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Original article

A study on ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of human embryonic stem cells[☆]



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ABSTRACT

As a series of endeavors to establish suitable measures for the sound development of regenerative medicine using human stem cell-based products, we studied scientific principles, concepts, and basic technical elements to ensure the quality and safety of therapeutic products derived from the processing of human embryonic stem cells (hESCs), taking into consideration scientific and technological advances, ethics, regulatory rationale, and international trends in human stem cell-derived products. This led to the development of the Japanese official Notification No. 0907-6, "Guideline on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Human Embryonic Stem Cells," issued by Pharmaceuticals and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, on September 7, 2012. The present paper addresses various aspects of products derived from hESCs, in addition to similar points to consider that are described previously for allogeneic human stem cell-based products. Major additional points include 1) establishment of hESCs; 2) establishment of stable and well-characterized cell banks of hESCs and relevant intermediate cell products; 3) concerns about the presence of undifferentiated cells in final products, which may result in ectopic tissue formation and/or tumorigenesis; and 4) concerns about undesirable immunological reactions caused by the final products. The ultimate goal of this series of guidelines on regenerative medicine is to provide suitable medical opportunities as soon as possible to the patients with severe diseases that are difficult to treat with conventional modalities. If these guidelines are interpreted and employed in a flexible and meaningful way in this context, they should serve as a useful means to achieve their goals.

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

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1. Background (chronology and focus of the research)

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

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

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

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

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

As a result of analyses conducted up to 2009, in accordance with the Pharmaceutical Affairs Law, and with clinical application of the products derived from human somatic stem cells, iPS cells, ES cells, and other relevant cells, the study group concluded that relevant guidelines should be tailored to specific cell sources and phenotypes (human autologous versus human allogeneic; 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 relevant 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 the desire for consistency in scientific principles and concepts, 2 interim reports on draft guidelines on autologous human somatic stem cell-based products and autologous human iPS cell-based products were prepared in 2009 according to Japanese Ministry of Health, Labour and Welfare Notification No. 0208003. Three other interim reports of draft guidelines on allogeneic human somatic stem cell-based products, allogeneic human iPS cell-based products, and human ES cell-based products were also prepared according to Japanese Ministry of Health, Labour and Welfare Notification No. 0912006. These 5 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 [2010], in Japanese). Thereafter, these articles were updated and published as 8 articles (*Journal of the Japanese Society for Regenerative Medicine*, 10, 86–152 [2011], in Japanese) that served as the basis for the final draft guidelines. After extensive

discussions with the study group and after public consultation, the Pharmaceutical and Food Safety Bureau of the Ministry of Health, Labour and Welfare of Japan issued 5 notifications on September 7, 2012, as described in the previous paper [1].

In the present paper, a continuation of the previous articles [1–4], 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 lineage 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 were recently evaluated in clinical trials in the USA. However, human ES cells are generated by destruction of human embryos. Because this approach raises ethical issues, the generation and use of human ES cells require 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” (Japanese Ministry of Education, Culture, Sports and Technology Notifications No. 156 of August 21, 2009, and No. 86 of May 20, 2010) and the “Guidelines for the Utilization of Human Embryonic Stem Cells” (Japanese Ministry of Education, Culture, Sports and Technology Notifications No. 157 of August 21, 2009, and No. 87 of May 20, 2010). In these 2 guidelines, basic issues concerning the protection of personal information and protocols for the derivation and use of human ES cells are defined from the standpoint of bioethics. According to the guidelines, the derivation and use of human ES cells from 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 or treatment, or products intended for medical use.

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

We developed a draft guideline containing the points to consider for ensuring the quality and safety 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 preexisting ES cells. However, in the future, another guideline also needs to be prepared for pharmaceuticals and medical devices derived from the processing of newly established human ES cells.

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 Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Allogeneic Human Cells” (Notification No. 0912006; from the Director of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan) in order to ensure the quality and safety of the final products. This is because the clinical application of products made from both cell types is allogeneic 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 the Japanese Ministry of Education, Culture, Sports, Science and Technology (No. 156 and

No. 157) and Japanese Ministry of Health, Labour and Welfare (No. 0912006) express different viewpoints, and their recommendations may therefore be contradictory. The 2 Ministry of Education, Culture, Sports, Science and Technology notifications focus on the protection of donors' personal information; the Ministry of Health, Labour and Welfare 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 issues.

Nevertheless, we thought that it would be helpful to prepare a draft guideline that outlines points to consider when developing pharmaceuticals and medical devices derived from newly established human ES cells. After receipt of informed consent from donors to use human ES cells as a raw material for cell- and tissue-based products, the requirements in "4. Chapter II. Manufacturing methods, 4.1. 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 "4. Chapter II. Manufacturing methods, 4.1. Raw materials and materials used in manufacturing, 3. Establishment of human ES cell lines and human ES cell-derived differentiated cell lines." Human ES cells are acceptable 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 2 notifications of the Ministry of Education, Culture, Sports, Science and Technology of Japan. This is indeed a measure to move ES cell-based therapies "from bench to bedside." We excluded the use of cloned ES cells from the draft guideline because many ethical arguments can arise.

Characterization and quality control of raw materials in biologics cannot be adequately performed because of a material's indistinct origin and complexity. Likewise, characterization and quality control of final products cannot be performed adequately because of a product's limited quantity and complex attributes. To minimize these problems as much as possible, it is very 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 of substrates for production of biologics at relevant stage(s) in the manufacturing process; these substrate can be extensively characterized and controlled and are of stable quality and constant processing of these substrates into the subsequent intermediate(s) and finally to a desired product is achievable.

The ideal base camp(s) in the sustainable manufacture of human 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 can 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 a 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. During establishment of 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 isolation, cultivation, and induction of differentiation 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 assess cell attributes such as morphology, cell population purity, 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 doubling 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 intended to guarantee consistency in quality and to ensure quality and safety of the final products. It should be emphasized that quality, safety, and constancy can be ensured via 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 are ensured and scientifically verified, it is 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 (e.g., intermediate cell lines) 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 (e.g., the potential for ectopic tissue formation and tumorigenesis). This concern is raised from one of the characteristics of ES cells and is therefore quite difficult to avoid and counteract. Elimination of the intrinsic characteristics of ES cells is a trade-off, at least in principle, and is thus considered quite difficult. Accordingly, in the future, it will be important to have a strategy for the development of safer final products that involves improved manufacturing processes and process controls rather than addressing safety issues at the ES cell level. Therefore, this draft guideline requires elimination of contamination with undifferentiated cells at the cell bank level and intermediate and/or final cell product level by thorough analysis or efforts to minimize potential contamination via development of efficient methods to eliminate or inactivate undifferentiated cells during the cell processing. Furthermore, selection of the appropriate administration method can minimize the safety risks. The guideline also explains 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 materials. R&D of examination techniques to predict the pluripotency and differentiation potential of each ES cell lineage and processing techniques to induce target cells efficiently and properly and to isolate differentiated cells from undifferentiated cells during the cell processing will open up novel opportunities for business.

We 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 are based on Japanese Ministry of Health, Labour and Welfare Notification No. 0912006.

When interpreting and implementing the present guideline, the following points should be taken into consideration. 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 help to achieve the specified goal in the most efficient and effective manner possible. Because situations, circumstances, and products will vary, the guideline addresses the points of concern in a comprehensive manner. Therefore, it is important to identify the relevant testing parameters and evaluation methods by taking into consideration, for example, the characteristics of the cells in question, such as the specific clinical objective and the method of application. Those that are applicable should be justified and implemented in an appropriate and flexible manner.

Several points should be kept in mind with respect to the development of medicinal products for regenerative medicine and the implementation of this guideline. The desired products are expected to show a potential as a novel therapeutic method as a result of relevant proof of concept (POC). Relevant data and/or information should establish that there are no critical concerns about 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 soon as possible to patients with severe diseases that are difficult to treat with conventional modalities. 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 human 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 for mankind. In this context, the role of the 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 efforts to deliver to patients revolutionary, cell-based products and therapeutic techniques as soon as possible.

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

(Notification No. 0907-6, issued by Pharmaceuticals and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, on September 7, 2012).

2. Introduction

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

At present, it is assumed that 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 information outlined below in “4. Chapter II. Manufacturing methods, 4.I. Raw materials and materials used in manufacturing, 1. In vitro fertilized embryos” as possible and ensure anonymity of the donors. After that, devise appropriate measures in accordance with “4. Chapter II. Manufacturing methods, 4.I. Raw materials and materials used in manufacturing, 3. Establishment of human ES cell lines and human ES cell-derived differentiated cell lines” and clearly explain their appropriateness. There are many types of human ES cell-based products and methods of clinical application. In addition, scientific progress in this field is incessant, while expertise and knowledge are constantly accumulating. Therefore, it is not always appropriate to consider the present guidelines all-inclusive and definitive. Consequently, when testing and evaluating each product, it is necessary to adopt, on a case-by-case basis, a flexible approach in accordance with the rationale that reflects the scientific and technological advances at that point in time.

2. The main purpose of evaluating the quality and safety of the desired cell products before conducting investigational clinical trials (e.g., at the time of “clinical trial consultation”) is to determine whether there are any quality and/or safety problems that would obviously hinder initiation of human clinical trials of the 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 consistency of the QA can be ensured within a definite range. Simultaneously, it is important to eliminate as much as possible any 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 an action. The remaining presumed risk factors should be weighed against the risks associated with not performing the trials on patients who suffer from diseases that are serious and life-threatening, or that involve marked functional impairment or a marked decrease in quality of life (QOL) resulting from the loss of a certain degree of a physical function or form, or for which existing therapies have limitations and do not result in a cure. Furthermore, it is important to entrust the patient with the right to make a decision after receiving all of the available information. When applying for approval of investigational clinical trials, applicants submit a provisional nonclinical data package, which is prepared rationally by taking into account product aspects and patient aspects including a balance between the risk of the product versus the risk facing the patient with/without treatment in question, in order to decide 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 among individual data packages; these differences cannot be definitively clarified in the present guidelines.

3. The items, test methods, criteria, and any other technical requirements described in the present 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 why the

background, selection, application, and the content and extent of evaluation are appropriate and scientifically rational.

3. Chapter I. General principles

3.1. Objective

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

3.2. 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 derived 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 type or tissue, such as propagation and/or differentiation, production of a cell line, activation of a cell by pharmaceutical or chemical treatment, alteration of a biological characteristic, combination with a noncellular component, and manipulation using genetic engineering, with the aim of preparing desired cell products to treat a patient or repair or regenerate tissue.

Isolation of a tissue, homogenization of a tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by γ -irradiation or other methods, freezing, thawing, and other such procedures that are 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 the market. This includes, in addition to the processing of cells and tissues, minimal manipulations such as isolation of a tissue, homogenization of a tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by γ -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 produced by a certain gene under constant environmental conditions.
5. “HLA typing”: Identification of the type of HLA (human leukocyte antigen), a human primary histocompatibility antigen.
6. “Donor”: A person who donates his/her own cells, which serve as a raw material for 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.

4. 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 to ensure the quality, safety, and efficacy of the final products and is important for guaranteeing consistency

in quality from the manufacturing perspective. It should be noted that quality, safety, and consistency are ensured by mutual complementary measures throughout the manufacturing process. It is very important that the measures be rational and serve the intended purpose. It is acceptable to omit a portion of the items listed below, if the 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.

4.1. Raw materials and materials used in manufacturing

1. In vitro fertilized embryos

- (1) Source and origin, and justification of their selection
Explain the source and origin of the in vitro fertilized embryos used to establish the human ES cell line and provide 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

Provide and explain 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 that is 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 a 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, the medical history, the health condition, test parameters related to any type of infection that may be transmitted, for example, via sampled gametes, immunological compatibility, and other characteristics and explain their appropriateness. If donor genomic or gene analysis is undertaken, it shall be performed in accordance with “Ethical Guidelines for Analytical Research on Human Genome/Gene,” issued jointly on February 8, 2013 (partially revised on November 25, 2014) by the Japanese Ministry of Education, Culture, Sports, Science and Technology; Ministry of Health, Labour 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), or parvovirus B19 shall be ruled out via physician-donor interviews and clinical laboratory tests, such as serological tests and nucleic-acid amplification tests. Infection with cytomegalovirus, Epstein-Barr (EB) virus, or West Nile virus shall also be ruled out, if necessary, via appropriate clinical laboratory tests.

In addition, further assess and determine the eligibility of donors by examining the medical history (mentioned below) of the donor, for example, through physician-donor interviews, and determine whether he/she 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
- A malignant neoplasm
- Serious metabolic or endocrine diseases
- Collagen and blood diseases
- Hepatic diseases
- Confirmed or suspected transmissible spongiform encephalopathy (TSE) or other brain disorders
- A 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 is 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 determined 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 progressed further, it is acceptable to perform the aforementioned studies and determine 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 explain their appropriateness.

(3) Records related to the donor

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

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

The collection of gametes that are 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 items (i) through (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, published on August 21, 2009, by the Japanese Ministry of Education, Culture, Sports and Science). Human ES cells are established (primary culture) using human embryos in vitro. Do not use ES cells established by preparing a human cloned embryo and then by using this cloned embryo to establish the ES cells (secondary establishment). In addition, do not use entosomal fertilized embryos.

(i) Eligibility of personnel and medical institutions collecting the 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 embryos in vitro. Describe steps taken to select embryos in a scientifically and ethically appropriate manner. Determine whether 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-up), and cross-contamination.

(iii) Informed consent of 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 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 undertaken when the test results indicate that a problem exists.

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

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 provide the justification. Describe in detail the measures to be taken to prevent erroneous sampling (mix-up), referring to “Safety Control in Infertility Treatments” (dated February 20, 2009, Notification No. 0220001, Equal Employment, Children and Families Bureau, Japanese Ministry of Health, Labour and Welfare).

(vii) Transportation methods

If gametes or in vitro embryos need to be transported, specify the containers used for transport and the transportation procedure (including temperature control) and provide the justification.

(viii) Preparation of records and record-keeping procedures

Written records for items (i) through (vii) above shall be prepared, and record-keeping procedures should be described in detail. If differentiated cells that were 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 (a set of acceptance criteria and analytical procedures). Proper quality control of these materials should be implemented.

When so-called Biological Products or Specific Biological Products (refer to Articles 2.9 and 2.10 of the Pharmaceutical Affairs Law) are used as raw materials, use the minimum amount required and strictly conform to the relevant laws and regulations, such as “Standards for Biological Raw Materials” (Notification No. 210, Japanese Ministry of Health, Labour and Welfare, 2003; a partially revised version was issued on

September 26, 2014). It is particularly important to adequately evaluate information related to the inactivation and elimination of viruses and to specify measures for encouraging retrospective survey and other studies. The technical requirements described in this paragraph should be taken into consideration when 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 via directed differentiation from ES cells.

(1) When culturing cells

- (i) Indicate the appropriateness of all media components including such as additives (e.g., serum, growth factors, and antibiotics), and reagents used for the treatment of cells and set specifications if necessary. Give consideration to the route of clinical application and other characteristics 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 using standards equivalent to those used with pharmaceuticals and pharmaceutical ingredients.
 - (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 using these serum components. If the use of serum or other such materials is unavoidable, consider the following points and investigate ways to prevent the contamination and the transmission of bacteria, fungi, viruses, and prions from serum and other related materials as well as treatment methods for their elimination, to the extent possible, from the final product.
 - (a) Clarify the origin of the serum or other components.
 - (b) Make strenuous efforts to minimize the risk of prion infection, e.g., by strictly avoiding the use of serum from areas or regions with known outbreaks of bovine spongiform encephalopathy (BSE).
 - (c) Use these batches of serum only after confirming that they are not contaminated with viruses or other pathogens by conducting appropriate tests to prove the absence of specific viruses and mycoplasma that originate in animal species.
 - (d) Perform appropriate procedures to inactivate and eliminate bacteria, fungi, and viruses to an extent that does not affect the activation and growth of the cells. For example, to avoid the risks associated with latent viral contamination, perform combinations of heat

treatment, filtration, γ -irradiation, and/or ultraviolet light treatment, if needed.

- (e) Preserve and store a portion of the serum used in order to monitor cultured cells for viral infections, to monitor onset of viral diseases among the patients, and measure antigen production in response to a component of the heterologous serum used.
 - (iv) When using feeder cells, conduct a quality evaluation while referring to “Derivation and Characterization of Cell Substrates Used for the Production of Biotechnological/Biological Products” (Pharmaceutical Notification No. 873, issued July 14, 2000, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare), “Guidelines on Public Health Infection Issues Accompanying Xenotransplantations” (Notification No. 0709001, issued July 9, 2002, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labour and Welfare), and “Guidelines on Epithelial Regenerative Therapy Using 3T3J2 Strain or 3T3NIH Strain Cells as Feeder Cells” based on “Guidelines on Public Health Infection Issues Accompanying Xenotransplantations” (Notification No. 0702001, issued July 2, 2004, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labour and Welfare) in order to prevent contamination of feeder cells and the transmission of bacteria, fungi, viruses, and prions. Indicate methods for the inactivation of the 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 is 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 at the initial stages of processing is deemed indispensable, attempt to decrease their use at subsequent steps as much as possible, and clearly indicate the appropriateness of their use from perspectives such as the scientific rationale, estimated residual amounts in the final product, and the effects on the patient. If it has been determined that an antibiotic can be adequately eliminated, its use does not need to be restricted. On the other hand, if a patient has a 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 the processing and that may contaminate the final product, choose components that do not have any harmful biological effects.
- (2) When combining cells with noncellular components
- (i) Quality and safety of noncellular raw materials
 - If the final product consists of cells and noncellular

components, such as a 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, the form and function in the final product, and evaluation of their quality, safety, and efficacy from the standpoint of the presumed clinical indication. If using materials that are absorbed by the body, perform the necessary tests on the degradation products to address 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, issued February 13, 2003, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare), describe the test results, and provide justification for the use of such raw materials. The use of information obtained from scientific literature is encouraged.

(ii) Interactions with target cells

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

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

(3) When cells are subjected to genetic modification

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

- (i) For the target gene (the 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, and other relevant information.
- (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, their properties, and procedures (transgenic method, origin and properties of the vector, and method of obtaining the vector used for introduction of the transgene) needed to produce the transgenic construct.
- (v) Structure and characteristics of the transgene construct.
- (vi) Control and preparation methods for cell and virus banks that are used to prepare vectors and transgenic constructs.

For manufacturing methods for transgenic cells, refer to Chapter 2 and other sections of “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 Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare on November 15, 1995. In addition, state clearly the appropriateness of the establishment of a cell line 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 (and other aspects) of genetic recombination-derived organisms and related organisms, 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” 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 contribute either chemically or functionally 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 best of your ability, 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 at the stage of the *in vitro* fertilized embryo 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 duration, and other characteristics 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 in question by taking into consideration cell characteristics, such as cell population purity, morphological features, results of HLA typing, phenotype-specific markers, karyotype, DNA fingerprinting, cell growth properties, and pluripotency and set acceptance criteria for the selected critical quality attributes. In addition, demonstrate the number of passages or cell divisions within 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 and technological limits on sample analysis. Thus, it is considered acceptable to perform a limited study 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 on the ES cell line itself to determine if any factors that are 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 from which to manufacture ES cell-based products, it is preferable to perform the tests on 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 necessary 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 taken, 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, methods of differentiation induction and methods for the isolation, culture, and establishment of the target cell line as well as media, culture conditions, culture period, the yield, and other characteristics and provide justification for the manufacture of a human ES cell-based product.

To ensure that the quality of the differentiated cell line remains stable and consistent, identify the critical quality attributes of the cells in question by taking into consideration various cell characteristics, such as cell population purity, morphological features, results of HLA typing, phenotype-specific markers, karyotype, DNA fingerprinting, cell growth properties, and differentiation potency and set acceptance criteria for the selected critical quality attributes. In addition, demonstrate the number of passages or cell divisions within 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 and technological limits on sample analysis. Thus, it is

considered acceptable to perform a limited study 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 possibly 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, issued in 2003 by the Japanese Ministry of Health, Labour and Welfare; a partially revised version was issued on September 26, 2014). 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, or parvovirus B19 in the human ES cell-derived differentiated cell line should be ruled out by relevant testing. Infection with cytomegalovirus, EB virus, and/or West Nile virus should also be ruled out, if necessary, by testing. If the genetic data on 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, potency, and other characteristics, establish the storage method and validity period, and make clear their appropriateness, taking into consideration the duration of storage and the distribution and storage form. In particular, when freezing and thawing, determine whether the process of freezing and thawing has an effect on stability and any other parameter of the cell line. Evaluate storage during the standard storage period and confirm the margin of stability to the extent possible. However, this does not apply when using cells immediately after their establishment. During transport of a human ES cell line or human ES cell-derived differentiated cell line, the containers used for the transport and the transportation procedure (including temperature control) shall be specified and their appropriateness clearly indicated.

5. Preparation of records and record-keeping procedures

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

4.2. Manufacturing process

When manufacturing 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 control

Indicate whether a lot control procedure is applied for final products and intermediate products. If any lot control procedure is adopted, establish standardized procedures for the makeup and control of the lot, which may include the lot size, labeling/numbering, testing method and acceptance criteria.

2. Manufacturing method

Provide an outline of the manufacturing method, from the

preparation of in vitro embryos from collected gametes to the establishment of human ES cells, differentiated cells, if any, 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 for assessment of eligibility 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, and cell viability assays. (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 is officially permitted by government regulations.)

(2) Establishment of a human ES cell line

Clarify its role in the manufacturing method that the manufacturer adopted [refer to 4. Chapter II. Manufacturing methods, 4.I. Raw materials and materials used in manufacturing, 3. Establishment of human ES cell lines and human ES cell-derived differentiated cell lines (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 4. Chapter II. Manufacturing methods, 4.I. Raw materials and materials used in manufacturing, 3. Establishment of human ES cell lines and human ES cell-derived differentiated cell lines (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 suitability. 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 duration, the yield, and other characteristics and explain their appropriateness to the best of your ability.

To ensure that the quality of the intermediate cell line remains stable and consistent, identify the critical quality attributes of the cells by taking into consideration various cell characteristics, such as cell population purity, morphological features, phenotype-specific markers, karyotype, cell growth properties, and differentiation potency, and set acceptance criteria for the selected critical quality attributes. In addition, demonstrate the number of passages or cell divisions within 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 and technological limits on sample analysis. Thus, it is considered acceptable to perform a limited study 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 point (6) below.

(5) Preparation of cells that constitute a principal component and serve as an 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

items to be described include induction of differentiation, isolation, and culture of the desired cells, and the media, culture conditions, culture duration, the yield, and other characteristics 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. Provide justification for each. Refer to “Derivation and Characterization of Cell Substrates Used for the Production of Biotechnological/Biological Products” (Pharmaceutical Notification No. 873, issued July 14, 2000, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare) and other documents. It is acceptable to omit a portion of the test items if, for a valid reason, the cells were evaluated properly at an upstream point in the process.

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

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

(8) Preparation of records and record-keeping procedures

Written records for items (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 and an active ingredient of the final product

For cells that are a principal component of the final product, analyze their attributes, such as cell population purity (to control contamination by undifferentiated cells or nontarget 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 markers. In addition, characterize their biological functions, if necessary. Furthermore, to evaluate the suitability of the culture duration 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 is acceptable to perform these studies preliminarily, using test samples obtained from donors in place of the real products that will be prepared for a clinical trial. On the basis of these results, identify the critical cell characteristics that should be used when applying the product to the treatment of a patient. Although comprehensive cell characterization is always desirable, it is not always possible to characterize the cells fully because there are quantitative and technological limits on sample analysis. Thus, it is acceptable to perform a limited study to the extent possible. When cell processing, such as growth within the body, is anticipated after a clinical application, clearly demonstrate the functions expected by describing the specified criteria with respect to the passage number or number of cell divisions.

4. The form and packaging of the final product

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

5. Storage and transport of the final product

If an intermediate or final products need to be stored and

transported, the storage procedure and duration, the containers used for transport, and the transportation procedure (including temperature control) shall be stated and their suitability clearly indicated (refer to Chapter III).

6. Consistency of the manufacturing procedure

To assess the consistency of the manufacturing process using each lot obtained from different production runs, determine whether they differ significantly with respect to the number of cells, cell viability, and cell characteristics (such as relevant markers of phenotype and genotype, functional characteristics, and the percentage of the desired cells) and from the point of view of the application method and the intended clinical use of the product. It is acceptable to use test samples obtained from donors in place of the products that will be prepared for a clinical trial. Evaluation of intermediate products may provide insight into the suitability of the cells used as raw materials and the validity of the manufacturing process up until the intermediate-product stage and can provide an appropriate guidepost en route to the final product. Therefore, it may be reasonable to adopt such an approach, where necessary and appropriate.

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

7. Changes in the manufacturing process

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

4.3. Quality control of the final product

1. Introduction

The overall quality control strategy for human ES cell-based products includes specifications (a set of acceptance criteria and analytical procedures) for the final products, quality control of raw materials for each therapeutic application to each patient, verification of the suitability of the manufacturing process, maintenance of consistency, and proper quality control of any intermediate products.

One of the most critical issues surrounding human ES cell-based products is contamination of the cells with other undifferentiated cells. It is preferable that the absence of contamination by nontarget undifferentiated cells is verified, as thoroughly as possible, at the intermediate-product stage.

Specifications will differ among final products, depending on the type and properties of the desired cells and tissues, manufacturing methods, the intended clinical use, method of application, stability, and test methods available. These differences shall be taken into consideration when set the acceptance criteria and test procedures. In addition, specifications shall be set and justified from the standpoint of achieving the purpose of quality control as a whole, by taking into consideration the mutually complementary relationships among 1) verification of the suitability of the manufacturing process, 2) the method for maintaining consistency, and 3) quality control of the raw materials and intermediate products. The purpose of the assessment at the initiation of clinical trials is to confirm that the product in question is unlikely to pose significant quality/safety problems for use in investigational clinical trials. Therefore, it is possible to set provisional specifications with allowance for

some variation on the basis of the measured values obtained for a few test specimens, as long as one can explain about the relationship between the results of clinical tests and the quality attributes after the clinical trial. However, testing for sterility and the presence of mycoplasma is essential. It should be noted that the quality control strategy, including specifications, should be enriched and developed as the clinical trial progresses.

2. Quality control of the final product

Refer to the general quality control parameters and tests shown below, set necessary and appropriate specifications for the final product, and provide the rationale for these specifications.

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

(1) Cell number and cell viability

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

(2) Tests of identity

Confirm that the cells are the intended target cells by assessing important characteristic(s), such as morphological characteristics, biochemical markers, immunological markers, characteristic products, and other appropriate genotypic or phenotypic features.

(3) Tests of purity

To test the purity of the cell population in a final product, if necessary, set the test parameters, test methods, and acceptance criteria for evaluating and controlling nontarget cells, such as undifferentiated cells, cells that exhibit abnormal growth, transformed cells, and contaminating cells, taking into consideration such parameters as the origin of the target cells and tissues, the culture conditions, and other parameters of the manufacturing process, such as quality control of intermediate products. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

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

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

(5) Tests for process-related impurities

For substances that may be present in the final product as, for example, contaminants, residues, newly generated products or degradation products; that potentially originate from raw materials, noncellular components, media ingredients (including feeder cells), chemical reagents, or any other process-related materials; and that may have deleterious effects on quality and safety (for example, albumin derived from fetal calf serum; and antibiotics), it is necessary to 1) prove that the substance is not present in the final product by taking into consideration the results of process evaluations related to the elimination of the substance or the results of in-process substance control or 2)

establish appropriate tests to control permissible levels of the substance in the final product. When selecting substances to be tested and setting their acceptance criteria, their appropriateness should be explained. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

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

Sterility should be ensured throughout the entire manufacturing process by evaluating test samples. The sterility (negative results of tests for common bacteria and fungi) of the final product should be demonstrated before its use in patients. Appropriate tests confirming the absence of mycoplasma should also be performed. A validated nucleic-acid amplification test can be used. If the results of the sterility tests and other assays of the final product can be obtained only after the product is administered to the patient, methods for dealing with the lack of sterility detected after administration should be established beforehand. In such cases, demonstrate by testing that the intermediate products are sterile and that sterility has been strictly maintained in all processes leading to the final product. If a product from the same facility and same process has already been used in patients, its sterility must be confirmed by testing all patients. If complete closure (hermetic seal) of a product that is a part of a lot has been ensured, tests on representative samples are sufficient. When tests need to be conducted for each application and if the results of sterility tests and other assays can be obtained only after administration of the product to the patient, the decision to administer the product will be based on the most recent data. However, even in such cases, sterility tests and other assays of the final product shall be conducted.

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

(7) Endotoxin tests

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

(8) Virus tests

Use titer tests to detect viruses in the intermediate and final products and confirm that administration of the ES cell-based products does not adversely affect the patient, when using cells that are not banked as raw materials or during manufacturing processes; that are from donors not tested during the infection window; and in which HBV, HCV, HIV, or HTLV can propagate. If components of biological origin are used in the manufacturing process, it may be necessary to test the final product for viruses originating from those components. However, whenever possible, it is preferable to determine that there is no contamination by testing or process evaluation at the original component stage.

(9) Specific biological tests

In some instances, it will be necessary to consider specific (quantitative or qualitative) biological testing that takes into account the cell type, intended clinical use, or

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

(10) Potency assay

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

(11) Mechanical compatibility tests

For products that require a certain degree of mechanical strength, set acceptance criteria for mechanical compatibility and durability that take into account the site of application. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

5. Chapter III. Stability of human ES cell-based products

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

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

6. Chapter IV. Preclinical safety testing of human ES cell-based products

To the extent that they are scientifically reasonable and technically possible, relevant animal tests and/or in vitro tests may be performed in order to address safety concerns associated with a human ES cell-based product. For noncellular constituents and process-related impurities, safety concerns should be addressed as thoroughly as possible by physicochemical analyses not animal testing. In addition, the presence of undifferentiated cells in the final product and their potential to cause ectopic tissue formation, tumorigenicity, or malignant transformation pose important safety concerns. To reduce the risk of contamination by such cells, conduct a thorough analysis, to the extent possible, at the cell bank and/or at the intermediate-product stage; alternatively, develop and utilize methods that effectively separate, remove, and/or inactivate contaminating undifferentiated cells during the manufacturing process. Careful selection of the route of target cell administration and other parameters may also be the way to minimize safety risks.

Meaningful results are not always obtained when products of human origin are tested in experimental animals. Thus, there may

be a scientific rationale for preparing products of animal origin and testing them in appropriate experimental animals if such a test system is expected to generate useful information. In such a case, consider using an animal model that is suitable for the target disease. (For example, monkeys may be suitable for studies of neurological diseases, and pigs and/or dogs may be suitable for studies of cardiovascular diseases.) However, because cells with characteristics identical to those of cells that constitute a human ES cell-based product cannot necessarily be obtained from nonhuman animal species, even if the preparation procedures are the same, and because a product of animal cell origin manufactured using identical processes will not necessarily be comparable to a human cell product, applicants should conduct a feasibility study before adopting, conducting, and evaluating such tests. When performing animal experiments using ES cell-based products obtained from nonhuman animal species, explain why extrapolation to humans is appropriate. Depending on the case, consider test systems that employ cells, and clearly explain the suitability of the test system.

Presented below are items and points to consider and to refer to when confirming preclinical safety of a product. These are provided as examples for illustration purposes; they are not intended to prescribe tests for which there is no rational basis. Conduct necessary and appropriate tests, taking into account the characteristics of the product, such as its intended clinical use, and evaluate and discuss the results in a comprehensive manner.

1. For cells expanded beyond the limit set for routine cultivation (defined by duration of culture, the population doubling level, or the passage number of the cells), demonstrate that undesirable alterations other than the intended transformation and abnormal proliferation of nontarget cells have not occurred.
2. It may be necessary to quantify special physiologically active substances produced by the cells and tissues and to discuss their effects when they are administered to patients. In some cases, significant amounts of active substances including cytokines and growth factors would be produced by the cells and this may result in undesirable effects on the patients.
3. From the standpoint of product safety, examine and discuss the potential effects of the product on a patient's healthy cells and tissues and the consequences.
4. Investigate and discuss the possibility of ectopic tissue formation by target cells and/or by contaminating undifferentiated cells and the potential consequences when the product is administered to the patients. Discuss this topic in a comprehensive manner, taking into account the type and characteristics of the product, such as the route of administration, the target diseases, and the validity of the test system.
5. Investigate and discuss the possibility of undesirable immunological reactions caused by the product and/or the expression product of a transgene and the consequences thereof.
6. Using an appropriate animal model or other system, investigate and discuss the possibility of tumor formation, including benign tumors and/or malignant transformation, by the final product or an intermediate product. These studies should be performed suitably by taking into account the type and characteristics of the product, the number of cells and route of administration, mode of application (e.g., cell sheet or cell suspension), cell engraftment site, target diseases, validity of the tests systems, and other relevant issues. If there is a possibility of tumorigenicity or malignant transformation, provide justification for the use of the product in question, taking into consideration the relationship with the anticipated efficacy. (Note: The most important aspect of a tumorigenicity test is to accurately assess the tumorigenicity of a final product that will be used in patients. However, it is conceivable that tumorigenicity will need

to be evaluated using cells from the intermediate product because the cells comprising the final product cannot be used for various reasons, such as the impossibility to obtain a sufficient number of cells. Furthermore, in tumorigenicity tests on animal models, various conditions such as cell dispersion and cell adhesion to the scaffolding, cell density, and the administration site are not necessarily identical to those for the final product. There are also differences in sensitivity depending on the species, strain, and immunological state of the animal. The tumorigenicity of the final product should be evaluated taking into consideration these circumstances in a comprehensive manner. The risks to the patient arising from tumorigenicity of the final product should be rationally evaluated based on the balance between any risks and the benefits to the patient as a result of treating the disease.)

7. If an exogenous gene is introduced into certain cells during the manufacturing process and if it may function or remain as a residue in the final product, conduct tests in accordance with "Gene Therapy Pharmaceutical Guidelines." In particular, if viral vectors are used, conduct quantitative tests to determine if any replication-competent viruses are present and provide justification for the test method employed. Describe the safety of the transgene and its products on the basis of their characteristics. For cells, discuss the possibility of changes in cell growth and the risk of tumor formation, including benign tumors and malignant transformation. Whenever using a vector that can get inserted into a chromosome, consider the necessity of evaluating abnormal proliferative characteristics and/or tumorigenicity and implementing long-term follow-up.
8. Consider conducting rationally designed general toxicological tests if the product, including an animal-derived product, is easily obtained and doing so will produce useful information regarding its clinical application. When conducting general toxicological tests, refer to "Guidelines for Toxicology Studies on Pharmaceuticals," which is an appendix in the document "Guidelines on Toxicology Studies Required for Regulatory Approval for the Manufacture or Import of Pharmaceuticals" (Drug Evaluation Notification 1:24, issued September 11, 1988, New Drug Division/Evaluation Licensing Division, Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare).

7. Chapter V. Studies supporting the potency or efficacy of human ES cell-based products

1. A well-designed study on experimental animals and/or cells should be performed in order to demonstrate, to a scientifically reasonable and technically possible extent, functional expression, sustainability of effects, and/or the anticipated clinical efficacy (proof of concept) of a human ES cell-based product.
2. For transgenic cells, demonstrate the expression efficiency, sustainability of expression, and biological activity of the desired products derived from the transgene. Discuss rationale of the transgene expression products as active ingredients for anticipated clinical efficacy (proof of concept) of the human ES cell-based product in question.
3. Where appropriate products derived from the processing of animal ES cells and/or animal models of a disease are available, use them to study the potential therapeutic efficacy of the product.
4. At the beginning of the clinical trial, detailed experimental studies will not necessarily be required if scientific literature and/or other information supports the prediction that the potency or efficacy of the product in question will be markedly superior to that of a different therapeutic method.

8. Chapter VI. Pharmacokinetics of human ES cell-based products

1. Pharmacokinetic studies of the internal behavior of transgene expression products or cells/tissues that constitute the final products (these studies may include assessment of their absorption and distribution in experimental animals), should be performed to an extent that is technically possible and scientifically reasonable extent. Thereby, these experiments are expected to estimate the survival of cells/tissues administered to patients and the duration of their effects and to determine whether the intended efficacy is successfully achieved. (Note: Testing methods may include histological studies, human Alu sequences amplification by polymerase chain reaction (Alu-PCR), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), and bioimaging.)
2. For human ES cell-based products, clarify, in animal studies, the rationale for the administration method. In particular, extrapolate from animal experiments, the systemic distribution of the cells after systemic administration and discuss the distribution from the point of view of clinical usefulness. (Note: Although it is unclear exactly where the cells adhere with each administration route, local administration is presumed to be preferable to systemic administration. However, if the benefits to patients can be explained, it is acceptable to use systemic administration. In any case, an administration method that minimizes the distribution of an ES cell-based product to organs other than the target organ would be a rational choice. Even if the cells do localize to a site other than the intended transplantation site, this administration method may be used if no adverse effects result. Arrhythmia caused by osteogenesis of cells that ectopically localize to the heart is an example of an adverse effect that can result from ectopic localization.)
3. When the cells or tissues are directly applied or targeted to a specific site (e.g., tissue) where they are expected to act, clarify the localization, and discuss the effect of the localization on the efficacy and safety of the product.

9. Chapter VII. Preliminary analysis of clinical trials

The main purpose of the present guideline is to outline points to consider when evaluating the quality and safety of human ES cell-based products, either at the time of application for marketing authorization or at the beginning of an investigational clinical trial. In the latter case, it is necessary to determine, while taking into consideration the clinical usefulness, whether there are any quality or safety problems that might impede the initiation of human clinical trials. Thus, quality and nonclinical safety assessments for the decision to initiate an investigational clinical trial of the product in question should be considered in reference to the points outlined below. Any known risk factors associated with product quality and safety should be eliminated to the extent possible using up-to-date scientific and technological methods, and the scientific appropriateness should be clearly described. Any remaining risks should be weighed against the risks associated with not performing the trials on patients that suffer from diseases that are serious and life-

threatening, that involve marked functional impairment or a marked decrease in quality of life (QOL) resulting from the loss of a certain degree of a physical function or form, or for which existing therapies have limitations and do not result in a cure. Furthermore, it is necessary to entrust the patient with the right to make a decision after receiving all of the available information, including all information on identified/presumed risks and anticipated benefits.

1. Target disease.
2. Subjects and patients who should be excluded as participants.
3. Details of the therapy to be performed on the subjects, including the application of human ES cell-based products and drugs used concomitantly. (Note: If it is anticipated that drugs to maintain, enhance, and/or induce the function of the administered or transplanted cells will be co-administered, verify the intended activity of the drugs either *in vitro* or *in vivo*.)
4. The rationale for conducting the clinical trials in light of existing therapeutic methods.
5. Plan on explaining the clinical trial to the patients, including the currently known risks and benefits of the product.

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

Disclosures

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

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