control of raw materials for each different application to each individual patient, verification of the appropriateness of the manufacturing process and maintenance of its consistency, as well as proper quality control of intermediate products, if any. One of the most critical issues in case of iPS(-like) cell-based products is a measure to ensure absence of contamination of the cells by undifferentiated cells other than the desired cells. Verification of the absence of contamination by non-target undifferentiated cells is desirable, as much as possible, at the intermediate product stage.

Because specifications for the final product differ 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 available test methods, differences that depend on cell or tissue handling shall be taken into sufficient consideration when setting acceptance criteria and test procedures. In addition, 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 1) the verification of the suitability of the manufacturing process, 2) the method of maintaining consistency, and 3) quality control of the raw materials and intermediate products.. The purpose of the assessment for initiating clinical trials is to confirm that the product in question can be deemed to have no significant quality/safety problems for use in investigational clinical trials. Therefore, it may be possible to set provisional specifications, with allowances for some variation, based on values measured using 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 the quality control strategy, including specifications, shall be enriched and developed in tandem with the progress of clinical trials.

2. Quality control of the final product
Refer to the general quality control parameters and tests described below, set appropriate specifications for the final product, and justify the rationale for the specifications set.
Set appropriate acceptance criteria and test procedures for individual products that do not comprise a lot as well as for individual products that do comprise a lot, because each individual lot is typically the unit subjected to quality control.

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

(2) Tests of Identity
Confirm that the cells are the intended target cells using markers for important cell characteristic 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
To test the purity of cells in a final product, set the test parameters, test methods, and acceptance criteria for evaluating and controlling non-target cells, such as undifferentiated cells, cells exhibiting abnormal growth, transformed cells, and the presence of any contaminating cells, considering 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 the 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 the appropriate and permissible dose-limiting tests for any potential undesirable physiologically active substances derived from target cells, the significant presence of which in the product is presumed clearly to impact the safety of the patient. At the 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 either to prove that the substance is not present in the final product using the results of process evaluation for the elimination of the substance or the results of in-process control of the substance, or to establish appropriate tests to control the amount of the substance in the final product within permissible levels. When selecting substances to be tested and setting their acceptance criteria, their appropriateness should be explained and justified.
At the 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 test can be used. If the results of the sterility and other

tests of the final product can only be obtained after administration to the patient, the proper measures for dealing with potential non-sterility should be established beforehand. In such an instance, the intermediate products must be demonstrated to be sterile, and sterility should be strictly controlled in all processes leading up to the final product. If a product from the same facility and same process has already been used in patients, its sterility must be confirmed by testing it in all patients. If complete closure (hermetic seal) of an individual lot of the product has been assured, tests using only representative samples are sufficient. When tests must be conducted for each different application, and if the results of sterility and other tests can only be obtained after administration to the patient, the determination of whether or not application should proceed will be determined based on the most recent data. However, even in this instance, sterility tests and other tests on the final product shall be conducted.

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

(7) Endotoxin test

Perform an endotoxin test, considering the impact of potential contaminant in the samples. The acceptance criteria do not necessarily depend on the actual measured values. It is recommended to set acceptance criteria considering the safety ranges given in the Japanese Pharmacopoeia and/or any other relevant compendia based on a single dose of the final product. Endotoxin testing can be established as an in-process control test; however, in such cases, establish criteria, including validation results, and justify their appropriateness.

(8) Virus tests

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

(9) Efficacy tests

In some instances, it will be necessary to consider efficacy testing that takes into consideration the cell type, intended clinical use, or distinctive characteristics of the cells. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on 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 is responsible for the efficacy or the essential effect of an iPS(-like) cell-based product in its intended clinical use, 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, etc. for phenotype products from the desired cells or for an expression product secreted from the cells when a gene has been introduced. At the 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 dynamic strength, set acceptance criteria to confirm mechanical compatibility and durability that take into account the site of application. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria based on actual measured values from a small number of test samples.

Chapter III Stability of Autologous Human iPS(-like) Cell-based Products

Taking into full consideration the storage and distribution periods and the storage form, perform suitable stability testing on autologous human iPS(-like) cell-based products and/or critical intermediate products based on cell viability, potency, 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 affect the stability or acceptance criteria of the product. Where necessary and possible, it is recommended to conduct stability studies on products whose manufacturing or storage periods exceeds the normal period, 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 production.

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

Chapter IV Preclinical Safety Testing of Autologous Human iPS(-like) Cell-based Products

Relevant animal tests and/or *in vitro* tests may be performed to elucidate concerns regarding the safety of an autologous human iPS(-like) cell-based product when it is scientifically reasonable and technically possible. Safety concerns regarding non-cellular constituents and process-related impurities should be resolved, as far as possible, using physicochemical analyses and not animal testing. In addition, the presence of undifferentiated cells in the final product and their potential to cause ectopic tissue formation, tumorigenicity, or malignant transformation is a safety concern. Therefore, it is necessary to reduce the risk of contamination with such cells as much as possible via thorough

analysis at the cell bank and/or 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 the manufacturing process. Furthermore, the administration route for the target cells may be selected to aid in the minimization of the safety concerns.

Animal testing of products of human origin do not always yield meaningful results. Thus, there may be a scientific rationale for preparing product models of animal origin and testing with appropriate experimental animals, if more useful information may be obtained. In such a case, consider conducting tests using suitable animal models for each target disease. (Note: For example, monkeys may be suitable for nervous system diseases, while pigs and/or dogs may be suitable for cardiovascular diseases.) However, because use of identical procedures in non-human animals will not necessary yield cell groups that possess identical characteristics to cells that constitute an autologous human iPS(-like) cell-based product, and because a product of animal cell origin manufactured using identical processing, including culture conditions, etc. will not necessarily be comparable to a human cell product, careful feasibility studies are required beforehand when adopting, conducting, and evaluating such studies. When conducting animal experiments using iPS(-like) cell-based products obtained from non-human animal species, explain the suitability 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.

The examples below present points to consider when confirming the preclinical safety of a product. These are merely examples for illustration and are not meant to suggest conducting tests with no rational basis. Conduct necessary and appropriate tests, taking into account the characteristics of the product, intended clinical use, etc., 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 demonstrate that transformations other than the intended transformation and abnormal proliferation of non-target cells have not occurred.

2. It may be necessary to conduct quantitative assays for particular physiologically active substances produced by the cells and tissues and to discuss their effects when given to patients. In some cases, significant amounts of active substances, including cytokines and growth factors, would be produced by the cells, potentially resulting in undesirable effects on the patients.

3. Examine and discuss the potential effects and safety consequences of the product on the normal cells and tissues of a patient.

4. Investigate and discuss the possibility and potential safety consequences of the formation of ectopic tissue by cells in the product and/or contaminating undifferentiated cells when the product is given to the patient. Discuss in a comprehensive manner, taking into account the type and characteristics of the product, the route of administration, target diseases, appropriateness of the test system, etc.

5. Investigate and discuss the possibility and safety of undesirable immunological reactions to the product and/or expression product of a transgene, and the relevant safety concerns.

6. Using an appropriate animal model or other system, investigate and discuss the possibility of tumor formation including benign tumors and/or malignant transformation of cells in the final product or an intermediate product. Upon testing, take into account the type and characteristics of the product, number of cells and route of administration, mode of application (e.g., cell sheet, cell suspension, etc.), cell engraftment site, target diseases, appropriateness of the tests systems, etc. If there is a possibility of tumorigenicity or malignant transformation, justify the appropriateness of the use of the product in question and its rationale, considering the relationship with the anticipated efficacy. (Note: The most important aspect of a tumorigenicity test is to accurately assess the tumorigenicity of a final product that will be used in patients. However, it is conceivable that tumorigenicity will need to be evaluated using cells from an intermediate product because the cells comprising the final product cannot be used for various reasons, such as the inability to obtain a sufficient number of cells. Furthermore, in tumorigenicity tests using animal models various conditions, such as cell dispersion and cell adhesion to scaffolding, cell density, and administration site, are not necessarily identical to those for the final product. Sensitivity may differ depending on the species, strain, and immunological state of the animal. The tumorigenicity of the final product should be evaluated with comprehensive consideration of these circumstances. The risks to the patient arising from tumorigenicity of the final product should be rationally evaluated based on the balance between any risks and the benefits to the patient by treating the disease.)

7. If an exogenous gene is introduced into cells during the manufacturing process, and if it may function or remain as a residue in the final product, conduct tests in accordance with the "Gene Therapy Pharmaceutical Guidelines," published as Notification 1062 by the Ministry of Health and Welfare on November 15, 1995. In particular, if virus vectors are used, test quantitatively to determine the potential presence of any replication-competent viruses and justify the appropriateness of the test method employed. Describe the safety of the transgene and its products based on their characteristics. For cells, discuss the possibility of changes in cell growth or tumor

formation, including benign tumors and malignant transformation. Whenever a vector, which may be inserted into a chromosome is used, consider the necessity of evaluating possible occurrences of abnormal proliferative characteristics and/or tumorigenicity due to insertion mutation in the cells, and of implementing long-term follow-up for clinical applications.

8. Consider conducting rationally designed general toxicology tests, if the product, including an animal-derived product is easy to obtain, and if doing so will generate useful information regarding its clinical application.
When conducting general toxicology tests, refer to the "Guidelines for Toxicology Studies on Pharmaceuticals," which is an appendix to the document entitled "Guidelines on Toxicology Studies Required for Regulatory Approval for the Manufacture or Import of Pharmaceuticals" (Drug Evaluation Notification 1:24, Ministry of Health and Welfare, issued September 11, 1988).

Chapter V Studies Supporting the Potency or Efficacy of Autologous Human iPS(-like) Cell-based Products

1. A well-designed study using experimental animals and/or cells should be performed in order to demonstrate the functional expression, sustainability of effect, and/or anticipated clinical efficacy (proof-of-concept) of an autologous human iPS(-like) cell-based product to the scientifically reasonable and technically possible extent.

2. For transgenic cells, demonstrate expression efficiency and sustainability of expression and biological activity of desired products from the (transgene), and discuss the feasibility of the anticipated clinical efficacy (proof-of-concept) of the human iPS(-like) cell-based product in question.

3. Where appropriate models of products derived from processing of animal iPS(-like) cells and/or disease model animals are available, use them to study the potential therapeutic efficacy of the product.

4. At the beginning of the clinical trial, detailed experimental studies will not necessarily be required if the potency or efficacy of the therapy employing the product in question is expected to be markedly superior to other therapeutic methods, and if this can be justified by means of scientific literature and/or other available information.

Chapter VI Pharmacokinetics of Autologous Human iPS(-like) Cell-based Products

1. Pharmacokinetic studies of the internal behavior of cells/tissues that constitute the final products or expression products of transgenes, which may include absorption and distribution in experimental animals, should be performed to the extent technically possible and scientifically reasonable. Thereby, it is expected to estimate the survival of cells/tissues administered to patients and the duration of their effect, and

determine if the intended efficacy is sufficiently achieved. (Note: Testing methods may include histological studies, Alu-PCR, MRI, PET, SPECT, and bioimaging).

2. Clarify, using animal studies, the rationale for the administration method for the autologous human iPS(-like) cell-based products. In particular, extrapolate from animal experiments, the systemic distribution of cells after systemic administration, and discuss the distribution from the point of view of clinical usefulness. (Note: Although it is unclear exactly where the cells adhere for each administration route, it is assumed that local administration may be preferable to systemic administration. However, if the benefits to patients can be explained in a rational manner, it may be acceptable to use systemic administration. In any case, an administration method that minimizes distribution of iPS(-like) cell-based product to organs other than target organ is preferred. Even if the cells localize to a site other than the intended transplantation site, the administration method may be acceptable if patients experience no adverse effects. For example, a disadvantage caused by ectopic differentiation may be, for example, arrhythmia caused by osteogenesis of some types of cells that ectopically locate to the heart.

3. When the cells or tissues are directly applied or alternatively targeted to a specified site (tissue, etc.) where they can be expected to perform their actions, clarify the localization and discuss the effect of the localization on the efficacy and safety of the product.

Chapter VII Referring to Clinical Trials

The main purpose of the present guidelines is to address points to consider for evaluating the quality and safety of autologous human iPS(-like) cell-based products at the time of application for marketing authorization as well as at the beginning of investigational clinical trials. In the latter case, it is necessary to evaluate whether any quality or safety problems exist that might pose an obstacle to initiating human clinical trials, taking into consideration, the product's clinical usefulness. Thus, quality and non-clinical safety evaluations for determining to initiate the investigational clinical trials of the product in question should refer to the points outlined below.Any presumed known risk factors associated with the product's quality and safety should be eliminated, as much as possible, using up-to-date science and technology, and the scientific appropriateness should be clearly described. Any remaining risks should be weighed against the risks associated with not performing the trials in patients that suffer from diseases that are serious and life-threatening, that involve marked functional impairment, or a marked decrease of QOL resulting from the loss of a certain degree of physical function or form, and for which existing therapies have limitations and do not provide cures. Furthermore, it is also critical to entrust to the patient the right to make a decision after providing all of the information available, including all

information on identified/unidentified risks and anticipated benefits.

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

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

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C.12.6 ヒト（同種）iPS（様）細胞加工医薬品等の品質及び安全性の確保に関する研究の関する研究の経緯と視点及びガイドライン英文版

Study on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from Processing of Allogenic Human Induced Pluripotent Stem Cell (-Like Cells)

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Background (Chronology and Focus of the Research)

The details of a series of the present study have been described in a previous paper[1-3]. The present paper provides a summary of points that are closely related to those in the first.

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

In Japan, translational research in regenerative medicine is advancing rapidly. In particular, much work has been conducted on product development using human stem cells, i.e,, somatic stem cells such as mesenchymal stem cells, embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells. It is therefore urgent to prepare relevant guidelines for the evaluation of products expected to be developed in the near future. Identifying the technical, medical and ethical conditions necessary for utilizing of various types of stem cell at an early stage of development is vital for their rapid application in clinical settings.

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

The objective of the study group is to promote the development of products

derived from human stem cells through investigation and research into scientific and technological advances, their ethical validity, regulatory rationale, and international trends in human stem cell-derived products, and and to establish and implement appropriate safety evaluation criteria.

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

In 2009, two interim reports on draft guidelines on autologous human somatic stem cell-based products and autologous human iPS (-like) cell-based products were developed based on the existing MHLW Notification No. 0208003 and on the above considerations. Three other interim reports detailing draft guidelines were also developed for allogenic human somatic stem cell-based products, allogenic human iPS (-like) cell-based products and human ES cell-based products based on MHLW Notification No. 0912006. These five sets of draft guidelines, still in the interim stage, were presented as the subjects of thorough discussions from a variety of viewpoints. They were widely circulated among the interested parties as articles published in a relevant scientific journal to elicit readers' comments [Hayakawa T., et al.: Regenerative Medicine (Journal of the Japanese Society for Regenerative Medicine), 9, 116-180, in Japanese]. Thereafter, these articles were updated and published as a series of eight articles (Journal of the Japanese Society for Regenerative Medicine), 10, 86-152(2011)), which would form the basis of the final draft guidelines. After extensive discussions with the study group and implementation of public consultation, the Pharmaceutical and Food Safety Bureau of MHLW issued five Notifications on September 7, 2012, as described previously [1].

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

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

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

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

Based on the concept mentioned above, these guidelines refer to both "human iPS-like cells" and "human iPS cells" and provisionally define these two types of cells as follows.

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

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

Raw materials in biologics cannot be sufficiently characterized or quality controlled due to their indistinct origin and complexity; the same holds

true for final products due to their limited quantity and complex quality attributes. To minimize these concerns, it is most important to ensure constancy and robustness of the manufacturing process in the production of all types of biologics. The core technical elements required is to establish base camp(s), i.e. to prepare biologics production substrates at relevant stage(s) in the manufacturing process, which can be extensively characterized and controlled a, which are of stable quality, and from which constant processing to the subsequent intermediate(s) and finally to a desired product is achievable.

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

For products derived from human iPS cells or iPS-like cells (here after referred to as iPS(-like) cells), the presence of undifferentiated cells in final products is a major safety concern, i.e., ectopic tissue formation and tumorigenesis. However, because this is one of t iPS(-like) cells' strongest characteristics, it is quite difficult to avoid. Elimination of intrinsic characteristics of iPS(-like) cells is a trade-off at least in principle, and is thus considered very difficult. Accordingly, it is significant to have strategy and tactics to develop safer final products by improving manufacturing process and process control more properly rather than discussing safety issues at iPS(-like) cell level. These draft guidelines, therefore, require the demonstration of potential free of undifferentiated cell contamination at the level of an iPS(-like) cell-derived bank and/or intermediate cell products thorough analysis, or an effort to develop efficient methods to eliminate or

inactivate undifferentiated cells in the course of cell processing. Furthermore, selection of administration methods will help to minimize safety concerns. These guidelines also describes the importance of technical development to generate and characterize iPS(-like) cell-derived somatic stem cells, which may lead to safe, stable, characteristically well defined and appropriate raw materials. In addition, the need for R&D on examination techniques to predict the pluripotency and differentiation potential of each iPS(-like) cell and processing techniques to induce target cells efficiently and properly, and to isolate differentiated cells from undifferentiated cells during processing will provide novel business opportunities.

These draft guidelines include discussion of all of the above-stated aspects of iPS(-like) cells. iPS(-like) cells possess pluripotency and self-replication abilities exceeding those of normal somatic stem cells and can therefore differentiate into a variety of cell types depending on the processing techniques used. Such iPS(-like) cell-based products will be clinically applied heterologously, i.e., in an environment that diffe from the environment where the cells perform their natural endogenous function. Concerns related to these points have been included in these human iPS(-like) cell guidelines in comparison with MHLW Notification No. 0208003 and MHLW Notification No. 0912006.

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

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

It should be emphasized again that our primary goal is to offer suitable medical opportunities as rapidly as possible to patients suffering from severe diseases are difficult to treat

using conventional medicine. The present guideline should further this purpose. Therefore, it is important to interpret and employ these guidelines flexibly and meaningfully in this context. Stringent observance of these guidelines without primary consideration of the patient and his/her specific situation should be avoided.

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

Guidelines on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from Processing of Allogenic Human Induced Pluripotent Stem(-Like) Cells

(September 7, 2012)

Introduction

1. The present guidelines outline the basic technical elements for ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of allogenic human induced pluripotent stem (iPS) cells or allogenic human iPS-like cells (excluding autologous human iPS cells and autologous human iPS-like cells). These products are hereinafter referred to as allogenic human iPS(-like) cell-based products or merely as "desired cell products".
Allogenic human iPS(-like) cell-based products are obtained by artificially inducing the differentiation of various types of iPS(-like) cells generated artificially from allogenic human somatic cells; they are used directly or after further processing. There are many different types of manufacturing methods, intermediates, types and characteristics of desired cell products, and methods of clinical application. In addition, the scientific progress in this field is constantly advancing and experience and knowledge in this field are constantly accumulating. Therefore, it is not always appropriate to consider the present guidelines all inclusive and definitive. Consequently, when testing and evaluating each individual product, it is necessary to take, on a case-by-case basis, a flexibleapproach based on a rationale that reflects 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 to initiating human clinical trials of the iPS(-like) cell-baced products in question, whether certain quality attributes (QA) of the product are understood sufficiently to establish the relationship between clinical findings and the QA, and whether consistency of the QA can be ensured within a definite range. Simultaneously, it is important to eliminate any known risk factors associated with the product quality and safety in so far as possible using up-to-date science and technology, and to describe the scientific appropriateness of the results of such action. The remaining unidentified risk factors should be weighed against the risks associated with not performing the trials in patients who suffer from diseases that are serious and life-threatening, that involve marked functional impairment, or a marked decrease in quality of life (QOL) resulting from loss of a certain degree of physical function or form, or for which existing therapies have limitations and do not provide cures. Furthermore, it is also important to entrust to the patient the right to make a decision, after providing all of the information available. When applying for investigational clinical trials, applicants can submit a reasonably-prepared provisional non-clinical data package, which is prepared reasonably by taking into account product aspects and patient aspects including a balance between risk of product *vs* risk of patient with/without treatment in question, ; for determining to initiate investigational clinical trials, on the premise that data package for ensuring quality and safety at the time of marketing application/registration will be enriched in line with the guidelines as the clinical trialprogress.

Finally, applicants are encouraged to discuss with the Pharmaceuticals and Medical Devices Agency (PMDA) the type and extent of data that may be needed to initiate an individual clinical trial. Because of differences in product origin, target disease, target patients, application sites, application methods, and processing methods, there may be numerous variations between individual data packages that cannot be definitively clarified in the present guidelines.

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

Chapter I. General Principles

I. Objective

The present guidelines outline the basic technical elements for ensuring the quality and safety of

pharmaceuticals and medical devices derived from processing of allogenic human induced pluripotent stem (iPS) cells or allogenic iPS-like cells (excluding autologous human iPS cells and autologous iPS-like cells). These products are hereinafter referred to as allogenic human iPS(-like) cell-based products or merely as the "desired cell products".

II. Definitions

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

1. "Human induced pluripotent stem cells (iPS cells):" Cells that are generated from somatic cells through artificial reprogramming by introducing genes or proteins, or by chemical or drug treatment, or cells that are obtained from such cells through cell division, and they possess the ability to differentiate into endoderm, mesoderm, and ectoderm, and furthermore, maintain the ability to self-renew or have a similar ability.
2. "Human induced pluripotent stem-like cells (iPS-like cells):" Cells that are generated from somatic cells through artificial dedifferentiation by introducing genes or proteins, or by chemical or drug treatment, or cells that are obtained from such cells through cell division, and they at least possess the ability to differentiate into some type of endoderm, mesoderm, or ectoderm, and furthermore, maintain the ability to self-renew or have a similar abitily.
3. "Processing of cells and tissues:" Any processing of a cell or tissue, such as propagation and/or differentiation, production of a cell line, activation of a cell by pharmaceutical or chemical treatment, alteration of a biological characteristic, combination with a noncellular component, or manipulation by genetic engineering, with the aim of preparing desired cell products to treat a patient, or repair or regenerate tissues.
Isolation of tissue, disintegration of tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by gamma irradiation or other methods, freezing, thawing, and similar procedures regarded as minimal manipulations are not considered to be processing.
4. "Manufacture:" Actions undertaken before the final product (an allogenic human iPS(-like) cell-based product) is released to market. This includes, in addition to processing of cells and tissues, minimal manipulations such as separation of tissue, disintegration of tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by gamma irradiation or other methods, freezing, thawing, and other procedures that do not change the original properties of the cells or tissues.
5. "Phenotype:" A morphological or physiological characteristic that is expressed by a certain gene under constant environmental conditions.
6. "HLA typing:" Specifying the type of HLA (human leukocyte antigen), a human primary histocompatibility antigen.
7. "Donor:" Persons who donate their own somatic cells, which serve as the raw material for an allogenic human iPS(-like) cell–based product.

8. "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.
9. "Protein transductant:" A construct that contains a target protein and elements such as reagents necessary for introducing the target protein into a target cell.

Chapter II Manufacturing Method

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

I. Raw Materials and Materials Used in Manufacturing

1. Human somatic cells that serve as raw materials

(1) Source and origin, justification of their selection

Explain the source and origin of the somatic cells used as raw materials when establishing the human iPS(-like) cell line, and justify the reasons for selecting these somatic cells.

(2) Characteristics and eligibility of somatic cells serving as raw materials

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

Explain and justify the reasons for selecting the somatic cells used as raw materials with reference to characteristics of their biological structure and function, such as morphological characteristics, growth characteristics, biochemical indicators, immunological indicators, specific substances produced, HLA typing, and other suitably chosen and appropriate genotype or phenotype indicators.

This should lead to the identification of the main cell characteristic indicators that are to be employed when preparing the somatic cells as raw materials. It is recognized that quantitative technological limits to sample analysis will affect the extent to which such studies can be performed..

(ii) Donor selection criteria and eligibility

Indicate that the donors was selected in an appropriate and ethical manner and that the proper procedure was followed. Establish selection criteria and eligibility criteria that take into consideration age, sex, ethnic

characteristics, genetic characteristics, disease history, health condition, test parameters related to any type of infection that may occur via cell and/or tissue samples, immunological compatibility, etc.,and justify their appropriateness. If donor genome or gene analysis is undertaken, they shall be performed in accordance with "Ethical Guidelines for Human Genome/Gene Analysis Research," issued jointly on December 28, 2004 and revised on December 1, 2008 by the Japanese Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labor and Welfare, and Ministry of Economy, Trade and Industry.

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

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

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

Alternatively, 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 cells (intermediate products or cell banks) derived from processing of iPS(-like) cells in investigations, after having justified their appropriateness.

(3) Records related to the donor

Retain complete records related to the donor s in order that any information necessaryto ensure the safety of somatic cells used as raw materials can be verified. Concrete measures shall be described. For patients and donors of test samples, it is sufficient to prepare and retain only specific information that relates to the intended use of the cells.

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

(i) Eligibility of personnel and medical institutions collecting samples

Describe the technical requirements for personnel and medical institutions that collect the cells and tissues.

(ii) Suitability of the sampling site and sampling method

Describe the rationale for selecting the cell and tissue sampling sites and the sampling method, State how the selected sites selected are scientifically and ethically appropriate. For cell and tissue sampling 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, provided by the donor of the cells or tissue.

(iv) Protection of donor privacy
Indicate the measures adopted to ensure the protection of t 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 at the time of cell or tissue sampling, describe the details of the tests, as well as any interventions taken when test results indicated a problem existed.

(vi) Storage method and measures to prevent erroneous sampling (mix-ups)
If the somatic cells collected must be stored for a defined period of time, set the storage conditions and storage period, and justify their appropriateness (validity). Describe in detail the measures and procedures to be followed to prevent erroneous sampling (mix-ups).

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

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

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

Describe any raw materials other than target cells and tissues as well as other materials used in the manufacturing process, and indicate their appropriateness for their intended use, and if necessary establish their specifications (acceptance criteria and analytical procedures). Proper quality control for these materials should be implemented.
When so called ' Biological Products' or 'Specific Biological Products' (refer to Article 2.9 and 2.10 of the Pharmaceutical Affairs Law) are used as raw materials, the amounts used should be kept to the minimum amount required and should strictly obey the relevant laws and notifications, such as the "Standards for Biological Raw Materials" (Notification Number 210, Japanese Ministry of Health, Labor, and Welfare, 2003. Note that a new version will soon be issued). It is particularly important to sufficiently evaluate information related to the inactivation and elimination of