Provisional Translation (as of September 2025)*

# Guidance on Evaluation of the Treatment of Severe Heart Failure Using Human (Allogeneic) iPS Cells-derived Cardiovascular Cells Multilayered Cell Sheets

## 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 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) Cell sheet: A sheet-like structure of cells that are directly or indirectly connected to each other.

(2) Cell bank: A system consisting of a substantial number of containers, each containing contents

*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 uniform composition, stored under defined conditions. Each container represents an aliquot 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) Biomaterials: Materials that come into direct contact with living organisms.

(6) Multilayered cell sheet: A graft that has a three-dimensional structure formed by multilayering cell sheets using a biological material, etc.

(7) Cardiovascular cells: Cells that develop during myocardial or vascular differentiation. (cardiomyocytes, fibroblasts, smooth muscle cells, vascular endothelial and wall cells, etc.)

**5. Points to Consider for Evaluation**

For the time being, this evaluation guidance is intended to apply to the evaluation of multilayered cell sheets containing cardiovascular cells as a human (allogeneic) iPS cell-based product (hereinafter referred to as “multilayered cell sheets”) 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 cardiovascular cells through the defined manufacturing process.

For iPS cells established by introducing reprogramming genes into human somatic cells, it is

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

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 multilayered cell sheets of the final product.

(2) Matters requiring special attention in the manufacturing process

In the manufacture of multilayered cell sheets (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.

Production of multilayered cell sheets is expected to involve multiple processes, including extended culture, differentiation induction, cell sheet formation, cell sheet layering, and packaging and packing processes. In-process inspections are recommended at each stage.

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 multilayered cell sheets for transplantation from the frozen cells (cell thawing, cell sheet production method, multilayering 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 multilayered cell sheets (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 cardiovascular cells is influenced by many parameters related to cell conditions and culture, potentially leading to variability in the proportion of cardiovascular cells in the final product. In addition, cell cultures for cell sheet formation (cell sheet-forming culture) may also cause a variation in the proportion of cells contained in the sheet. Therefore, during the manufacturing process up to the final product, it is recommended to control the proportion and the number of component cells within an appropriate range that does not affect the cell quality. Measures for such control should be specified.

f) Establishment of cell sheet formation process

It is recommended that cell sheets be formed using previously established methods, such as technology using temperature-responsive culture surfaces. The number of cells to be seeded should be predefined to be sufficient to ensure the formation of cell sheets with adequate yield.

g) Establishment of cell sheet layering process

In the layering process of the formed cell sheets, manual or mechanical layering is required to

be performed aseptically. To perform this layering process consistently, predefine the standard operating procedure, and validate the consistency of the step.

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

When part of the manufacturing process is shared 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 multilayered cell sheets (final product). For example, a possible transplantation method involves administration of the required number of cells in a multilayered state, containing iPS-derived cardiovascular cells (as the final product), to an appropriate site on the heart.

Points to consider for the quality control of multilayered cell sheets 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 with substitute samples, such as products manufactured in parallel etc., and provide justification.

If long-term storage of the multilayered cell sheets 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 and cell morphology

It is recommended to confirm that the final product has the intended description by visual inspection and to record its morphology. When the final product is multilayered cell sheets, the requirements may be established based on the visual inspection of multilayered cell sheets (e.g.,

multilayered membrane-like structures) or 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 multilayered cell sheets, measuring the number of cells and viability within the multilayered cell sheets may be 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 cell count and viability in the cell sheet before multilayering may be used as surrogate markers.

c) Confirmation of cell specificity

Determine the expression levels of representative marker molecules specific to each cardiovascular cells population constituting the final product using flow cytometry and other methods. These molecules include cardiac troponin T (cardiomyocytes), VE-cadherin (vascular endothelial cells), and platelet-derived growth factor receptor β (vascular wall cells). The expression levels may be assessed using other methods such as mRNA expression analysis and cellular immunostaining. Beyond these analyses alone, it is recommended to evaluate the specificity of cardiovascular cells populations and the proportion of cells exhibiting each specificity using multiple different methods.

When the final product is multilayered cell sheets, evaluating the specificity of cells within the multilayer structure may be technically challenging. In such cases, surrogate markers that support specific indicators within the structure may be used. The justification for selecting the markers should be provided. For example, cell specificity (e.g., cardiac troponin T expression) in the cell sheet before multilayer formation may be used as a surrogate marker, provided that a correlation between pre- and post-multilayer formation has been demonstrated.

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 multilayered cell sheets, this can be assessed by measuring intracellular calcium activity or 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 multilayered cell sheets, measuring the function of the sheets may be 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) in the cell sheet before multilayer formation may be used as a surrogate marker, provided that a correlation between pre- and post-multilayer 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. For the evaluation of genetic stability, 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.

g) Evaluation of the suitability of the cell sheet multilayer process

It is recommended to evaluate the presence or absence of cell necrosis associated with internal ischemia which may be caused by multilayering.

(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 they 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. In addition, it should be demonstrated in advance that the noncellular components have no adverse effect on the quality of the final product through the freeze-thaw step. The transportation solution should also be appropriately selected when transporting the final product in an unfrozen state.

When multilayered cell sheets as the final product are transported in a multilayered 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, cell type, and/or biomaterials contained. Therefore, the optimal combination of these factors should be determined for each product to ensure stability.

(5) Biocompatibility of noncellular biomaterials and final products

For noncellular biomaterials, which constitute the final product as subcomponents, 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 biomaterials, 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.

For noncellular biomaterials related to the product, provide information on the quality and safety of noncellular biomaterials 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 biomaterials 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,

[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).

particularly the tissue surrounding the application site. Also, for biocompatibility of non-cell biomaterials, 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 multilayered cell sheets 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 between a treatment group to which multilayered cell sheets are transplanted and a control group (non-treatment or sham surgery group). The rationale for the evaluation period should also be explained. Evaluate the transplanted multilayered cell sheets and their delivered efficacy over time, including the identification of the localization of cells within the sheets 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 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 cardiovascular cells 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

Perform a pathological examination of the transplantation site of multilayered cell sheets to evaluate the conditions of the site and surrounding tissues. For example, the following aspects may be investigated: Engraftment of multilayered cell sheets at the transplantation site, presence or absence of fibrotic degeneration and inflammatory cell infiltration around the application site, and changes in the transplantation site and surrounding tissues (morphology, thickness, number of cells, differentiation status, etc.). In addition, if present, investigate the structural elements necessary to maintain the morphology, properties, and function of the transplanted multilayered cell sheets (e.g., vascular network) using immunohistochemistry or other techniques. It is also recommended to pathologically evaluate local inflammation, as necrotic cells associated with internal ischemia due to multilayer may induce a local inflammatory response after transplantation.

(ii) Evaluation of proarrhythmia

The proarrhythmia 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 the transplantation of cardiomyocyte sheets 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 for emerging tumor cells in immunocompromised animals. When conducting a tumorigenicity test, 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 test 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 onto the heart surface is to verify whether the cells in the final product are not tumorigenic within the microenvironment corresponding to the transplantation site in humans.

(v) Evaluation of the method of application, administration procedure, and dose, etc. of multilayered cell sheets

It is recommended to consider the appropriate transplant dose of multilayered cell sheets (e.g., based on the surface area of the sheets, number of layers, or number of sheets) in relation to the

site or area of infarction or dilatation, etc., as well as the transplantation procedure, etc. 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.

(vi) Cardiac functional assessment

Cardiac evaluation should include systolic and diastolic function assessment by cardiac ultrasonography and contrast-enhanced MRI, etc. If necessary, left ventricular cavity shortening, left ventricular wall motion, and other parameters should also be evaluated. Blood flow should be evaluated, as necessary, after transplantation of the multilayered cell sheet product. Evaluation methods may include, for example, 18F-fluorodeoxyglucose Positron Emission Tomography (FDG-PET) or echocardiography. The duration of efficacy should also be investigated. If improved cardiac function is expected to be primarily related to secreted factors derived from the transplanted multilayered cell sheet, it is necessary to confirm the engraftment of cardiovascular cells derived from the sheet by histopathological examination, etc.

(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. The symptoms and prognosis of heart failure vary depending on the severity. To narrow down the population appropriate for efficacy evaluation, the inclusion/exclusion criteria should include the disease severity of the target population, taking into consideration the availability, efficacy, and safety of existing treatments. 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 extrapolating 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 diagnostic classification and its validity as well as 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, etc. 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 is 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, 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 according to the underlying heart disease.

(ii) Determination of sample size and control group

The sample size should be determined based on scientific rationale, aligning with the study objectives, hypotheses to be tested, achievement criteria, and study design. The establishment of a control group is discussed below, not limited to human (allogeneic) iPS cells-derived

cardiovascular cells multilayered cell sheets but also 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 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. 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. Monitoring and comparing cardiac function parameters, clinical symptoms, etc. over time in the same patient after intervention is one approach to eliminate variations in patient characteristics for efficacy evaluation. For evaluating individual patient data collected in clinical studies, a well-devised approach enabling objective comparison should be implemented, such as separating the operator and the evaluator and establishing a third-party committee.

(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 heart failure are avoidance of cardiac events, such as death and hospitalization; 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. Also, the QOL 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. Based on the evaluation items described in "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), 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, echocardiography, ejection fraction and ischemic change as measured by cardiac MRI (for ischemic disease), left ventricular end-systolic volume index (LVESVI), hemodynamic evaluation, and evaluation using biomarkers such as N-terminal pro-brain natriuretic peptide (NT-proBNP). 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. In addition to the above, establishing safety endpoints, such as the incidence of clinical cardiac events, is considered important for evaluating heart failure improvement from various perspectives and in a comprehensive manner to elucidate the mechanisms supporting the product's efficacy and safety.

(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. Arrhythmia
VI. Pneumonia
VII. Respiratory failure
VIII. Deep vein thrombosis/pulmonary infarction
IX. Drug-induced hypersensitivity syndrome
X. Aggravation of cardiac failure

(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.