Guidance on Evaluation of the Treatment of Severe Heart Failure Using Human (Allogeneic) iPS Cells-derived Cardiomyocyte Spheroids

1. Introduction

The fundamental technical requirements for ensuring the quality and safety of products derived from the processing of allogeneic human induced pluripotent stem cells (iPS cells) (hereinafter referred to as “human (allogeneic) iPS cell-based product”) are stipulated in the “Guidelines on ensuring quality and safety of products derived from processed cell and tissue (Allogeneic iPS (-like) cells)” (PFSB Notification No. 0907-5, issued by the Director of Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated September 7, 2012).

In addition to the fundamental technical requirements mentioned above, this guidance provides points to consider that are specific to regenerative medical products intended for the treatment of severe heart failure, among human (allogeneic) iPS cell-based product, (referring to regenerative medical products as defined in Article 2, paragraph (9) of the “Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices” (PMD act) (Act No. 145 of 1960), hereinafter the same applies).

2. Subject

This guidance covers the points to consider when evaluating the quality, efficacy, and safety of human (allogeneic) iPS cell-based products, particularly regenerative medical products intended for transplantation to the heart and engraftment in the myocardium for the treatment of severe heart failure, as well as the basic technical requirements.

3. Scope

Given its intention for human (allogeneic) iPS cell-based product with technologies that are markedly advancing, this guidance presents the points that should be considered at present. It is not necessarily intended to be exhaustive. Therefore, there are revised based on further technological innovation and accumulation of knowledge in the future, and are not binding on the content of applications.

When evaluating products, it is necessary to respond flexibly with a scientific rationale after fully understanding the characteristics of individual product.

In addition to this guidance, other relevant guidelines of both domestic and international should also be referred.

Furthermore, it is recommended to consult with Pharmaceuticals and Medical Devices Agency (PMDA) regarding the evaluation required for individual product.

4. Definitions

(1) Cardiomyocyte spheroid: A sphere-shaped mass of cardiomyocytes.

(2) Cell bank: A system consisting of a substantial number of containers, each containing contents of uniform composition, stored under defined conditions. Each container represents an aliquot

*This English translation of the Japanese Administrative Notice is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail.

of a single pool of cells (as defined in ICH Q5D “Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products, PMSB/ELD Notification No. 873, issued by the Director of Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare, dated July 14, 2000).

(3) Cross-contamination: Contamination between samples. It means contamination between raw materials used for production, between intermediates, etc. For example, cells derived from a cell bank may be contaminated with cells derived from another cell bank. Alternatively, raw materials before inactivation may be mixed with those after undergoing virus inactivation.

(4) Surrogate marker: A substitute marker that is established in advance to correlate with the target parameter when direct measurement is difficult.

(5) Structure: A graft that contains cells and has a three-dimensional structure such as a sheet or spherical shape.

(6) Cardiac cells: Cells that develop during myocardial differentiation. (cardiomyocytes, fibroblasts, smooth muscle cells, and vascular endothelial cells)

5. Points to Consider for Evaluation

For the time being, this evaluation guidance is intended to apply to the evaluation of sphere-shaped masses of cardiomyocytes as a human (allogeneic) iPS cell-based product (hereinafter referred to as “cardiomyocyte spheroids”) which is derived from allogeneic human iPS cells (cell line) already established as raw material for regenerative medical products. The cell line is received at the manufacturing site as the primary raw material, where a cell banking system is established and processed into multilayered product. In cases where human (allogeneic) iPS cells are newly established from somatic cells in the manufacturing site of regenerative medical products and are intended to be used as the raw materials for manufacturing of regenerative medical products while referring to this evaluation guidance, please also refer to “Guidelines on ensuring quality and safety of products derived from processed cell and tissue (Allogeneic iPS (-like) cells)” (PFSB Notification No. 0907-5 issued by the Director of Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated September 7, 2012) etc.

(1) Raw materials, etc.[1]

iPS cells to be used as raw materials, etc. should be allogeneic human iPS cells that have been established as raw materials to develop a cell banking system for regenerative medical products and also have been confirmed or can reasonably be expected to have the ability to differentiate into cardiomyocytes and other cardiac cells through the defined manufacturing process.

For iPS cells established by introducing reprogramming genes into human somatic cells, it is recommended to rule out the presence of residual transgenes. If the presence of residual transgenes cannot be ruled out, it should be confirmed that the residual transgenes have no adverse effect on the quality and safety of cardiomyocyte spheroids of the final product.

[1]For definition, refer to the Standards for Biological Raw Materials (MHLW Notification No. 210, 2003).

(2) Matters requiring special attention in the manufacturing process

For manufacturing cardiomyocyte spheroids (final product), specify the manufacturing method, and provide justification by verifying, to the extent possible, the following aspects to ensure consistent quality.

(i) Presence or absence of lot composition and specifications

It should be clarified whether the final and intermediate products consist of multiple lots. If they comprise a lot, the details of the lot should be specified.

(ii) Manufacturing method

A description should be provided of the history from the acceptance of the iPS cell line as raw materials at the manufacturing site to the establishment of a cell banking system for human iPS cells as the starting material and an outline of the manufacturing method from the starting material to the final product through advanced differentiated cells. The specific processing steps, necessary process controls, and quality control measures should also be detailed.

a) Acceptance inspection

Regarding the iPS cell line as the raw material, establish the tests (inspections) items for acceptance at the manufacturing site (e.g., visual inspection, microscopic examination, viability, cell characterization [phenotypic, genetic traits, specific functions, etc.], and tests for the absence of contamination by bacteria, fungi, viruses, etc.) and acceptance criteria for each item. If the result is positive, verify the presence or absence of contamination in the iPS cell line stock and during transportation, and obtain a new iPS cell line.

In cases where, for technical reasons, it is appropriate to perform the inspection after part of the process has been completed, perform it at an appropriate time after the iPS cell line has been accepted. For example, after receiving a frozen allogeneic human iPS cell line based on the Certificate of Analysis issued at the time of raw material production using the cell line, an additional test may be conducted at the time of thawing for culture expansion. At a stage prior to initiating clinical trials, measured values from test samples obtained up to that stage should be presented, and the provisional values derived from these observations should be provided.

b) Cell banking

The method for preparing cell banks from the iPS cell line accepted at the manufacturing site and the methods for characterization and storage, maintenance, control, and renewal of cell banks, as well as other procedures related to each operation process and testing, should be detailed, along with their validity. Refer to ICH Q5D etc. However, omitting certain attributes from testing is acceptable if justified by their evaluation in the more upstream process.

c) Preparation of cells as a component of the final product

The methods for preparing cells as a component of the final product from the iPS cell line received at the manufacturing site as raw materials, etc., along with its cell bank (e.g.,

differentiation method, separation and culture of target cells, culture medium at each stage, culture conditions, culture period, yield, etc.), should be specified. Additionally, their validity should be provided to the extent possible. If the final product is supplied as a frozen product, specify the cell freezing method and the method for preparing a cardiomyocyte spheroid suspension for transplantation from the frozen cells (cell thawing, final dosing formulation method, etc.), and justify to the extent possible.

d) Measures to prevent mix-up and cross-contamination during the manufacturing process

Since the prevention of mix-ups and cross-contamination is important during the manufacturing process of cardiomyocyte spheroids (final product), specify preventive measures in the in-process control.

e) Establishment of cell culture process

It has been suggested that the culture process of differentiation to cardiac muscle is influenced by many parameters related to cell conditions and culture, potentially leading to variability in the proportion of cardiomyocytes and residual undifferentiated iPS cells in the final product. For a product intended for transplantation to the heart and engraftment in the myocardium long term to regenerate functional myocardial tissue, it is recommended to control the cell growth and the proportion of differentiated cells within an appropriate range that does not affect the cell quality during the manufacturing process up to the final product, while incorporating a purification process to remove undifferentiated iPS cells. Measures for such control should be specified.

f) Establishment of process conditions for manufacturing across multiple cell processing centers and for cell processing within hospitals

When the manufacturing process is completed across multiple cell processing centers, the transportation condition of intermediate products between centers should be predetermined and monitoring of intermediate products should be performed to verify whether the conditions for the release, acceptance, and transportation, etc. meet the requirements. In addition, when cell processing is performed in the hospital after shipment of the final product, the processing conditions should be predetermined in advance, and the rationale for implementation should be provided.

(3) Quality control of the product

Define the transplantation method of cardiomyocyte spheroids (final product). For example, a possible transplantation method involves direct administration of the required number of iPS cell-derived cardiomyocytes in a cardiomyocyte spheroid state (as the final product) to the heart.

Points to consider for the quality control of cardiomyocyte spheroids include, for example, those described below; however, alternative or additional tests may be adopted as necessary and appropriate. In addition, it is necessary to explain the rationale for selecting each test item and to validity the test methods. Regarding the control limits for in-process control and specification values of quality specifications at a stage prior to initiating clinical trials, actual measured values

from test samples obtained up to that stage should be presented, and provisional control limits and specification values derived from these observations should be provided.

If it is technically challenging to conduct specification tests on the product to be released or its parts, conduct the specification tests using surrogate markers or substitute samples, such as products manufactured in parallel etc., and provide justification.

If long-term storage of cardiomyocyte spheroids of the final product is technically challenging, the results of specification testing may be unavailable by the time of their use. In such cases, the tests may be conducted using substitute samples obtained during the manufacturing process, and the product may be released based on the results. However, it is required to validate the release based on substitute samples and to conduct the tests using samples of the final product to confirm the results.

a) Confirmation of the description

It is recommended to confirm that the final product has the intended description previously demonstrated by visual inspection and to retain its record. When the final product is cardiomyocyte spheroids, the requirements may be established based on the visual inspection of their structure (e.g., cell mass) and color (e.g., white to pale yellow).

b) Number of cells and viability

Requirements should also be established for the number of cells and viability. To determine the number of cells, a portion of the final or intermediate product is taken to prepare a cell suspension. The number of cells in the suspension is counted using a validated method (such as a hemocytometer or cell counter). Cell viability can be determined by counting the number of living and dead cells using a validated method (e.g., trypan blue dye exclusion or fluorescent dye method). When the final product is cardiomyocyte spheroids, measuring the number of cells and viability within the spheroids is technically challenging. In such cases, surrogate markers that support the number of cells and viability within the structure may be used. The validation for selecting the markers should be provided. For example, the number and viability of cardiomyocytes before spheroid formation may be used as surrogate markers, provided that a correlation between pre- and post-cardiomyocyte spheroid formation has been demonstrated in advance.

c) Confirmation of cell specificity

Determine the expression level of cardiac troponin T, etc. using flow cytometry, etc. to measure cardiomyocytes constituting the final product.

The expression levels of marker molecules indicating the specificity of primary and other component cells in the final product may be assessed using mRNA expression analysis, cellular immunostaining, and flow cytometry, etc. Beyond these analyses alone, it is recommended to evaluate the specificity of cardiomyocytes and the proportion of cells exhibiting each specificity using multiple different methods.

When the final product is cardiomyocyte spheroids, evaluating the specificity of cells within

the spheroid structure is technically challenging. In such cases, surrogate markers that support specific indicators within the structure may be used. The validation for selecting the markers should be provided. For example, cell specificity (e.g., cardiac troponin T expression) before spheroid formation may be used as a surrogate marker, provided that a correlation between pre- and post-cardiomyocyte spheroid formation has been demonstrated in advance.

d) Functional assessment

Demonstrate either during the manufacturing process or on the final product that the product has functional characteristics as cells compatible with the therapeutic use. For example, when the final product is cardiomyocytes, this can be assessed by expression cardiomyocyte markers using mRNA expression analysis, cellular immunostaining, and flow cytometry, as well as observing pulsation.

If cell-derived cellular secreted factors, etc. are assumed to be related to the efficacy of the final product, the feasibility of their assessments should be considered.

When the final product is cardiomyocyte spheroids, measuring the function of the spheroids is technically challenging. In such cases, a specific indicator within the structure may serve as a surrogate marker of function. The validation for selecting the markers should be provided. For example, cell specificity (e.g., cardiac troponin T expression) before spheroid formation may be used as a surrogate marker, provided that a correlation between pre- and post-cardiomyocyte spheroid formation has been demonstrated in advance.

e) Confirmation of absence of undifferentiated cells

The presence of undifferentiated cells may be evaluated by quantification of marker genes using quantitative PCR, cell immunostaining, measurement of expression quantification of undifferentiated cell marker antigens using flow cytometry, etc. It also includes back culturing in which the final product is cultured for a certain period under the culture conditions for undifferentiated iPS cells, etc. Among these, an analytical method with sufficient detection power for evaluation should be selected, taking the number of transplanted cells into account. If possible, it is recommended to assess the presence or absence of undifferentiated cells using different methods.

Since the presence of undifferentiated iPS cells does not necessarily correspond with tumorigenicity, refer to the Nonclinical Studies section for tumorigenicity test.

f) Evaluation of chromosomal and genomic structures

If possible, the chromosomal and genomic structures of the final product should be evaluated. It is recommended to analyze the chromosome karyotype structure using Giemsa staining (G-banding) of chromosomes, etc. The genomic structure may also be evaluated at a whole genome level using microarray analysis, etc. When conducting a genetic stability study, also refer to “Guidelines on the Detection of Undifferentiated Pluripotent Stem Cells and Transformed Cells, Tumorigenicity Test and Genetic Stability Evaluation on Human Cell Processed Products”

(PSEHB/MDED Notification No. 0627-1 by the Director of Medical Device Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare, dated June 27, 2019), etc.

(4) Stability test of the product

For the final product or important intermediate products, stability tests should be conducted under actual storage conditions using surrogate markers that support cell viability and efficacy, considering the storage and distribution periods and the storage status. The storage method and expiration date should be established, and their validation should also be provided. Notably, when the product is stored frozen and then thawed, demonstrate the impact of the freeze-thaw procedure on the post-thawing culturable period and the product quality. If necessary, extended storage beyond the standard production or storage period should also be considered to establish the stability limit to the extent possible. However, this does not apply if the product is used immediately after completion of manufacturing.

When starting materials, intermediate products, and final products are transported, the respective conditions and procedures (including the container, transportation solution, and temperature control) should be specified, and a validation should be provided. If the cells are transported in a frozen state, the medium, cryopreservation liquid, cryoprotective agent, and other materials used for freezing should be appropriately selected, as well as those used in the manufacturing process. The transportation solution should also be appropriately selected when transporting the final product in an unfrozen state.

When cardiomyocyte spheroids as the final product are transported in a spheroid state, the storage condition and expiration date should be established based on the evaluation of transportation stability (e.g., effects of temperature, vibration, atmospheric pressure change), in addition to storage stability. Select an appropriate container, storage solution, and transportation configuration. The appropriate storage form, temperature conditions, transportation solution, and other factors required to maintain product stability may vary depending on the product form and/or cell type. Therefore, the optimal combination of these factors should be determined for each product to ensure stability.

(5) Biocompatibility of noncellular materials and final products

For noncellular materials related to the product, provide information on the quality and safety of those that constitute the final product as subcomponents or those used concomitantly at the time of product application (e.g., encapsulation membranes and fibrin glue), as well as those that come into contact with cells during the manufacturing process. Also, provide information on the biocompatibility and other interactions between these materials and cells in the product and the patient's cells. In addition, the final product as a whole should be evaluated for interaction with the patient's cellular tissue, particularly the tissue surrounding the application site. For noncellular materials as subcomponents of the final product, appropriate information should be collected regarding their degradation characteristics during the manufacturing process (in the culture medium) and in the body, their reabsorption characteristics in the body, and the safety of their

degradation products. In particular, when bioabsorbable materials are used, necessary tests should be conducted on their degradation products. For biocompatibility of noncellular materials, refer to ISO10993-1, JIS T 0993-1, ASTM F748-04, and "Amendment of Basic Principles of Biological Safety Evaluation Required for Application for Marketing Approval to Medical Devices" (PSEHB/MDED Notification No. 0106-1 dated January 6, 2020[2]), etc.

(6) Nonclinical studies

When evaluating the efficacy and safety of cardiomyocyte spheroids by applying them to animals, prepare disease model animals as necessary, considering the target disease. For the animal models used, the validation for their selection, the validity of the test system, and the extrapolation of the results to humans should be provided. To evaluate efficacy and safety, comparative studies should be considered, including groups to which cardiomyocyte spheroids are transplanted, a control group with a control substance, and, if necessary, a sham surgery group. The rationale for the evaluation period should also be explained. Evaluate the transplanted cardiomyocyte spheroids and their delivered efficacy over time, including the identification of the localization of the spheroids at the transplantation site, to assess the relationship between the localization and efficacy. Since animal studies encompass the evaluation of the method of application, the application procedure in animals should reflect the intended clinical use (e.g., open-chest surgery and endoscopic surgery) to the greatest extent feasible. The safety and efficacy are evaluated separately using respective methodologies. For example, safety may be evaluated primarily based on items (i) to (iv), while efficacy may be evaluated comprehensively based on items (v) and (vi). Alternative or additional test items may be adopted as necessary and appropriate. When producing cardiomyocytes and other cardiac cells (final product) with comparable quality attributes from multiple iPS cell banks that have been established using the same method after HLA typing, etc. and have been demonstrated to have comparable quality attributes as the raw materials for the final product, it is acceptable to demonstrate the proof of concept (POC) using the final product produced from a representative cell line.

(i) Morphological evaluation

The efficacy of the treatment for replacing cardiomyocytes is thought to result from the reinforcement of contractility by the engrafted myocardium, derived from transplanted cardiomyocytes, in cooperation with the host myocardium. It has been suggested that there is a correlation between the amount of engrafted myocardium (e.g., its proportion relative to the infarcted site or the entire left ventricle, as assessed histopathology) and the improvement of left ventricular ejection fraction. Perform a pathological examination of the transplantation site to evaluate the conditions of the site and surrounding tissues. For example, the following aspects may be investigated: Engraftment of cardiomyocyte spheroids at the transplantation site, presence or absence of fibrotic degeneration and inflammatory cell infiltration around the transplantation site,

[2] This notification has been replaced with Complete Revision of "Revision of Basic Principles of Biological Safety Evaluation Required for Application for Market Approval of Medical Devices" (PSB/MDED Notification No. 0311-1 dated March 11, 2025).

and changes in the transplantation site and surrounding tissues (morphology, thickness, number of cells, differentiation status, etc.).

(ii) Evaluation of proarrhythmia

The proarrhythmic potential should be evaluated using animals considered suitable for that purpose (e.g., monkeys, dogs, and pigs), as universally accepted animal models have not been established. For example, long-term electrocardiogram (ECG) data, such as Holter ECG recordings, in each group before and after transplantation may be compared to determine the presence or absence of arrhythmia and its severity.

(iii) Serological evaluation

Renal function, hepatic function, myocardial disorders, etc. should be evaluated using commonly used marker factors.

(iv) Evaluation of tumorigenicity

When evaluating the tumorigenicity of iPS cells-derived regenerative medical products, there should be awareness that the correlation or causal relationship between the tumorigenicity of iPS cells as raw materials, etc. and that of the final product has not been elucidated. In other words, in clinical application, it must always be noted that the evaluation of tumorigenicity of iPS cell-based products as final products is the most important, but not iPS cells as raw materials, etc. Therefore, it is useful to evaluate tumorigenicity test using the final product and a test system with a known detection limit in immunocompromised animals. When conducting a tumorigenicity, also refer to “Guidelines on the Detection of Undifferentiated Pluripotent Stem Cells and Transformed Cells, Tumorigenicity Test and Genetic Stability Evaluation on Human Cell Processed Products” (PSEHB/MDED Notification No. 0627-1 by the Director of Medical Device Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare, dated June 27, 2019), etc.

Tumorigenicity testing as part of nonclinical safety evaluation is preferably conducted by transplantation to immunocompromised animals (such as NOG mice or NSG mice) because of their high susceptibility.

It is recommended that the number of transplanted cells is calculated by multiplying the intended clinical dose by the safety factors for species and individual variations. However, the possibility that the total volume of transplanted cells may significantly affect the microenvironment at the transplantation site and become an artifact when transplanted into animals should be fully considered. In other words, it is important to determine the number of cells to be administered, considering that the purpose of tumorigenicity test via transplantation to the heart is to verify whether the cells in the final product have tumorigenic potential in the microenvironment corresponding to the transplantation site in humans.

(v) Evaluation of the method of application and administration procedure of cardiomyocyte spheroids

Consider the appropriate transplantation procedure (e.g., based on the number of cells) in relation to the site of infarction, dilatation, or other lesions. When a device is used for transplantation, safety measures should be considered from the design stage of the device, such as preventing insertion beyond a certain depth to ensure safe transplantation. For studies deemed necessary and scientifically valid for clinical application, such as the safety of the transplantation procedure and short-term response at the transplantation site after the procedure, it is recommended to conduct them by using suitable experimental models, for example, medium- or large-sized animals, depending on the purpose. It is recommended to transplant the maximum possible number of cells in clinical trials into pigs with hearts of similar size to those of humans, using the same transplantation device and procedure as in clinical trials. This aims to confirm whether the cells can be safely transplanted into the myocardium and whether there is no leakage of cells into the cardiac cavity.

(vi) Cardiac functional assessment

It is important that cardiac evaluation includes systolic and diastolic function assessment at and around the transplantation site by cardiac ultrasonography and contrast-enhanced MRI, etc. If necessary, left ventricular cavity shortening, left ventricular wall motion, left ventricular ejection fraction, and other parameters should also be evaluated.

(7) Clinical studies (clinical trials)

(i) Study population

To select a population suitable for evaluating efficacy and safety in clinical studies, the inclusion and exclusion criteria and evaluation criteria should be established after specifying the expected clinical positioning of the therapy using the widely accepted diagnostic criteria, severity classifications, etc. However, regarding the efficacy and safety in patients who are excluded from the study due to their disease severity, it is also necessary to consider the possibility of generalizing the results obtained in the clinical study and collecting relevant information through additional clinical studies, etc.

a) Inclusion criteria

When a clinical study is conducted for severe heart failure, consider the appropriate timing to initiate therapeutic intervention and disease severity based on the product characteristics. In the acute phase, existing therapy is usually chosen as the first-line treatment. Given the product's attributes, such as cell culture, the target phase is expected to be in the chronic phase. Mild cases may be adequately controlled with existing therapy, even if the product is not used. In the most severe cases, there may be no appropriate options other than heart transplantation, or invasive treatment may be challenging to perform. Note that the severity should be appropriately defined based on the timing of intervention and duration of treatment for heart failure, the New York Heart Association (NYHA) class and left ventricular ejection fraction (LVEF) value, according to the characteristics of the product being evaluated.

b) Exclusion criteria

When establishing exclusion criteria, it is important to consider the risks associated with the use of the product being evaluated. The use of allogeneic cells is expected to cause a certain level of immune rejection in the heart, even when HLA type matching is considered; thus, the use of immunosuppressants may be unavoidable. The use of immunosuppressants is required to suppress immune rejection of allogeneic human cell-based products. In patients in whom the use of these agents is not allowed or are contraindicated due to underlying diseases, controlling the immune response is challenging, raising safety concerns, and posing difficulties with product evaluation. Therefore, such patients are deemed unlikely to be included in clinical studies. It is also considered inappropriate to include patients with an allergy or hypersensitivity to immunosuppressive agents. In patients with malignant tumors as underlying disease, the safety evaluation is expected to be difficult, considering the use of immunosuppressants and product characteristics including tumorigenicity and other risks. Therefore, it should be considered that such conditions be included in the exclusion criteria. In addition, consideration should be given to individuals at risk conditions other than the target disease, who are typically not appropriate for inclusion in clinical studies, such as those with active infections, pregnant women, and children.

c) Elderly and young patients

Since severe heart failure commonly occurs in the elderly (65 years or older), efficacy and safety should be evaluated based on the “Studies in Support of Special Populations: Geriatrics” (PAB/NDD Notification No. 104 issued by the Director of New Drug Division, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, dated December 2, 1993) and the “Q&A about the Studies in Support of Special Populations: Geriatrics” (Administrative Notice issued by the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated September 17, 2010). However, since the severity of heart failure symptoms is not always age-dependent, the necessity of including the elderly/non-elderly as an allocation factor should be considered based on the presence or absence of existing diseases and other factors. In addition, regarding young patients (under 20 years of age), since the pathological condition of heart failure varies depending on the underlying heart disease, particularly congenital heart disease, consideration should be given to separating the inclusion and evaluation criteria or conducting separate clinical studies.

(ii) Determination of sample size and control group

The sample size should be determined aligning with the study objectives, hypotheses to be tested, and study design. It should be appropriately planned based on the clinical positioning and mechanism of action of the product. The establishment of a control group is discussed below as generally applicable to regenerative medical products in this disease area.

As a general rule, to appropriately evaluate the safety and efficacy of the product while minimizing various influencing factors, a control group receiving conservative therapy for severe heart failure is considered appropriate. On the other hand, considering the disease severity in the target population, establishing an appropriate control group may not be feasible. Therefore, the use

of external controls or registry data from patients with heart failure of similar severity may be acceptable for evaluation purposes. However, a simple comparison with published information, such as published papers, is insufficient from the viewpoint of a well-controlled comparison, and the data used as controls should be carefully examined. With reference to the “Basic Principles on Utilization of Registry for Approval Applications” (Joint PSEHB/PED Notification No. 0323-1, and PSEHB/MDED Notification No. 0323-1, by the Director of Pharmaceutical Evaluation Division, and by the Director of the Medical Device Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare, dated March 23, 2021), consider the following: the information used should be collected prospectively. Both patient populations included in clinical studies and those sourced from registries should have adequate information on patient characteristics to ensure that the effects of at least known confounders can be eliminated by matching using propensity scores or by weighted estimation. The ethics and reliability of the collected data should be adequately ensured.

(iii) Efficacy evaluation

In general, endpoints that have been established for reliability and validity and widely used internationally are selected as primary efficacy endpoints. Changes from baseline in the endpoint, the proportion of patients with improvement, etc. at specific time points will be used for efficacy evaluation. Secondary efficacy evaluation is helpful not only for examining the validation of the results of the primary endpoint but also more extensively investigating the clinical significance of the results obtained. For tests subject to subjective bias or expected to show variations in results due to the variations in the use of measuring devices, appropriate strategies should be implemented to minimize between-evaluator variation, such as providing evaluator education and training. Particularly in global clinical trials, care should be taken to ensure that evaluation methods do not differ between participating regions. It is also necessary to assess the eligibility of evaluators prior to initiating clinical studies.

Preferably, also refer to the descriptions in the “Revision of the Guidelines on Clinical Evaluation of Anti-Heart Failure Drugs” (PFSB/ELD Notification No. 0329-18 issued by the Director of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated March 29, 2011).

a) Primary endpoint

The true endpoints of the treatment of severe heart failure are avoidance of cardiac events, such as death and hospitalization; avoidance of multiple cardiac events, including major adverse cardiovascular events (MACE); and improvement of clinical conditions, such as the quality of life (QOL) including the activity of daily living (ADL). However, QOL improvement involves multiple factors and may not necessarily correlate with the efficacy of this product as assessed based on its characteristics and is strongly influenced by subjective elements of bias. Therefore, using QOL as the primary endpoint is expected to make evaluation difficult. At present, parameters that can be objectively quantified and directly measure ischemic changes and improvements in cardiac function over a short time may be used as surrogate endpoints.

The cardiac function parameters described in the “Revision of the Guidelines on Clinical Evaluation of Anti-Heart Failure Drugs” (PFSB/ELD Notification No. 0329-18 issued by the Director of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated March 29, 2011) should also be considered as endpoints for the investigational product. In addition, evaluation of the true long-term endpoints is essential. Follow-up studies should be designed to enable long-term data tracking and collection and should also be planned in advance to enable the discussion or verification of the product’s efficacy relative to external controls or registry data.

b) Secondary endpoints

Secondary endpoints include efficacy measures to supplement the primary endpoint. Endpoints related to cardiac function should be specified as secondary endpoints to supplement the primary endpoint. These include, for example, New York Heart Association (NYHA) Functional Classification, ejection fraction measured by echocardiography or cardiac MRI, left ventricular end-systolic volume index (LVESVI), and evaluation using biomarkers such as N-terminal pro-brain natriuretic peptide (NT-proBNP) and brain natriuretic peptide (BNP). In addition, to assess improvements in ADL and QOL, physical activity evaluation, such as 6-minute walking distance (6 MWD) and Symptom Assessment Scale (SAS), exercise tolerance assessment, and comprehensive QOL assessments (e.g., Euro-QoL 5-dimension [EQ5D] and MOS 36-Item Short-Form Health Survey [SF-36]) should be considered for inclusion as secondary endpoints. Disease-specific QOL and patient-reported outcome (PRO) (e.g., Kansas City Cardiomyopathy Questionnaire [KCCQ] and Minnesota Living with Heart Failure Questionnaire [MLHFQ]) should also be considered to be included. In addition to the above, endpoints may be added according to the underlying disease of heart failure.

It is also important to confirm that the transplanted myocardium has been engrafted and that the engrafted myocardium reinforces the host myocardial contraction. In the future, it is recommended to establish a method for measuring engraftment of the transplanted myocardium by quantifying the myocardium at the transplantation site. Cardiac MRI, echocardiography, and myocardial single photon emission computed tomography (SPECT) may be useful for this evaluation. Summed rest score (SRS) from myocardial SPECT is also considered useful in ischemic heart disease. In the future, it is recommended to establish a method for quantifying the myocardium at the transplantation site. It is also necessary to establish an analytical method to determine whether the transplanted myocardium enhances contractility in cooperation with the existing myocardium.

(iv) Safety Evaluation

An adverse event is any untoward medical occurrence in a patient administered a medicinal product (including a regenerative medicine product, hereinafter the same in this section) and whether or not related to the administration of the investigational product. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal clinical test results), symptom, or disease temporally associated with the use of a medicinal product. If any adverse event is observed, document the name of the adverse event, its severity, outcome, confirmed times

of onset and outcome, use of the investigational product (including drugs, biologics, and cell-based products, hereinafter referred to as the same in this section), specific treatment provided, and its contents will be recorded in the case report form. In addition, it should be evaluated whether the adverse event is serious and its causal relationship with the investigational product.

In clinical studies, special attention should be paid to collecting adverse events characteristic of cell transplantation and those related to the pathological conditions of heart failure, such as the following: Attention should also be paid to adverse events caused by immunosuppressants used after allogeneic cell transplantation. In particular, renal impairment is considered a significant adverse event.

Significant adverse events

I. Tumorigenesis
II. Infection
III. Rejection
IV. Adverse events associated with transplantation procedure (bleeding, occurrence of fatal arrhythmia, etc.)
V. Fatal arrhythmia
VI. Pneumonia
VII. Respiratory failure
VIII. Deep vein thrombosis/pulmonary infarction
IX. Drug-induced hypersensitivity syndrome
X. Aggravation of cardiac failure

Item IV is an adverse event of concern when cardiomyocytes are transplanted into the host myocardial wall using an injection needle. It is required to administer cardiomyocytes while monitoring myocardial wall thickness using echocardiography, etc. to prevent the needle from inserting beyond a certain depth. For item V, arrhythmia, such as ventricular tachycardia, may occur in the host myocardium for a certain period after cardiomyocyte transplantation. As a safety measure, patients will be hospitalized for certain period and continuous ECG monitoring during this period after transplantation. The duration of hospitalization will be determined based on findings from nonclinical studies, etc. Measures such as arrhythmia detection by a loop-type implantable electrocardiograph and the use of a wearable cardioverter defibrillator (WCD) may be one option.

(v) Concomitant medications and rehabilitation handling

a) Concomitant medications

It is recommended to avoid, as much as possible, medications that may affect the efficacy and safety evaluations because they make assessments difficult. However, given the severity of the target disease and with reference to the most recent guidelines of relevant academic societies, standard treatments, including digitalis, diuretics, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, beta-blockers, aldosterone antagonists, angiotensin receptor-neprilysin inhibitors (ARNIs), and sodium-glucose cotransporter 2 (SGLT2) inhibitors may be

continued without changing the dosage and administration during the study period, except when the patient's condition is unstable such as during the perioperative management period. In such cases, the details of standard treatments during the study period should be clearly defined prior to initiating the study. Specify that the details and reasons must be documented and retained if medications that may affect efficacy evaluation are inevitably added, changed, or have their dosage and administration modified (including frequency of use for as-needed medications).

b) Rehabilitation handling

Rehabilitation is a factor that influences functional recovery after heart failure. In clinical studies, the impact of individual differences in rehabilitation therapy on efficacy evaluation should be considered. If a rehabilitation program is performed after therapeutic intervention, an appropriate plan should be developed, considering an objective cardiac evaluation to ensure no bias between groups.