intended to be considered, selected, applied and evaluated for serving each intended purposes, and do not necessarily require the most stringent level of interpretation and practice. In accordance with the purpose of the present guideline, applicants are encouraged to explain and justify that consideration of the background, selection, application and the content and extent of evaluation are appropriate to their own purpose and scientifically rational.

Chapter I. General Principles

I. Objective

The present guidelines outline the basic technical elements to ensure the quality and safety of pharmaceuticals and medical devices derived from processing of human embryonic stem cells (ES cells). These products are hereinafter referred to as ES cell-based products or merely as "desired cell products".

II. Definitions

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

1. "Human embryonic stem cells (ES cells)": Cells that are collected from a human embryo or cells that are obtained from such cells through cell division or for cells that are not embryo itself, and that possess the ability to differentiate into endoderm, mesoderm, and ectoderm, and furthermore, maintain the ability to self-renew or the similar ability.
2. "Processing of cells and tissues": Any processing of a cell or tissue, such as pharmaceutical or chemical treatment, altering a biological characteristic, combining with a noncellular component, manipulation by genetic engineering, and so on, with the objective of propagation and/or differentiation of a cell or tissue, cell activation, and production of a cell line, with the aim of treating a patient, or repairing or regenerating tissue.
The isolation of tissue, disintegration of tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, sterilization by washing or gamma irradiation, freezing, thawing, and such similar procedures regarded as minimal manipulations are not considered to be processing.
3. "Manufacture": Actions undertaken up until the final product (a human ES cell-based product) is released to market. This includes in addition to processing of cells and tissues, minimal manipulations such as the isolation of tissue, disintegration of tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, sterilization by washing or gamma irradiation, freezing, thawing, and other procedures that are performed without any change in the original properties of the cells or tissues.
4. "Phenotype": A morphological or physiological characteristic that is expressed by a certain gene under constant environmental conditions.
5. "HLA typing": Specifying the type of HLA (human leukocyte antigen), a human primary histocompatibility antigen.
6. "Donor": Persons who donate their own cells that serve as the raw material of a human ES cell-based product. Persons who provide sperm and unfertilized eggs.
7. "Transgenic construct": A construct

which contains a vector for introducing a target gene (specific gene encoding a desired protein or RNA) into a target cell, the target gene, and the coding sequences of the elements essential for the expression of the target gene.

Chapter II Manufacturing Methods

Describe all important and relevant information concerning the manufacturing method, taking into account the items listed below. This information will contribute to ensuring the quality, safety, and efficacy of the final products, and is important for guaranteeing the consistency of the quality from a manufacturing perspective. It should be noted that assurance of the quality and safety, and their consistency is achieved by mutually? complementary measures throughout the manufacturing method as a whole, and it is most important that the measures are rational and serve the intended purpose. It may be acceptable to omit a portion of the items listed below, after providing the appropriate scientific basis, with respect to the quality tests or controls of the final product or intermediates, or control of the manufacturing process, if the quality and safety, and their consistency, can be assured.

I. Raw Materials and Materials Used in Manufacturing

1. In vitro fertilized embryos

(1) Source and origin, justification of their selection

Explain the source and origin of the in vitro fertilized embryos that are used to establish the human ES cell line, and justify the reasons for selecting these in vitro embryos.

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

(i) Features of biological structure and function, and selection criteria
Explain and justify the reasons for selecting the in vitro embryos used as raw materials based on the characteristics of their biological structure and function, such as for example, morphological characteristics, growth characteristics, and other suitably chosen and appropriate indicators.

(ii) Ethical propriety of donor selection
If a new human ES cell line intended for clinical use is established after publication of the present guidelines, indicate that the selection of donor was carried out in an appropriate and ethical manner and that a proper procedure was taken 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 prior to publication of this guideline, it is the responsibility of the manufacturer of the human ES cell-based product to prove (demonstrate? or clarify?) that selection of the donor was carried out in an appropriate and ethical manner and that a proper procedure was taken.

(iii) Donor selection criteria and eligibility

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

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

In addition to the above, further investigate and decide their eligibility as a donor by examining the past history mentioned below of the donor through physician-donor interviews and so on, and if they have ever had a blood transfusion or undergone a transplantation procedure.

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

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

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

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

raw materials or intermediate products and to justify their appropriateness.)

(3) Records related to the donor

All records related to the donor shall be complete and kept so that any information necessary with respect to ensuring the safety of an in vitro embryo can be verified. Concrete measures shall be described to the greatest extent possible.

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

The collection of gametes used to establish a human ES cell strain(line?), preparation of in vitro embryos, and their storage and transport should be carried out in accordance with (i) to (viii) below. The establishment and distribution of a human ES cell lineshould be conducted in accordance with "Guideline on the Establishment and Distribution of Human ES Cells'Indicate (Notification Number 156, Japanese Ministry of Education Culture, Sports, and Science, published on August 21, 2009). Human ES cells are established (primary establishment) using human in vitro embryos. Do not use ES cells established by preparing a human cloned embryo and then using this cloned embryo to establish the ES cells (secondary establishment). Also, do not use entosomatic fertilized embryos.

(i) Eligibility of personnel and medical institutions collecting samples
When preparing and using human in vitro embryos, describe the technical requirements for personnel and medical institutions that collect the male and female gametes.

(ii) Suitability of embryo collection method
Describe the method used to prepare the in vitro embryos, and describe how the embryos were appropriately selected both scientifically and ethically, and that appropriate procedures were taken. For the gamete collection methods and in vitro fertilization methods, indicate the suitability of the equipments and drugs used, and the measures adopted to prevent microbial contamination, erroneous sampling (mix-ups), and cross contamination.

(iii) Informed consent from donors
Describe the details of the informed consent, including the clinical application, of the donors of the gametes.

(iv) Protection of donor privacy
Indicate the measures adopted to ensure protection of the privacy of the donor.

(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 for test results that indicated a problem existed.

(vi) Storage method and measures to prevent erroneous sampling (mix-ups)
If the gametes or in vitro embryos prepared need to be stored for a definite period of time, set the storage

conditions and storage period, and justify the appropriateness (validity) for their setting. Describe in detail the measures and procedures to be taken to prevent erroneous sampling (mix-ups), referring to "Safety Control in Infertility Treatments" (Dated February 20, 2009, Notification 0220001, Equal Employment, Children and Families Bureau, Japanese Ministry of Health, Labor and Welfare).

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

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

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

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

When so called ' Biological Products' or 'SpecificBiological Products' (refer to Article 2.9 and 2.10 of Pharmaceutical Affairs Law) are used as raw materials, the amounts used should be kept to the minimum amount required and should strictly obey the relevant laws and notifications, such as "Standards for Biological Raw Materials" (Notification Number 210, Japanese Ministry of Health, Labor, and Welfare, 2003). It is particularly important to sufficiently evaluate information related to the inactivation and elimination of viruses, as well as to indicate measures for ensuring retrospective and other studies. The technical requirements described in this paragraph should be taken into consideration when the process of preparation of in vitro embryos from the raw materials (gametes) into ES cells, and of directed differentiation from ES cells into ES cell-derived differentiated cells and the final products in question include any relevant elements/concerns be applied ???.

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

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

(a) The ingredients and water used in media should be of high quality and high biological purity, and whose quality is controlled at standards equivalent to those for pharmaceuticals and pharmaceutical raw materials.
(b) Provide information on not only the main ingredients used in media, but all components, as well as the rationale for their selection, and if necessary, the quality control and other procedures. However, widely known and commercially available media products such as DMEM, MCDB, HAM, and RPMI are regarded as one raw material.
(c) Conduct sterile 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 believed to be controlled in 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. For products that may be used repeatedly in particular, investigate as much as possible ways to avoid using these serum components. If the use of serum or other such material is unavoidable, give consideration to the following points, and investigate ways to prevent the contamination and spread of bacteria, fungi, viruses, and abnormal prions from the serum and other products, as well as treatment methods for their elimination, to the greatest extent possible, from the final product.

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

(iv) When using feeder cells, conduct quality evaluation while referring to "Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products" (Pharmaceutical Notification Number 873, Ministry of Health, Labor, and Welfare, July 14, 2000), "Guidelines on Public Health Infection Issues Accompanying Xenotransplantations" (Notification 0709001, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labor, and Welfare, issued July 9, 2002), and "Guidelines on Epithelial Regenerative Therapy Using 3T3J2 Strain or 3T3NIH Strain Cells as Feeder Cells" based on "Guidelines on Public Health Infection Issues Accompanying Xenotransplantations" (Notification 0702001, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labor, and Welfare, issued July 2, 2004) in order to prevent the contamination and spread of bacteria, fungi, viruses, and abnormal prions from the feeder cells, and indicate the methods for the inactivation of cell division potential and conditions such as cell density when using the feeder cells. However, for example, if the feeder cells or equivalent cells are being used in the manufacture of a cell or tissue product that has already been used clinically and whose characteristics and microbiological safety have already been assessed and confirmed, it may be possible to omit the virus tests or parts of other tests by demonstrating the appropriateness of using these cells.

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

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

(vii) For media components that may be contained in the final product and other components that are used in manipulation, choose components that do have any harmful biological effects.

(2) When combining with noncellular components

(i) Quality and safety of noncellular raw materials

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

and beads, describe in details the quality and safety of the noncellular components.

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

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

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

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

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

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

substance derived from a desired cell is anticipated and the objective is segregation of the application site and the desired cells or undifferentiated cells, confirm that the cells do not leak out caused by the degradation, etc. of noncellular components.

(3) When cells undergo genetic modifications
When genes are introduced into cells, provide the details concerning the following items.

(i) For the target gene (specific gene encoding a desired protein or RNA), information related to its structure, origin, method by which it was obtained, cloning methods, and for cell bank of the target gene, methods of preparation, control, and renewal and so on.
(ii) Nature of the transgene.
(iii) Structure, biological activity, and properties of the desired gene products
(iv) All raw materials, properties, and procedures (transgenic method, and origin, properties, and method of obtaining vector used in gene introduction) needed to produce the transgenic construct.
(v) Structure and characteristics of the transgene construct.
(vi) Control and preparation methods for cell and virus banks in order to prepare vectors and transgenic constructs.

For the 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 of "Concerning Guidelines for Ensuring the Quality and Safety of Gene Therapy Pharmaceuticals" (hereinafter referred to as "Gene Therapy Pharmaceutical Guidelines"), published as Notification 1062 by the Ministry of Health and Welfare on November 15, 1995. Also, state clearly the appropriateness of the establishment in accordance with the appendix of the same notification.

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

Regardless of what is mentioned above, if a gene introduced into cells is used as a reagent in the manufacturing process and does not either chemically or functionally make up part of the final product, it is acceptable to just describe how the quality and safety of the gene conform to the intended use, based on the most up-dated knowledge.

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

In order to maintain the stability and consistency of the quality of the human ES cell line, identify critical quality attributes of the cells from among the various cell characteristic indicators(characteristics?) (for example; cell purity, morphological features, HLA typing, phenotype specific markers, karyotype, DNA fingerprinting, cell growth properties, pluripotency, etc.) and set acceptance criteria for them. Also demonstrate the potent number of passages or of cell divisions within which cells can be proliferated with keeping their quality in terms of the criteria specified. Although comprehensive cell characterization is always desirable, it is recognized that there may be difficult to perform the study fully since there are quantitative limits to samples as well as technological limits Then, it is acceptable to consider to perform the study to some extent possible.

If information related to infections in donors cannot be obtained due to donor anonymity or other reasons, infection of the human ES cell line established due to hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), adult human T-lymphotropic virus (HTLV), or parvovirus B19 should be ruled out by testing. Infection due to cytomegalovirus, EB virus, or West Nile virus should also be ruled out, if necessary, by testing. If the genetic traits of a donor cannot be obtained, it is necessary to analyze the genetic information of the ES cell line itself to determine if any factors related to genetic diseases are present. Although it is acceptable to perform these tests at the stage of a differentiated cell line from the perspective of a material with which to manufacture a drug, it is preferable to perform the tests in the ES cell line from the objective of establishing a human ES cell line.

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

It should be noted that in some cases the establishment of a cell line that has progressed from the differentiation stage from human ES cells (differentiated cell line: bank) by a facility, may be important in terms of the stable manufacture of a safe final product, as well as rather scientifically rational procedure. When such a measure is chosen, describe the intended use at the facility and explain the advantages

and appropriateness in the manufacture of a human ES cell-based product. If a cell line that exhibits a different phenotype is established in stages, describe the methods (for example, differentiation induction methods, methods for the isolation, culturing, and cell line establishment of the target cells, as well as the media, culture conditions, culture period, yield, and so on at each stage until establishment of the cell line) until the establishment of each respective cell line, and justify the appropriateness in the manufacture of a human ES cell-based product.

In order to maintain the stability and consistency of the quality of the differentiated cell line, identify critical quality attributes of the cells from among the various cell characteristic indicators (for example; cell purity, morphological features, HLA typing, phenotype specific markers, karyotype, DNA fingerprinting, cell growth properties, pluripotency, etc.) and set acceptance criteria for them. Also demonstrate the potent number of passages of cell divisions within which cells can be proliferated with keeping their quality in terms of the criteria specified. Although comprehensive cell characterization is always desirable, it is recognized that there may be difficult to perform the study fully since there are quantitative limits to samples as well as technological limits Then, it is acceptable to consider to perform the study to some extent possible.

The conditions that must be fulfilled are identical for imported ES cell strains and differentiated cell strains established from long-established ES cell strains, etc. However, it is possible a raw material that does not meet the stipulations of the "Guidelines for Organism-derived Raw Materials" and for which the establishment and maintenance processes are vague or unclear may have been used. The propriety of using such cell strains will be determined by separate review and evaluation for each individual product in consultation with the Pharmaceuticals and Medical Devices Agency (PMDA). (Note: If sufficient infection-related information for the human ES cell-derived differentiated cell strain that will be used cannot be obtained, infection due to hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), adult human T-lymphotropic virus (HTLV), and/or parvovirus B19 should be ruled out by testing. Infection due to cytomegalovirus, EB virus, and/or West Nile virus should also be ruled out, if necessary, by testing. If the genetic traits of a donor cannot be obtained, it is necessary to 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 appropriate stability tests based on cell viability and potency etc., of the cells, establish storage method and validity period, and make clear their appropriateness,

taking into the due consideration duration of storage and distribution and storage form. In particular, when freezing and thawing, confirm whether the process of freezing and thawing have effect on stability and any criterion of the cell line, if needed. Evaluate storage over standard storage period, and confirm the margin of stability to the extent possible. However, what are mentioned above are not adopted when using the cells immediately following establishment.

When transportting human ES cell line or human ES cell-derived differentiated cell line, the containers used for transport and the transportation procedure (including temperature control, etc.) shall be determined and their appropriateness clearly indicated.

5. Preparation of records and keeping procedures
Written records for 2. through 4. above should be prepared and proper keeping procedures for the records shall be clearly described.

II. Manufacturing Process

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

1. Lot composition and lot control
Indicate whether or not a lot is made up of final products and intermediate products. If a lot is composed of both final and intermediate products, establish standardized procedures concerning the make up and control of the lot.

2. Manufacturing method
Provide an outline of the manufacturing method from the preparation of in vitro embryos from collected gametes through to the establishment of human ES cells and cells that have progressed to the differentiation state, and then to the final product, and 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 to assess appropriateness of the human ES cells or human ES cell-derived differentiated cells, respectively, that will serve as the raw materials, taking into account the nature of the cells and its intended use. These may include, for example, visual test, microscopic examination, recovery factor of target cells, cell viability, characterization, of cells and tissues, microbiological tests, and so on. At the stage of initiating clinical trials, provide the actual measured values obtained up until that point with test samples, and propose provisional a set of acceptance criteria based on these values.

(2) Establish of human ES cell line
Clarify its role in a manufacturing method that a manufacturer adopted.)(Refer to chapter II-I.-3(1).)

(3) Establish of human ES cell-derived differentiated cell line
Clarify its role in a manufacturing method that a manufacturer adopted, if any.(Refer to chapter II-I.-3(2).)

(4) Establishment of intermediate cell line derived from a human ES cells
When the manufacturer of a human ES cell-based product establishes a cell line (intermediate cell line) as an intermediate product from an ES cell line or a differentiated cell line that has been received, explain its advantages and appropriateness. If a cell line that exhibits a different phenotype is established in stages, describe the methods (for example, differentiation induction methods, methods for the isolation, culturing, and cell line establishment of the target cells, as well as the media, culture conditions, culture period, yield, and so on at each stage until establishment of the cell line) until the establishment of each respective cell line, and justify their appropriateness to the extent possible.

In order to maintain the stability and consistency of the quality of the intermediate cell line, identify critical quality attributes of the cells from among the various cell characteristic indicators (for example; cell purity, morphological features, phenotype specific markers, karyotype, cell growth properties, pluripotency, etc.) and set acceptance criteria for them. Also demonstrate the potent number of passages or of cell divisions within which cells can be proliferated with keeping their quality in terms of the criteria specified. Although comprehensive cell characterization is always desirable, it is recognized that there may be difficult to perform the study fully since there are quantitative limits to samples as well as technological limits. Then, it is acceptable to consider to perform the study to some extent possible.

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

(5) Preparation of cells that make up principal component of the final product as an active ingredient
Describe the methods, either directly from a human ES cell-derived differentiated cell line or via an intermediate cell line that is derived from a human ES cell line, to prepare the cells that serve as the active ingredient of the final product. The methods to be described include induction of differentiation, isolation, and culturing of the desired cells, and the media, culture conditions, culture period, yield, and so on used 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 process of manufacturing human ES cell products, describe the details of the rationale for preparing the cell bank, the methods used to prepare the cell bank, characterization of the cell bank, the storage, maintenance, and control methods, renewal methods, as well as any other processes and tests performed, and justify the appropriateness of each. Refer to "Derivation and Characterisation of Cell Substrates Used for Production

of Biotechnological/Biological Products" (Pharmaceutical Notification Number 873, Japanese Ministry of Health, Labor, and Welfare, July 14, 2000) and other documents. Nevertheless, it is acceptable to omit a portion of the test items, if there is rational reason that the cells have been already evaluated properly at an upstream point in the process.

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

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

(8) Preparation of records and keeping procedures

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

3. Characterization of cells that make up principal component of a final product as an active ingredient

Analyze various attributes of the cells such as cell purity to control contamination by undifferentiated cells or non-target cells, the cell viability, morphological characteristics, growth characteristics, biochemical indicators(makers?), immunological indicators(makers?), distinctive substances produced from cell, karyotype, differentiation potency, and other appropriate genotypic and phenotypic indicators(makers?) of cells that make up principal component of the final product. Also characterize with respect to biological functions, where necessary. Furthermore, in order to evaluate the appropriateness of the culture period and stability of the cells, use appropriate cell characteristics to prove there have been no unintended changes in cells cultured for duration beyond the proposed culture period. When performing these studies, it is acceptable to study and verify beforehand using test samples obtained from donors who are not patients. Based on these results, it is necessary to identify the critical cell characteristics that should be used when applying the product to a patient. Although comprehensive cell characterization is always desirable, it may not always be possible to perform the study fully since there are quantitative limits to samples as well as technological limits. Then, it is acceptable to just perform the study to the extent possible. When cell processing like growth within the body is anticipated after clinical application, clearly demonstrate the functions expected using the passage number or number of cell divisions based on specified criteria.

4. Form and packaging of final product

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

5. Storage and transport of final product

If intermediate or final product needs to be stored and transported, storage

procedure and duration, the containers used for transport and the transportation procedure (including temperature control, etc.) shall be stated and their appropriateness clearly indicated. (Refer to chapter-III)

6. Consistency of the manufacturing procedure

When manufacturing human ES cell-based products, assess beforehand whether or not during the manufacturing process and for each individual product there has been any significant differences between each production (each lot) with respect to the number of cells, cell viability, and cell characteristics (such as relevant markers of phenotype, appropriate markers of genotype, functional characteristics, and the percentage content of desired cells) from the point of view of intended clinical use of the product. It is acceptable to use test samples obtained from donors who are not patients in place of the real products that will be prepared for clinical trial. Evaluation using intermediate products may provide a good reflection of the appropriateness of cells and tissues used as raw materials and the validity of the manufacturing process up until the point of intermediate products, as well as also being an appropriate guidepost leading up to the final product. Therefore, it may be reasonable to adopt such approach, where necessary and appropriate.

When the cryopreservation period or time of cell cultivation last to a long term during the manufacturing process, perform sterilization tests and so on at constant intervals to confirm that the sterility has been ensured.

7. Changes in manufacturing process

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

III. Quality Control of Final Product

1. Introduction

The overall quality control strategy of human ES cell-based products include specification of final products, quality control of raw materials for each different application to each individual patient, verification of the appropriateness of the manufacturing process and maintenance of consistency thereof, as well as the proper quality control of intermediate products, if any.

One of the most critical issues on ES cell-based cell products is a measure to ensure there has been no contamination of the cells by undifferentiated cells other than the desired cells. It is preferable that no contamination by non-target undifferentiated cells is verified, as much as possible, at the intermediate product stage.

Since specifications for the final product are to be different depending

upon the type and properties of the desired cells and tissues, manufacturing methods, intended clinical use and method of application for each product, stability, and test methods that can be available, these differences that depend on the cell or tissue being handled shall be taken into sufficient consideration when setting acceptance criteria and test procedures. Also, specifications shall be set and justified from the perspective of achieving the purpose of quality control as a whole by taking into consideration the mutually complementary relationships between the verification of the suitability of the manufacturing process and the method of maintaining consistency and quality control of the raw materials and intermediate products. The purpose of the assessment for initiating of clinical trials is to confirm that product can be deemed to have no significant quality/safety problems for using investigational clinical trials. Therefore, it may be possible to set provisional specifications with allowances for some variation on the basis of the values measured on a few test specimens, as long as one can argue the relationships between the results of clinical tests and such quality attributes after clinical trials. However, testing for sterility and presence of mycoplasma is essential. It should be noted that quality control strategy including specifications shall be enriched and developed along with the progress of clinical trials.

2. Quality control of the final product

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

Set appropriate acceptance criteria and test procedures for the individual products that do not make up a lot and for the lot consisting of the products that do make up a lot since normally each individual lot is the unit subjected to quality control.

(1) Cell number and cell viability

The number and viability of cells as being active ingredient in the final product or if needed, in an appropriate intermediate product in the manufacturing process should be determined. At beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on actual measured values from a small number of test samples.

(2) Tests of Identity

Confirm that the cells are the intended target cells by means of important cell characteristic markers selected from among the morphological characteristics, biochemical markers, immunological markers, characteristic products, and other appropriate genotypes or phenotypes of the intended target cells and tissues.

(3) Tests of Purity

If necessary, set the test parameters, test methods, and acceptance criteria for evaluating and controlling the purity of cells with respect to non-target cells, such as

undifferentiated cells, cells exhibiting abnormal growth, transformed cells, and the presence of any contaminating cells, taking into consideration the origin of the target cells and tissues, the culture conditions and other parameters of the manufacturing process, quality control of intermediate products, and so on. At beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on actual measured values from a small number of test samples.

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

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

(5) Tests for process-related impurities

For substances that may be present in the final product as contaminants, residues, or as newly generated products or degradation products, etc., potentially originating from raw materials, non-cellular components, media ingredients (including feeder cells), chemical reagents, or any other process-related materials, and that may have deleterious effect on the quality and safety (for example, albumin derived from fetal calf serum, antibiotics, etc.), it is necessary to either prove that the substance is not present in the final product by taking into consideration the results of process evaluation related to elimination of the substance or the results of in-process control of the substance, or alternatively establish appropriate tests with which to control permissible levels for the substance in the final product. When selecting substances to be tested and setting their acceptance criteria, their appropriateness should be explained and justified.

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

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

The sterility of the final product should be sufficiently assessed to ensure sterility throughout the entire manufacturing process using test samples. The sterility (negative for common bacteria and fungi) of the final product should be demonstrated in tests before use in a patient. Appropriate tests confirming the absence of mycoplasma should also be carried out. A validated nucleic acid amplification method can be used. If the results of the sterility and other tests on the final product can only be obtained after administration to the patient, the methods for dealing with non-sterility after administration should be established beforehand. In such an instance, demonstrate by testing that the intermediate products are sterile, and the sterility should be

strictly controlled in all processes up until the final product. If a product from the same facility and same process has already been used in patients, its sterility had to be confirmed by testing in all patients. If complete closure (hermetically sealed) of the product comprising a lot has been assured, tests using only representative samples are sufficient. When tests need to be conducted for each different application and if the results of sterility and other tests can only be obtained after administration to the patient, whether or not application should be done or not will be determined based on the most recent data. However, even in such an instance, sterility tests and other tests on the final product shall be conducted.

While it is desirable that every possible effort be made so that antibiotics are not used in cell culture systems, if they are used, adopt measures to ensure that the antibiotics do not influence the sterility tests.

(7) Endotoxin tests

Carry out the endotoxin test, taking into consideration the impact of the contaminant in the samples. The acceptance criteria do not necessary depend on the actual measured values. It is recommended to set acceptance criteria taking into consideration the safety ranges given 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 criteria, including validation results, and justify their appropriateness.

(8) Virus tests

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

(9) Efficacy tests

In some instances, it will be necessary to consider efficacy testing that takes into consideration cell type, intended clinical use, or distinctive characteristics of the cells. At beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on actual measured values from a small number of test samples.

(10) Potency tests

If the secretion of a specific physiologically-active substance from the cells or tissues accounts for the efficacy or the essential effect of an ES cell-based product, establish test

parameters and/or acceptance criteria related to the substance in order to demonstrate the intended effect. Set acceptance criteria for potency, amount produced, and so on for phenotype products or for a desired product secreted from cells when a gene has been introduced. At beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on actual measured values from a small number of test samples.

(11) Mechanical compatibility tests

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

Chapter III Stability of Human ES Cell-based Products

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

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

Chapter IV Preclinical Safety Testing of Human ES Cell-based Products

Relevant animal tests and/or in vitro tests may be performed in order to elucidate safety concerns on a human ES cell-based product to a scientifically reasonable and technically possible. The type, characteristics and intended clinical use of the individual final product should be critical elements when considering the scope and protocol of the non-clinical safety study and demonstrating the propriety thereof.

Non-cellular constituents and process-related impurities should be evaluated as much as possible by physicochemical analyses but not tests using animals. Also, there is an important safety concern regarding the presence of undifferentiated cells in the final product and their potential to cause any ectopic tissue formation, tumorigenicity, or malignant transformation. For this, it is necessary to strive as much as

possible to reduce the risk of contamination of such cells by thorough analysis to the greatest extent possible at the cell bank and/or intermediate product stage, or alternatively by developing and utilizing methods that effectively separate, remove, and/or inactivate these contaminating undifferentiated cells from the target cells during manufacturing process. Furthermore, selection of route of administration and so on for the target cells may also be a useful measure to minimize any safety concerns.

It is not always true that meaningful results can be obtained by testing of products of human origin with experimental animals. Thus, where more useful information is expected to be obtained by preparing product models of animal origin and conducting tests with appropriate experimental animals, there may be a scientific rationale for using such a type of test system. In such a case, consider conducting tests using suitable animal models for each target diseases. (Note: For example, monkeys may be suitable for nervous system diseases, while pigs and/or dogs may be suitable for cardiovascular diseases). However, since cell groups that possess identical characteristics to cells that constitute a human ES cell-based product will not necessarily be obtained from non-human animal species even though the preparation procedures are same as human, and since an animal cell origin product manufactured using identical culture conditions and so on will not necessarily be comparable to a human cell product, careful study beforehand are needed when adopting, conducting, and evaluating such tests. When conducting animal experiments using ES cell-based products obtained from non-human animal species, explain the feasibility of extrapolation. Depending on the case, consider test systems that employ cells, and clearly explain the appropriateness of the test system when conducting tests using this kind of approach.

Presented below are examples of items and points to consider that should be referred to, if necessary, when confirming the preclinical safety of a product. These are merely examples for illustration purposes and are not suggesting tests with no rational basis be carried out. Conduct necessary and appropriate tests, taking into account the characteristics of the product, intended clinical use, and so on, and evaluate and discuss the results in a comprehensive manner.

1 For cells expanded beyond the defined limit for cultivation (by a period of time population doubling level of the cells, or passage level of the cells) for routine production clearly demonstrated that transformations other than the target transformation and abnormal proliferation of non-target cells have not occurred.
2 It may be necessary to conduct quantitative assays of some special physiologically-active substances produced by the cells and tissues and discuss their effects when given to patients. In some cases, significant amounts