consistent, identify the critical quality attributes of the cells by describing various cell characteristics, such as cell purity, morphological features, HLA typing, phenotype-specific markers, karyotype, DNA fingerprinting, cell growth properties, pluripotency etc., and set acceptance criteria for them. In addition, demonstrate the potent number of passages or cell divisions through which the cells maintain their quality in terms of the criteria specified. Although comprehensive cell characterization is always desirable, it is recognized that full characterization may be difficult because there are quantitative limits and technological limits to sample analysis. Thus, it is considered acceptable to perform the studies to the extent possible.

The conditions that must be fulfilled are identical for imported ES cell lines and differentiated cell lines derived from already existing ES cell strains that were established before the publication of the relevant official guidelines and notifications. However, there may be cases where a certain raw material was used or potentially used, for which the establishment and maintenance processes are vague or unclear and thus might not meet the stipulations of the "Standards for Biological Raw Materials" (Notification No. 210, Japanese Ministry of Health, Labor, and Welfare, issued 2003; note that a new version will soon be published). Because the propriety of using such cell lines will be reviewed and evaluated on a case-by-case basis, consultation with the Pharmaceuticals and Medical Devices Agency (PMDA) is encouraged. (Note: Even when sufficient infection-related information for the human ES cell-derived differentiated cell line that will be used cannot be obtained, the presence of HBV, HCV, HIV, adult HTLV, and parvovirus B19 in the human ES cell-derived differentiated cell line should be ruled out by relevant testing. Infection due to cytomegalovirus, EB virus, and/or West Nile virus should also be ruled out, if necessary, by testing. If the genetic traits of a donor cannot be obtained, analyze the genetic information of the ES cell line itself to determine if any factors related to genetic diseases are present.)

4. Storage and transport of human ES cell lines and human ES cell-derived differentiated cell lines

For human ES cell lines and human ES cell-derived differentiated cell lines, perform the appropriate stability tests to assess cell viability and potency etc., establish the storage method and validity period, and make clear their appropriateness, taking into the consideration the duration of

storage and the distribution and storage form. In particular, when freezing and thawing, assess whether the process of freezing and thawing has an effect on stability and any criterion of the cell line. Evaluate storage over the standard storage period and confirm the margin of stability to the extent possible. However, this does not apply when using cells immediately following their establishment. When transporting a human ES cell line or human ES cell-derived differentiated cell line, the containers used for transport and the transportation procedure (including temperature control, etc.) shall be set and their appropriateness clearly indicated.

5. Preparation of records and record-keeping procedures

Prepare written records for 2 through 4 above, and clearly describe the record-keeping procedures.

II. Manufacturing Process

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

1. Lot composition and lot control

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

2. Manufacturing method

Provide an outline of the manufacturing method, from the preparation of in vitro embryos from collected gametes and through the establishment of human ES cells, differentiated cells, and the final product. Describe the technical details of the process and necessary process control and product quality control.

(1) Tests upon receipt

Establish a battery of tests as well as acceptance criteria with which to assess the appropriateness of human ES cells or human ES cell-derived differentiated cells that will serve as the raw material, taking into account the nature of the cells and their intended use. These may include, for example, visual tests, microscopic examination, cell viability assays, etc. (Note that the receipt of an ES cell line at an institution where ES cell-based products will be manufactured is permitted only when the clinical use of the said ES cells are officially permitted by government rules.)

(2) Establishment of a human ES cell line

Clarify its role in the manufacturing method that the manufacturer adopted (refer to Chapter II, I. 3. (1)).

(3) Establishment of a human ES cell-derived differentiated cell line

Clarify its role in the manufacturing

method that the manufacturer adopted, if any (refer to Chapter II, I. 3. (2)).

(4) Establishment of an intermediate cell line derived from human ES cells

When the manufacturer of a human ES cell-based product establishes a cell line as an intermediate product (intermediate cell line) from an ES cell line or a differentiated cell line that has been received, explain its advantages and appropriateness. If a cell line that exhibits a different phenotype is established in stages, describe the procedure used to establish each respective cell line, including, for example, differentiation induction methods and methods for the isolation, culture, and establishment of the target cell lines, as well as media, culture conditions, culture period, yield, etc., and justify their appropriateness to the extent possible.

To ensure that the quality of the intermediate cell line remains stable and consistent, identify the critical quality attributes of the cells by describing various cell characteristics, such as 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 cell divisions through which the cells maintain their quality in terms of the criteria specified. Although comprehensive cell characterization is always desirable, it is recognized that full characterization may be difficult because there are quantitative limits and technological limits to sample analysis. Thus, it is considered acceptable to perform the studies to the extent possible.

If establishing a cell bank from the intermediate cell line in accordance with the criteria described above and utilizing said cell bank, refer to (6).

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

Describe the methods by which cells that serve as an active ingredient in the final product were prepared from a human ES cell-derived differentiated cell line or via an intermediate cell line derived from a human ES cell line. The methods to be described include the induction of differentiation, the isolation and culture of the desired cells, and the media, culture conditions, culture period, yield, etc. at each step. Describe the appropriateness of each method.

(6) Establishment of cell banks

When a cell bank is established at any stage during the manufacture of human ES cell-based products, describe the rationale for preparing the cell bank; the methods used to prepare the cell bank; the characteristics of the cell bank; and the storage, maintenance, control, and renewal methods, as well as any other processes and tests performed. Justify the appropriateness of each. Refer to "Derivation and Characterization of Cell Substrates Used for the Production of Biotechnological/Biological Products" (Pharmaceutical Notification No. 873, Japanese

Ministry of Health, Labor, and Welfare, issued July 14, 2000) and other documents. It is acceptable to omit a portion of the test items if, for a rational reason, the cells were evaluated properly at an upstream point in the process.

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

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

(8) Preparation of records and record-keeping procedures

Written records for (1) through (7) above should be prepared, and proper record-keeping procedures shall be clearly described.

3. Characterization of cells that are a principal component of the final product as an active ingredient

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

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 the final product

If intermediate or final products need to be stored and transported, the storage procedure and duration, the containers used for transport, and the