September 2, 2015

Medical Device and Regenerative Medicine Product Evaluation Division
Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

Report on the Deliberation Results

[Classification]	Human cellular/tissue-based products 2 Human somatic stem cell-processed products
[Non-proprietary name]	Human (autologous) skeletal myoblast-derived cell sheet
[Brand name]	HeartSheet
[Applicant]	Terumo Corporation
[Date of application]	October 30, 2014

[Results of deliberation]
In the meeting held on September 2, 2015, the Committee on Regenerative Medicine Products and Biotechnology made the following decision and concluded that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product may be approved and this approval is classified as a conditional and time-limited approval under the following conditions.

[Conditions for approval]
1. The applicant is required to ensure that the product is used by physicians and surgeons with adequate knowledge and experience in severe heart failure and thoracotomy at medical institutions with capacity for emergency response under a system that ensures appropriate patient control through laboratory tests, etc.
2. The applicant is required to conduct an approval condition-based post-marketing evaluation in all patients transplanted with the product during the period between the conditional and time-limited approval and reapplication for marketing approval.

[Duration of approval]
5 years

Review Report

August 17, 2015

Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following regenerative medical product submitted for registration are as follows.

[Brand name] HeartSheet

[Classification] Human cellular/tissue-based products 2 Human somatic stem cell-processed product

[Non-proprietary name] Human (autologous) skeletal myoblast-derived cell sheet

[Applicant] Terumo Corporation

[Date of application] October 30, 2014

[Shape, structure, ingredients, quantities, or definition]

The product is a regenerative medical product. The primary constituent part of the product is cryopreserved skeletal myoblast-derived cells filled in a dedicated container that have been cultured *ex vivo* after isolation from the patient's skeletal muscle harvested at a medical institution.

The secondary constituent parts of the product include equipment for transporting skeletal muscle harvested at a medical institution, equipment for transporting the patient's serum used as a component of culture medium for cell sheet forming, and other equipment and materials necessary for forming cell sheets from the cryopreserved skeletal myoblast-derived cells.

[Application classification] (1-1) New regenerative medical products

[Items warranting special mention]

None

[Reviewing office] Office of Cellular and Tissue-based Products

[Results of review]

Based on the overall evaluation of the submitted data, the Pharmaceuticals and Medical Devices Agency has concluded that the product is expected to have a certain level of efficacy in the treatment of patients with severe heart failure unresponsive to standard drug therapies or surgical operations and its safety is acceptable, as described in the Attachment. However, since only limited information is available, the efficacy of the product should be further evaluated and confirmed after marketing approval.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the indication or performance and dosage and administration or method of use as shown below, with the following conditions. The approval should be time-limited and conditional in accordance with Article 23-26 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics.

[Indication or performance]
Treatment of patients with severe heart failure due to ischemic heart disease unresponsive to standard treatments including drug and invasive therapies who meet all of the following criteria.

Eligibility criteria:
- NYHA class III or IV heart failure; and
- Resting left ventricular ejection fraction ≤35%

[Dosage and administration or method of use]
The product is used according to the following method.

Prior to the production of skeletal myoblast-derived cell sheets
1) Skeletal muscle is harvested from the patient. As a general procedure, the harvesting is performed according to the procedure for diagnostic muscle biopsy for neuromuscular diseases. Skeletal muscle should be harvested from the quadriceps, in principle, but may be harvested from any other appropriate site of the body depending on the patient's condition. The harvested skeletal muscle is delivered in a dedicated container to the facility designated by the marketing authorization holder.

2) Blood is collected from the patient to separate serum. The separated serum is delivered in a container to the facility designated by the marketing authorization holder.

Preparation and transplantation of skeletal myoblast-derived cell sheets
1) Using the secondary constituent parts, 5 skeletal myoblast-derived cell sheets (preferably 6 sheets including 1 spare) are prepared from the cryopreserved cells. Each step is taken aseptically.

2) Each of 5 skeletal myoblast-derived cell sheets is sequentially transplanted onto the surface of the heart. The transplantation procedure is usually performed through left thoracotomy.

[Conditions for approval]
1. The applicant is required to ensure that the product is used by physicians and surgeons with adequate knowledge and experience in severe heart failure and thoracotomy at medical institutions with capacity for emergency response under a system that ensures appropriate patient control through laboratory tests, etc.

2. The applicant is required to conduct an approval condition-based post-marketing evaluation in all patients transplanted with the product during the period between the conditional and time-limited approval and reapplication for marketing approval.

Attachment

Review Report (1)

June 12, 2015

1. Product Submitted for Registration

[Brand name] [redacted]

[Classification] Human cellular/tissue-based products 2 Human somatic stem cell-processed product

[Non-proprietary name] Human (autologous) skeletal myoblast-derived cell sheet

[Applicant] Terumo Corporation

[Date of application] October 30, 2014

[Shape, structure, ingredients, quantities, or definition]

The product is a regenerative medical product. The primary constituent part of the product is cryopreserved skeletal myoblast-derived cells filled in a dedicated container that have been cultured *ex vivo* after isolation from the patient's skeletal muscle harvested at a medical institution.

The secondary constituent parts of the product include equipment for transporting skeletal muscle harvested at a medical institution, equipment for transporting the patient's serum used as a component of culture medium for cell sheet forming, and other equipment and materials necessary for forming cell sheets from the cryopreserved skeletal myoblast-derived cells.

[Proposed indication or performance]

Maintenance or improvement of the condition of patients with severe heart failure due to ischemic heart disease

[Proposed dosage and administration or method of use]

1. Harvesting of skeletal muscle and transportation to the cell processing facility designated by the marketing authorization holder

   As a general procedure, the harvesting is performed according to the procedure for diagnostic muscle biopsy for neuromuscular diseases. Skeletal muscle should be harvested from the quadriceps, in principle, but may be harvested from any other appropriate site of the body depending on the patient's condition. The skeletal muscle is harvested from the patient by the following steps at least 7 weeks before the scheduled transplantation of skeletal myoblast-derived cell sheets.

   1) The skin is dissected along the muscle fiber. Then the subcutaneous fat layer is removed bluntly, and the quadriceps is incised to expose the skeletal muscle.

2) A required amount of muscle bundles (approximately 2-5 g) is harvested.

3) The harvested skeletal muscle is placed in a dedicated container with a patient identification label and the cap is tightly closed.

4) After hemostasis of the dissected site is confirmed, the quadriceps and the skin are sutured.

5) The skeletal muscle container is placed in a shipping container and delivered to the cell processing facility designated by the marketing authorization holder.

2. Collection of patient's serum and delivery to the cell processing facility designated by the marketing authorization holder

Serum is collected from the patient by the following steps.

1) Blood is collected from the patient and serum is separated from the blood, using vacuum blood collection tubes or serum bags, according to the instructions for use of the devices.

2) The separated serum is filled in a dedicated container, frozen (≤−20°C), and transported in a shipping container to the cell processing facility designated by the marketing authorization holder.

3. Preparation of skeletal myoblast-derived cell sheets to be transplanted to the patient

A total of 5 skeletal myoblast-derived cell sheets (or preferably 6 including 1 spare) are prepared according to the following procedures. All steps are taken aseptically.

1) The sheet-forming medium is added to temperature-responsive culture dishes and warmed at 37°C.

2) The cryopreserved cells are thawed at 37°C, diluted with a washing solution, and are centrifuged.

3) The sheet-forming medium is added to the centrifuged cells to prepare a cell suspension.

4) The sheet-forming medium is removed from the temperature-responsive culture dishes. The cell suspension is added to the dishes. The dishes are left to stand in a CO_2 incubator.

5) The sheet-forming medium is removed from the temperature-responsive culture dishes, and skeletal myoblast-derived cell sheets are detached by adding HBSS (+).

6) HBSS (+) is added to the temperature-responsive culture dishes to immerse the detached skeletal myoblast-derived cell sheets in HBSS (+).

7) The temperature-responsive culture dishes containing skeletal myoblast-derived cell sheets are packaged.

4. Transplantation of skeletal myoblast-derived cell sheets to the patient

The transplantation is performed according to cardiovascular surgery procedures. Skeletal myoblast-derived cell sheets are transplanted by the following steps.

1) The transplantation site is determined on the surface of the heart exposed by left thoracotomy.

2) Using an abdominal spatula, 5 skeletal myoblast-derived cell sheets are spread piece by piece over the surface of the heart, and sequentially transplanted onto the heart.

3) After the completion of the surgery, the unused spare skeletal myoblast-derived cell sheet is discarded.

The submitted data and the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined below.

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List of abbreviations

Abbreviation	Definition
ACE	Angiotensin-converting enzyme
ARB	Angiotensin receptor blocker
AT	Anaerobic threshold
BiPAP	Bilevel positive airway pressure
BNP	Brain natriuretic peptide
BSA	Bovine serum albumin
CABG	Coronary arterial bypass grafting
CPAP	Continuous positive airway pressure
CRT	Cardiac resynchronization therapy
CRT-D	Cardiac resynchronization therapy defibrillator
DMSO	Dimethyl sulfoxide
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
FAC	Fractional area change
FBS	Fetal bovine serum
HGF	Hepatocyte growth factor
IPAAm	N-isopropylacrylamide
LVAD	Left ventricular assist device
LVDd	Left ventricular internal dimension in diastole
LVDs	Left ventricular internal dimension in systole
LVEDA	Left ventricular end-diastolic area
LVEF	Left ventricular ejection fraction
LVESA	Left ventricular end-systolic area
MET	Metabolic equivalent
MHC	Myosin heavy chain
MR	Mitral regurgitation
Study M-51073-21	A Japanese clinical trial
NOG	NOD/Shi-*scid*,IL-2Rγ^{null}
NYHA	New York Heart Association
PCI	Percutaneous coronary intervention
PDGF	Platelet-derived growth factor
Peak VO_2	Peak oxygen uptake
SAS	Specific activity scale
SDF	Stromal cell-derived factor
SPECT	Single photon emission computed tomography
VEGF	Vascular endothelial growth factor
Patient serum	Serum derived from patient
PMDA	The Pharmaceuticals and Medical Devices Agency
Skeletal myoblast suspension	Cell suspension obtained by enzymatic treatment of cell sheets prepared from the product
Engraftment, etc.	Engraftment, subsequent changes and distribution, etc.
Standards for Animal-derived Raw Materials	Standards for Biological Ingredients 4. General Rules for Animal-derived Ingredients 4-3. Standards for Animal-derived Materials
Standards for Ruminant Animal-derived Raw Materials	Standards for Biological Ingredients 4. General Rules for Animal-derived Ingredients 1. Standards for Ruminant Animal-derived Materials
Porcine cell sheets	Porcine skeletal myoblast-derived cell sheets
Skeletal myoblast-derived cell sheets	Skeletal myoblast-derived cell sheets prepared from the product

2. Origin or History of Discovery and Usage Conditions in Foreign Countries, etc.

2.1. Outline of the product submitted for registration

[redacted] (the proposed product) is a combination product comprising (i) the primary constituent part, namely skeletal myoblast-derived cells cryopreserved after being isolated from the skeletal muscle of the patient's own quadriceps, etc., cultivated *ex vivo*, and suspended in a stock solution, and (ii) the secondary constituent parts: (a) a dedicated container (with a solution to keep the tissue during transportation) used to transport skeletal muscle harvested from the patient to the manufacturing site, (b) devices used for separating patient-derived serum (patient serum) and for the delivery of the patient serum to the manufacturing site, (c) culture media, etc. used to prepare skeletal myoblast-derived cell sheets from the cryopreserved cells, and (d) sheet preparation apparatuses.

This regenerative medical product is skeletal myoblast-derived cell sheets applied onto the surface of the heart through thoracotomy, used in a similar way as medical devices such as pericardial membrane patches. The primary constituent part, after arriving at the cell preparation facility attached to a medical institution, is processed into skeletal myoblast-derived cell sheets by a physician or a person under the supervision of the physician, and then used in treatment. Skeletal myoblast-derived cell sheets were shown to improve left ventricular systolic function in nonclinical studies in a mini-pig model. A clinical trial of skeletal myoblast-derived cell sheets yielded limited data on the efficacy of skeletal myoblast-derived cell sheets, but revealed clinically significant outcomes, suggesting that skeletal myoblast-derived cell sheets may suppress the progression of severe heart failure in patients with the disease, who are likely to suffer progressively deteriorating cardiac function. The outcomes included improvement in the comprehensive evaluation of left ventricular ejection fraction (LVEF), cardiac function classification by New York Heart Association (NYHA), and exercise tolerance, etc., as well as in the prevention of fatal events. So far, detailed mechanism of action of skeletal myoblast-derived cell sheets have not been elucidated, including the pharmacological actions of physiologically active substances produced from skeletal myoblast-derived cell sheets.

2.2. Development history, etc.

Currently, patients with severe heart failure who do not respond to standard drug therapies are treated with cardiac resynchronization therapy (CRT) and surgical therapies such as mitral valvuloplasty and left ventricular restoration (Guidelines for diagnosis and treatment of cardiovascular diseases, by the Japanese Circulation Society [2009 report of joint working group], Guidelines for treatment of chronic heart failure [2010 revised edition]). CRT is indicated for patients with moderate or severe heart failure, but approximately 30% of these patients are non-responders (*Curr Poin Cardiol.* 2006;21:20-26). Mitral valvuloplasty and left ventricular restoration are expected to improve clinical condition of the patients. However, these surgeries are indicated for a limited number of patients and are highly invasive. A left ventricular assist device (LVAD) is effective in patients with end-stage severe heart failure, but is approved only as a bridge to heart transplantation in patients scheduled to undergo the procedure. In Japan, because of the shortage of heart donors, heart transplantation is indicated only for patients with severe clinical condition who are unlikely to survive with conventional therapies, and consequently LVAD is indicated for only a small number of patients. Only limited therapeutic options are thus available for patients with severe heart failure who do not respond to standard drug therapies. The demand for the development of new therapies is increasing.

Studies on novel cell-based therapies for severe heart failure have been in progress since around 2000. To date, there have been reports on clinical studies on therapies using fibroblasts, skeletal myoblasts, bone marrow-derived cells, mesenchymal stem cells, etc. Several research institutions and companies, mostly in the US and Europe, released reports on therapies with autologous skeletal myoblasts. According to the reports, cells were injected to the myocardium by thoracotomy for coronary arterial bypass grafting (CABG) or LVAD implantation, or through a cardiac catheter (*Circulation.* 2008;117:1189-200, *JACC Cardiovasc Interv.* 2009;2:9-16). However, autologous skeletal myoblast-based products for the treatment of heart failure have not been approved or marketed in any country or region until now.

Before the submission of the clinical trial notification of the proposed product, an application was filed for accreditation of the quality and safety on January 29, 2010, according to the "Quality and Safety Assurance of Cell/Tissue Pharmaceuticals and Cell/Tissue-Derived Medical Devices" (PMSB

Notification No. 906, by the Director of Pharmaceutical and Medical Safety Bureau, dated July 30, 1999 [revised on May 18, 2009]), and confirmation was granted as a result of the discussion at the Committee on Biotechnology, Pharmaceutical Affairs and Food Sanitation Council (PFSB Notification No. 0131-5, by the Director of Pharmaceutical and Food Safety Bureau, dated January 31, 2011). Subsequently, the applicant conducted a clinical trial in patients with severe heart failure due to ischemic heart disease based on the advice given by PMDA at clinical trial consultations. This marketing application for the regenerative medical product is based on these clinical studies.

3. Manufacturing Process and Specifications

3.1. Constituent parts of the product

The primary constituent part is skeletal myoblast-derived cells from the patient's own skeletal muscle that are cultivated and suspended in the stock solution before being filled into containers in small portions. The myoblast-derived cells are then cryopreserved and delivered frozen. The stock solution contains human serum albumin and dimethyl sulfoxide (DMSO). The secondary constituent parts include (i) a container (filled with the tissue transport solution) for transporting the harvested skeletal muscle to the manufacturing site, (ii) devices used for separating patient-derived serum (patient serum) and for the delivery of the patient serum to the manufacturing site, (iii) culture media used for forming skeletal cell sheets from cryopreserved myoblast-derived cells (comprising washing solution and culture medium for sheet preparation, etc.), and (iv) sheet preparation apparatuses, including temperature-responsive culture dishes.

3.1.1. Manufacturing process

3.1.1.1. Manufacturing process

The manufacturing process of the proposed product comprises the production of the primary constituent part (cryopreserved cells) and the secondary constituent parts. The cryopreserved cells are stored in a liquid nitrogen container and, within [redacted] days, delivered to a medical institution. At the medical institution, the cryopreserved cells are processed into skeletal myoblast-derived cell sheets using the secondary constituent parts. The prepared sheets are transplanted into the patient.

3.1.1.1.1. Manufacturing process of the primary constituent part

The manufacturing process of cryopreserved cells comprises the preparation of solutions/media used for the cultivation of cells (solution for tissue transportation, [redacted]), receipt of the skeletal muscle, [redacted], filling/plugging, labeling/packaging, cryopreservation, testing, and packaging/labeling.

The manufacturing process of cryopreserved cells is the cultivation of autologous cells derived from the patient. Process validation has not been completely performed for this process because of the difficulty in optimizing process parameter for inter-individual variability. Instead, the process verification is performed for every production to confirm that the process is performed as intended; the quality of the proposed product is controlled in this manner [see Section 3.3.1.].

[redacted] processes were identified as critical process steps.

3.1.1.1.2. Manufacturing process of the secondary constituent parts

The manufacturing processes of (i) the skeletal muscle container (filled with the tissue transport solution) for the delivery of the harvested skeletal muscle at a medical institution to the manufacturing site and (ii) the washing solution and the culture medium used to prepare skeletal myoblast-derived cell sheets from the cryopreserved cells include the preparation of the solutions, sterile filtration, filling, plugging, packaging/labeling, and testing.

[redacted]

The manufacturing processes of serum separation devices and sheet preparation apparatuses include packaging, labeling, and testing.

3.1.1.2. In-process control tests and intermediate control

Table 3.1 shows the in-process control tests in the manufacturing process of the proposed product [see Section 3.3.1.].

The process control of the intermediate is not defined.

Table 3.1. In-process control tests of cryopreserved cells

3.1.2. Safety evaluation of adventitious agents

3.1.2.1. Skeletal muscle and patient serum

The raw material, 2 to 5 g-bundle of skeletal muscle, is isolated from the quadriceps or any other appropriate site of the patient body. The skeletal muscle is delivered to the manufacturing site in a dedicated container filled with the tissue transport solution, one of the secondary constituent parts.

Table 3.2 shows the acceptance criteria of skeletal muscle.

Table 3.2. Acceptance tests for skeletal muscle

Tests
Appearance of skeletal muscle (visual inspection)
Appearance of skeletal muscle container (visual inspection)
Virus test
[redacted]

Patient serum used for the cultivation of skeletal muscle-derived cells is collected at a medical institution using serum separation devices, secondary constituent parts, and is delivered to the manufacturing site. A portion of the patient serum is stored separately.

Skeletal muscle and patient serum are used to prepare skeletal myoblast-derived cell sheets after they are proved to conform to the Standards for Biological Ingredients (MHLW Ministerial Announcement No. 210, 2003) and after the patient is tested negative for viruses (HBV, HCV, HIV, and HTLV-1) by serologic tests or nucleic-acid amplification tests at the medical institution.

3.1.2.2. Human-derived or animal-derived raw materials other than skeletal muscle and patient serum

Table 3.3 shows human-derived or animal-derived raw materials used in the manufacturing process.

Table 3.3. Human-derived or animal-derived raw materials other than skeletal muscle and patient serum used in the manufacturing process

Use	Material or raw material		Animal species	Organ/Tissue	Country of origin
[redacted]	Collagenase	Peptone	Bovine	Bone and adherent tissue	US
			Porcine	Stomach	[redacted]
		Casein Peptone	Bovine	Milk	[redacted]
		Trypticase Soy Broth	Bovine	Milk	[redacted]
[redacted]	Fetal bovine serum (FBS)		Bovine	Blood	Australia, New Zealand
[redacted]	Human serum albumin		Human	Blood	

Collagenase derived from *clostridium histolyticum* bacteria is manufactured using medium containing bovine or porcine peptone. It is unknown whether the collagenase used to manufacture clinical trial batches was derived from the prohibited tissue stipulated by "1. Ruminant animal-derived Materials" in "4. General Rules for Animal-derived Raw Materials of the Standards for Biological Ingredients (Standards for Ruminant animal-derived Raw Materials)," or whether the manufacturing record is kept in accordance with the stipulation of "3. Animal-derived Materials" in "4. General Rules for Animal-derived Materials of the Standards for Biological Ingredients (Standards for Animal-Derived Raw Materials)." Therefore, after marketing approval, the collagenase in question will be replaced with one that conforms to the Standard for Biological Ingredients [see Section 3.3.3.].

Fetal bovine serum (FBS) is derived from the blood of healthy bovine fetuses of Australia or New Zealand origin and was gamma-ray irradiated (≥30 kGy) to inactivate pathogens. FBS has been demonstrated to meet the Standards for Biological Ingredients.

Human serum albumin products granted marketing approval is used.

3.1.3. Manufacturing process development (comparability)

Part of the manufacturing process was changed during the development of the product: During the clinical trial, the medical institution responsible for the preparation of skeletal myoblast-derived cell sheets was regarded as the manufacturing site of the proposed product. The prepared sheets were released as finished products from the institution. However, because of their short shelf-lives and vulnerability to external physical force during transportation, skeletal myoblast-derived cell sheets

cannot be delivered intact across Japan. The applicant therefore adopted a system in which cryopreserved myoblast-derived cells meeting release acceptance criteria are released to medical institutions so that skeletal myoblast-derived cell sheets are prepared in medical institutions where patients undergo transplantation. The secondary constituent parts thus include the culture media and sheet preparation apparatuses used to prepare skeletal myoblast-derived cell sheets in medical institutions. After all, the finished combination product comprise these secondary constituent parts as well as the primary constituent part.

3.1.4. Characterization

3.1.4.1. Characterization of cryopreserved cells

Prepared skeletal myoblast-derived cell sheets were subjected to characterization, while cryopreserved cells were subjected only to tests for specification compliance.

3.1.4.2. Characterization of skeletal myoblast-derived cell sheets prepared from the product

3.1.4.2.1. Assay of physiologically active substances

Using [redacted] obtained during the preparation of skeletal myoblast-derived cell sheets as the test sample, [redacted] types of physiologically active substances expected to be produced by skeletal myoblast-derived cells ([redacted]) were measured by enzyme-linked immunosorbent assay (ELISA). The assay showed the production of hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and stromal cell-derived factor (SDF)-1 [see Section 3.3.2.].

3.1.4.2.2. Changes in the cell morphology during skeletal myoblast-derived cell sheet preparation

During the preparation of skeletal myoblast-derived cell sheets from cryopreserved cells, a high density of cells are cultured. This may cause cell fusion and/or generation of multinucleated cells, and therefore the expression of [redacted] in the cells during cell sheet preparation was investigated. The cells started to show a cobblestone appearance [redacted] hours after the start of cell sheet preparation, with a minority of cells expressing [redacted]. After [redacted] hours, cell fusion and multinucleation became prominent, showing marked myotube formation. After [redacted] hours, the myotubes assumed a fibrous streamline structure.

3.1.5. Evaluation of manufacturing process

3.1.5.1. Test of purity (non-target cells)

Cryopreserved cells may contain non-target cells from raw material, such as endothelial cells, adipocytes, hematopoietic cells, and neurons. According to the applicant, spindle-shaped cells constituted the majority of cell populations during the culture process, and there were no cell populations with epithelial-like morphology. In addition, cell surface antigens on cryopreserved cells were resolved by flow cytometry by using antibodies against [redacted], a marker for skeletal myoblasts, and antibodies against [redacted] expressed specifically on fibroblasts. The flow cytometry revealed that the cryopreserved cells consisted of skeletal myoblasts and fibroblasts, with low contamination by fibroblasts.

3.1.5.2. Cytogenetic stability

Karyotype analysis was performed on cells obtained by enzyme treatment of skeletal myoblast-derived cell sheets prepared from cells during the early culture period (cell-doubling number, approximately [redacted]) and during the late culture period (cell-doubling number, approximately [redacted]). The karyotype of the cells during the early stage of the culture was identical with that of normal cells, whereas in cells in the late stage of culture, trisomy of chromosome [redacted] was observed in 3 of 100 cells in [redacted] of 3 batches.

Colony formation analysis using soft agar colony assay for cells during the late culture period demonstrated no abnormally growth in any of the batches, including the batches that showed chromosomal abnormalities.

3.1.6. Product control

Tables 3.4 and 3.5 show the specifications of the primary constituent part (cryopreserved cells) and the secondary constituent parts [see Section 3.3.1.].

No reference material is defined for either the primary or secondary constituent parts.

Table 3.4. Specifications for the primary constituent part (cryopreserved cells)

Tests	Method
Appearance	Visual inspection
Cell viability	Viable cell counting
[redacted]	[redacted]
Cell surface antigen (percentage of [redacted]-positive cells)	Flow cytometry
Sterility	Membrane filtration method
Mycoplasma	Nucleic acid amplification test method
Bacterial endotoxins	Japanese Pharmacopoeia

Table 3.5. Specifications for secondary constituent parts

Secondary constituent parts	Tests
Skeletal muscle container (filled with tissue transport solution)	Appearance
	pH
	Osmolarity
	Bacterial endotoxins
	Sterility
Culture media (washing solution)	Appearance
	pH
	Osmolarity
	Bacterial endotoxins
	Sterility
Culture media (sheet preparation medium)	Appearance
	pH
	Osmolarity
	Bacterial endotoxins
	Sterility
Sheet preparation apparatuses	Appearance
Serum separation devices	Appearance

3.2. Preparation of skeletal myoblast-derived cell sheets

3.2.1. Evaluation of the preparation method of skeletal myoblast-derived cell sheets

Critical operational parameters for the preparation of skeletal myoblast-derived cell sheets were defined based on the process parameters for skeletal myoblast-derived cell sheet preparation process that were used in the production of clinical trial batches (Table 3.6). An instruction manual for sheet preparation will be made based on these parameters and used to provide information and training for persons who prepare myoblast-derived cell sheets at medical institutions [see Section 3.3.5.]. The marketing authorization holder plans to prepare [redacted] cell sheets in parallel with skeletal myoblast-derived cell sheets to be transplanted into patients at medical institutions. The tests listed in Table 3.6 on the [redacted] sheets will be conducted by the marketing authorization holder.

Table 3.6. Process control parameters for skeletal myoblast-derived cell sheet preparation for clinical trial batches

Control parameters	
Process parameters	[redacted]
Tests for skeletal myoblast-derived cell sheets	Appearance
	Cell viability
	[redacted]
	Percentage of [redacted]-positive cells
	Sterility
	Mycoplasma
	Bacterial endotoxins

3.2.2. Process-related impurities

Among process-related impurities that may remain in prepared skeletal myoblast-derived cell sheets, medium components (FBS, [redacted]), [redacted], antibiotics (gentamicin sulfate and amphotericin B), a component in the stock solution (DMSO), and [redacted] monomer were identified as undesirable substances in terms of safety.

Each skeletal myoblast-derived cell sheet is meant for a single use. Human exposure to the estimated amount of residual process-related impurities pose little safety concern. The applicant thus considers that none of the process-related impurities need to be controlled by specifications. In order to raise the awareness of healthcare professionals, the applicant will inform healthcare professionals of the risk of FBS- and antibiotics-induced allergy and instruct them not to use skeletal myoblast-derived cell sheets in patients with a history of allergy to FBS or the antibiotics [see Section 3.3.4.]. The applicant explained the residual amount of each process-related impurity as shown below.

3.2.2.1. Medium components

3.2.2.1.1. FBS

FBS concentration was determined by ELISA using bovine serum albumin (BSA) as the index and [redacted] as the test sample. The residual BSA concentration was below the detection limit ([redacted] ng/mL).

3.2.2.1.2. [redacted]

[redacted], determined by ELISA using [redacted] as the test sample, was below the lower limit of quantitation ([redacted] pg/mL).

3.2.2.1.3. [redacted]

The residual selenium concentration, determined by [redacted] using [redacted] as the test sample, was below the lower limit of quantitation ([redacted] μg/mL).

3.2.2.1.4. [redacted]

[redacted], upon administration to humans, is rapidly [redacted] and metabolized to [redacted].[2] Therefore, the amount of [redacted] was determined by [redacted] using [redacted] as the test sample. [redacted]

[2] Survey report on the food effect evaluation related to agrochemicals, veterinary medical products, and feed additives for which tentative standards were established pursuant to the enforcement of the positive list system, published by Food Safety Commission of the Cabinet Office [redacted]

[redacted] was below the lower limit of quantitation ([redacted] ng/mL), and the mean [redacted] was [redacted] ng/mL.

3.2.2.2. [redacted]
The residual amount of [redacted] per skeletal myoblast-derived cell sheet at transplantation was evaluated. Taking into account the fact that [redacted] is diluted ≥[redacted] fold after washing, it was estimated to be present at ≤[redacted] nrPu[3] as [redacted], at ≤[redacted] pU as [redacted], and at ≤[redacted] nU as [redacted].

3.2.2.3. Antibiotics
According to the amount of antibiotics used during the manufacturing process, each skeletal myoblast-derived cell sheet contains residual gentamicin sulfate up to [redacted] mg and amphotericin B up to [redacted] mg.

3.2.2.4. Component in stock solution (DMSO)
The residual DMSO concentration in each sheet was estimated to be [redacted] ppm according to the liquid-exchange procedure in preparing skeletal myoblast-derived cell sheets.

3.2.2.5. [redacted] **monomer**
Residual [redacted] monomer concentrations in a temperature-responsive culture dish, determined by [redacted], were below the lower limit of quantitation ([redacted] μg/mL).

3.3. Outline of the review by PMDA
PMDA reviewed the submitted data as shown below. Since part of the data is still under review, the conclusion on the quality control will be described in the Review Report (2).

3.3.1. Verification
Cryopreserved skeletal myoblast-derived cells are derived from patients' own skeletal muscle; therefore the quality control of cryopreserved skeletal myoblast-derived cells are affected by individual differences among patients. Since it is difficult to evaluate whether the range of process parameters is fully optimized to control the individual differences, process validation has not been performed completely on the manufacturing processes. Furthermore, critical quality attributes have not been well identified in terms of the efficacy and safety of the proposed product and skeletal myoblast-derived cell sheets prepared from the product. PMDA thus asked the applicant to establish a verification-based quality control strategy to ensure that every production batch has the required quality, in view of the following points.

- Cryopreserved cells are released to medical institutions; the medical institutions then prepare skeletal myoblast-derived cell sheets and transplant them into patients. The applicant is required to confirm that the to-be-released cryopreserved cells are able to form skeletal myoblast-derived cell sheets of the required quality, by actually preparing skeletal myoblast-derived cell sheets or taking other appropriate measures.

- According to a quality attribute, cryopreserved cells must be skeletal myoblast-derived cells that have not been fully differentiated into muscle cells, instead of cells with myotubes. The muscle differentiation process of skeletal myoblasts is controlled by culture density during production. However, this quality attribute required for cryopreserved cells cannot be confirmed only by the expression of [redacted], which is constantly observed in all stages of muscle differentiation. Multiple markers should therefore be used to identify the stage of muscle differentiation occurring in the cell population.

The applicant's response:
Based on the results of the quality risk assessment, the manufacturing process parameters, in-process control tests, and specifications for the primary constituent part (cryopreserved cells) were re-defined as

[3] rPu (recombinant protease activity unit): 1.0 rPu refers to the amount of the enzyme that changes 1.0 mole of substrate acetyl-arginyl-p-nitroanilide (Ac-Arg-pNA) per minute at pH 8.0 and at 22°C ± 1°C.

shown in Tables 3.7 and 3.8, to ensure the required quality attributes of each production batch. Also, the manufacturing process parameters, in-process control tests, and specifications for the secondary constituent parts were re-defined as shown in Tables 3.9 and 3.10.

The in-process control tests of cryopreserved cells will include the determination of [redacted], which is assumed to change during muscle differentiation. In order to ensure that cryopreserved cells can produce skeletal myoblast-derived cell sheets of the required quality, [redacted] skeletal myoblast-derived cell sheets will be prepared to perform tests on [redacted] this procedure will be included in the specifications or in-process control tests. When a sufficient number of cells are obtained, more additional tests, including [redacted], will be performed.

Table 3.7. Manufacturing process parameters and in-process control tests for the primary constituent part (cryopreserved cells)

Process	Control items
[Step 1] [redacted]	In-process control tests
[Step 2] [redacted]	Control of raw materials
	In-process control tests
[Step 3] [redacted]	In-process control tests
[Step 4] [redacted]	In-process control tests
[Step 5] [redacted]	In-process control tests
[Step 6] [redacted]	Control of raw materials
[Step 7] [redacted]	Control of raw materials
[Step 8] [redacted]	Parameters
	In-process control tests
[Step 9] [redacted]	Parameters
	In-process control test
[Step 10] [redacted]	Parameters

Process		Control items
		In-process control tests
[Step 11]		Parameters
		In-process control tests
		Parameters
		In-process control tests
[Step 12]		Parameters
		In-process control tests
[Step 13]		Parameters
		In-process control test
[Step 14]		In-process control test
[Step 15]		Parameter
		In-process control tests

[redacted]

Table 3.8. Specifications for the primary constituent part (cryopreserved cells)

Tests	Method
Appearance	Visual inspection
Description	Visual inspection
Cell count	Viable cell counting
Cell viability	Viable cell counting
[redacted]	[redacted]
Cell surface antigen (percentage of [redacted]-positive cells)	Flow cytometry
Sterility	Membrane filtration method
Mycoplasma	Nucleic acid amplification test method
Bacterial endotoxins	Japanese pharmacopoeia
Check for skeletal myoblast-derived cell sheet formation	[redacted]

Table 3.9. Manufacturing process parameters and in-process control tests for the secondary constituent parts (skeletal muscle container and culture media)

Secondary constituent parts	Control items	
Preparation of tissue transport solution (skeletal muscle container)	Parameter	[redacted]
	In-process control tests	[redacted]
		Filter integrity
		Container integrity
Preparation of washing solution (culture media [washing solution])	Parameter	[redacted]
	In-process control tests	[redacted]
		Filter integrity
		Container integrity
Preparation of sheet preparation medium (culture media [sheet preparation medium])	Parameter	[redacted]
	In-process control tests	[redacted]
		Filter integrity
		Container integrity

Table 3.10. Specifications for the secondary constituent parts

Secondary constituent parts	Tests	Method
Skeletal muscle container (filled with tissue transport solution)	Appearance	Visual inspection
	Description	Visual inspection
	Identification	[redacted]
	pH	Japanese pharmacopoeia
	Osmolarity	Japanese pharmacopoeia
	Bacterial endotoxins	Japanese pharmacopoeia
	Sterility	Japanese pharmacopoeia
Culture media (washing solution)	Appearance	Visual inspection
	Description	Visual inspection
	Identification	[redacted]
	pH	Japanese pharmacopoeia
	Osmolarity	Japanese pharmacopoeia
	Bacterial endotoxins	Japanese pharmacopoeia
	Sterility	Japanese pharmacopoeia
	Mycoplasma	Nucleic acid amplification test method
Culture media (sheet preparation medium)	Appearance	Visual inspection
	Description	Visual inspection
	Identification	[redacted]
	pH	Japanese pharmacopoeia
	Osmolarity	Japanese pharmacopoeia
	Bacterial endotoxins	Japanese pharmacopoeia
	Sterility	Japanese pharmacopoeia
	Mycoplasma	Nucleic acid amplification test method
Sheet preparation apparatuses	Appearance	Visual inspection
	Bacterial endotoxins	Japanese pharmacopoeia
	Sterility	Check for sterilization parameters[a)]
Serum separation devices	Appearance	Visual inspection
	Bacterial endotoxins	Japanese pharmacopoeia
	Sterility	Check for sterilization parameters

[a)] A sterility test (Japanese pharmacopoeia) is performed for temperature-responsive culture dishes.

PMDA's view:
The proposed product is a human cell-based product derived from the patient's own skeletal muscle. Because of extremely limited experience in the production and clinical use of the product, efficacy- or safety-related critical quality attributes of the product are not yet to be fully determined. However, control parameters likely to potentially affect the quality of the product, as judged by the quality risk assessment based on good understanding of the manufacturing process, include (i) multiple cell markers used to identify the intended skeletal myoblast-derived cell population, (ii) process parameters, such as culture density, that ensure the attributes of skeletal myoblast-derived cells, and (iii) sheet-forming capacity of cryopreserved cells to be released to medical institutions. These parameters are defined to control quality attributes that are probably critical, and are included in the specifications, manufacturing process parameters, and in-process control tests. Accordingly, the verification plan proposed by the applicant will allow the quality of production batches to be consistent with clinical trial batches. PMDA thus accepts the proposed quality control strategy for the proposed product.

Nevertheless, critical quality attributes should be determined based on the evaluation of cumulative production data and the clinical efficacy and safety data; then the specifications for cryopreserved cells should be determined based on the critical quality attributes. The specifications, along with how to secure test samples and the establishment of feasible test methods, should be further discussed based on the results of verification.

3.3.2. Physiologically active substances produced by skeletal myoblast-derived cell sheets

PMDA asked the applicant to explain the relationship between physiologically active substances expected to be produced from skeletal myoblast-derived cell sheets and the efficacy and safety of the sheets based on the results obtained from the production of clinical trial batches [see Section 3.1.4.2.1.], and to explain the applicant's view on this matter including its plans for future investigation.

The applicant's response:
During the production of clinical trial batches, VEGF, HGF, and SDF-1 were produced from skeletal myoblast-derived cell sheets. However, the relationship between the production of these physiologically active substances and the efficacy and safety of skeletal myoblast-derived cell sheets remains unclear. After the market launch, the relationship will be further evaluated by quantitative measure of VEGF, HGF, and SDF-1 using [redacted] recovered from medical institutions.

PMDA's view:
Skeletal myoblast-derived cell sheets are derived from the patient's own skeletal muscle, and they have been produced or used in humans on a small number of occasions. Information should therefore be further collected on the types and volumes of physiologically active substances produced from skeletal myoblast-derived cell sheets, to determine whether they are critical quality attributes.

3.3.3. Safety of adventitious agents

The applicant plans to replace the collagenase currently used in the production of the primary constituent part with one that meets the Standards for Biological Ingredients. The conformity of the new collagenase to the standards and how the applicant deals with the change will be reported in the Review Report (2).

3.3.4. Safety of [redacted], a process-related impurity

PMDA asked the applicant to explain the safety of the estimated human exposure to [redacted] contained in the culture media and the stock solution.

The applicant's response:
The total volume of [redacted] in [redacted] medium, [redacted] medium, and [redacted] is [redacted] μg; the maximum human exposure is estimated to be approximately [redacted] ng. Some of vanadium compounds are reported to be positive in genotoxicity tests, while whether [redacted] is genotoxic or carcinogenic is unknown. Therefore, the safety of [redacted] in humans was evaluated based on the threshold of toxicological concern. The estimated exposure was far below the acceptance level of mutagenic impurities ([redacted] μg/day) corresponding to the excess lifetime cancer risk of 10^{-5}. This suggests a low safety risk in humans.

Given that skeletal myoblast-derived cell sheets prepared from the proposed product are meant for a single use, PMDA accepted the response of the applicant.

3.3.5. Preparation of skeletal myoblast-derived cell sheets at medical institutions

To allow medical institutions to consistently prepare skeletal myoblast-derived cell sheets as intended, persons in charge of sheet preparation should receive a training to become fully educated in the correct procedures. PMDA asked the applicant to explain how to train the persons involved.

The applicant's response:
The applicant prepared an instruction manual for the constituent parts used for sheet preparation. The manual describes procedures for preparing skeletal myoblast-derived cell sheets equivalent to those of the clinical trial batches, and points to consider during sheet preparation. Medical institutions that use the proposed product will receive a training based on the package insert and the instruction manual. The training will require trainees to prepare cell sheets as a simulation to familiarize them with the correct procedures. Persons in charge of sheet preparation will thus receive appropriate training.

PMDA accepted the response of the applicant. Medical institutions will prepare [redacted] cell sheets, along with skeletal myoblast-derived cell sheets to be transplanted into patients. The marketing authorization holder is responsible for testing the cell sheets. The applicant should build a system that allows test results to be provided to the medical institutions and enables the medical institutions to promptly take appropriate measures if the results suggest microbial contamination, etc.

4. Stability

4.1. Stability study on cryopreserved cells

A stability study was performed on cryopreserved cells in liquid nitrogen (gas phase) at ≤−150°C (Table 4.1). Throughout the study period of 135 days, no clear change was detected in the proposed specifications or in sheet-forming capacity.

Accordingly, a shelf life of 135 days has been proposed for cryopreserved cells when stored at ≤−150°C in a cryopreservation container.

Table 4.1. Stability study of cryopreserved cells

Production No.	Storage conditions	Storage period	Storage configuration
[redacted]	≤−150°C	135 days[a)]	Cryopreservation container (polypropylene container with polyethylene cap)

a) [redacted]

4.2. Stability study on skeletal myoblast-derived cell sheets

Skeletal myoblast-derived cell sheets prepared from the proposed product were tested for stability at 15°C, 20°C, and 25°C (Table 4.2). Throughout the study period of [redacted] hours, no clear change was detected in the proposed specifications.

Accordingly, a shelf life of 10 hours has been proposed for skeletal myoblast-derived cell sheets prepared from the proposed product when stored at 15°C to 25°C.

Table 4.2. Stability study of skeletal myoblast-derived cell sheets prepared from the product

Production No.	Storage conditions	Storage period	Storage configuration
[redacted]	15°C, 20°C, 25°C	[redacted] hours	Temperature-responsive culture dish

4.3. Stability study on secondary constituent parts

Stability studies were performed on the culture media and on the skeletal muscle container (filled with tissue transport solution), as shown in Table 4.3. Throughout the study period, no clear change was observed either in the culture media or in the container [see Section 4.4.].

Accordingly, a shelf life of 4 weeks has been proposed for the culture media and a shelf life of 12 weeks for the skeletal muscle container (filled with the tissue transport solution) when stored at 2°C to 8°C. (The culture media and the container are both secondary constituent parts.)

Table 4.3. Stability studies of culture media and skeletal muscle container

	Production No.	Storage conditions	Storage period	Storage configuration
Culture media (culture medium for sheet preparation)	[redacted]	2°C-8°C	4 weeks	Polystyrene container with polyethylene cap
Culture media (washing solution)				
Skeletal muscle container (filled with tissue transport solution)	[redacted]		12 weeks	Skeletal muscle container

4.4. Outline of the review by PMDA

The tissue transport solution filled in the skeletal muscle container was not assessed for the preservative effectiveness in the stability study. PMDA asked the applicant to explain the preservative effectiveness of the solution during the proposed shelf life.

The applicant's response:
The preservative effectiveness of the tissue transport solution was not evaluated in the stability study. The solution, a secondary constituent part, is released to medical institutions, which then send harvested

skeletal muscle in the solution to the manufacturing site. The acceptance tests of the skeletal muscle, performed in the manufacturing site, will therefore include preservative effectiveness test of tissue transport solution, to control the risk of microbial contamination. According to a report in the literature (*ASAIO J*. 2005;51:761-3.), the proposed concentration of gentamicin sulfate, one of the antibiotics contained in the tissue transport solution, is expected to reduce the risk of microbial contamination for up to 3 weeks. Therefore, a shelf life of the tissue transport solution is tentatively defined as 3 weeks, and will be finally determined based on the preservative effectiveness to be evaluated by a future stability study.

The applicant expects the stability of the transport solution to last for approximately 3 weeks based on the published report. Though understandable, PMDA considers that a shelf life should be determined based on a stability study of the secondary constituent parts. PMDA will therefore evaluate the appropriateness of the proposed shelf life of the tissue transport solution based on additional stability data that will be submitted later. PMDA's conclusion and the applicant's response to this matter will be reported in the Review Report (2).

5. Indication or Performance

5.1. Studies to support indication or performance

5.1.1. "Efficacy study on porcine skeletal myoblast-derived cell sheets in mini-pig model of heart failure" (Attached document 4)

The effect of porcine skeletal myoblast-derived cell sheets for improving cardiac function was investigated and premature ventricular contraction was analyzed using a female mini-pig model of chronic heart failure transplanted with [redacted] to [redacted] of the heart [for the evaluation of the risk of ventricular arrhythmia in this study, see Section 7.3.].

In this study, cell sheets prepared from porcine autologous skeletal myoblast-derived cells were transplanted onto the surface of the heart of the mini-pig model of chronic heart failure. The control group consisted of sham-operated animals not transplanted with the porcine cell sheets. Left ventricular end-diastolic area (LVEDA) and left ventricular end-systolic area (LVESA) were measured by echocardiography at baseline (before the preparation of chronic heart failure model), on the day the chronic heart failure model was established, on the day of cell sheet transplantation, and 1, 6, and 13 weeks posttransplantation. At the end of observation (13 weeks posttransplantation), a histopathological examination was performed on the heart and other tissues. According to the applicant, the preparation method of porcine cell sheets was not exactly the same as the manufacturing processes of the proposed product or the preparation method of human skeletal myoblast-derived cell sheets (made from the proposed product). However, the porcine cell sheets satisfied the process control parameters for human skeletal myoblast-derived cell sheet preparation (the parameters used in the production of clinical trial batches) [see Section 3.2.1.].

5.1.1.1. Evaluation by echocardiography

Based on LVEDA and LVESA on echocardiography at each measuring time point, left ventricular fractional area change (FAC), an index of the contractile function of the entire left ventricle, was calculated according to the following equation (Table 5.1).

$$FAC\ (\%) = (LVEDA - LVESA) / LVEDA \times 100$$

Table 5.1. FAC (mean ± standard error [SE]; unit, %)

Group (No. of animals)	Baseline	Day of model establishment	Day of transplantation	1 week posttransplantation	6 weeks posttransplantation	13 weeks posttransplantation
Porcine cell sheet transplantation group (6)	83.8 ± 1.0	41.5 ± 2.8	40.6 ± 2.0	55.3 ± 2.6	55.5 ± 2.3	63.6 ± 4.3
Control group (5)	83.0 ± 1.2	38.4 ± 2.1	38.5 ± 1.5	36.8 ± 1.8	40.8 ± 2.1	37.7 ± 3.1
All (11)	83.4 ± 0.7	40.1 ± 1.8	39.7 ± 1.3			

Also, change in FAC from the date of transplantation (baseline) to 1, 6, and 13 weeks posttransplantation was calculated (Table 5.2). The transplantation group showed increased FAC at 13 weeks, whereas the control group showed no change until 13 weeks.

Table 5.2. Change in FAC from the day of transplantation to 1, 6, and 13 weeks (mean ± SE; unit, %)

Group (No. of animals)	1 week posttransplantation	6 weeks posttransplantation	13 weeks posttransplantation
Porcine cell sheet transplantation group (6)	14.7 ± 2.8[a)]	14.9 ± 2.3[a)]	23.0 ± 2.6[a)]
Control group (5)	−1.7 ± 1.8	2.2 ± 2.6	−0.8 ± 2.4

[a)] Student's t-test between the two groups showed a significant difference (P <0.05).

5.1.1.2. Evaluation by histopathological examination

Immunostaining was performed to detect transplanted cells, using antibodies against [redacted] expressing on the surface of skeletal myoblasts. No [redacted]-positive cells were detected either in the epicardium (the surface of the heart where skeletal myoblast-derived cell sheet was transplanted) or in the myocardial layer. Immunostaining was also performed to evaluate angiogenesis, using antibodies against [redacted] expressing on the surface of [redacted] cells. No clear difference was observed between the transplantation group and the control group.

The fibrotic state of cardiac tissue was investigated by Masson's trichrome staining. The thickness and area of fibrosis in the cardiac muscle were smaller in the transplantation group than in the control group. The number of animals retaining the cardiac muscle on the heart wall was larger in the transplantation group than in the control group. The fractional area[4] of the fibrosis region in the cross section of the heart tended to be smaller in the transplantation group (12.2% ± 2.4%; mean ± SE) than in the control group (16.4% ± 2.1%; mean ± SE).

5.1.1.3. Efficacy evaluation of skeletal myoblast-derived cell sheets and the mechanism of cardiac function improvement

Neither engraftment nor differentiation of porcine cell sheets was observed in the cardiac tissue at 13 weeks posttransplantation, and increased FAC was observed in the transplantation group from 1 week posttransplantation. The applicant therefore considered that the improvement in cardiac function by skeletal myoblast-derived cell sheet transplantation, as shown by increased FAC, was not due to the long-term engraftment or differentiation of the transplanted cells, and that some sort of initial reaction of the transplanted cells played an important role in the improvement.

5.2. Outline of the review by PMDA

PMDA asked the applicant to explain the mechanism of skeletal myoblast-derived cell sheets for improving cardiac function, based on the results of quality tests conducted so far, published reports, etc.

The applicant's response:
In light of the observations 1) and 2) below, the effect of skeletal myoblast-derived cell sheets for improving cardiac function is considered due to angiogenic and antifibrotic effects on the cardiac tissue exerted by physiologically active substances (e.g., HGF, VEGF, and SDF-1) produced from the transplanted cells.

1) Physiologically active substances expected to be produced from skeletal myoblast-derived cell sheets was measured on the medium obtained during the preparation of skeletal myoblast-derived cell sheets as the test sample [see Section 3.1.4.2.1.]. The test measured [redacted] types of physiologically active substances expected to be produced from skeletal myoblasts, showing the production of HGF, VEGF, and SDF-1. However, in the efficacy study using the mini-pig model of heart failure, these humoral factors were not measured because of the lack of measurable materials (e.g., antibodies against porcine physiologically active substances) necessary for the evaluation. Therefore, it is unclear whether these physiologically active substances were produced from the transplanted skeletal myoblast-derived cell sheets.

2) In a rat model of myocardial infarction transplanted with skeletal myoblast-derived cell sheets (derived from syngeneic rats), increased expression of HGF and VEGF, physiologically active substances with angiogenic and antifibrotic activity, were seen on the heart at 1 week

[4] Fractional area of fibrosis region (%) = (stained area / cross sectional area) × 100

posttransplantation *(J Thorac Cardiovasc Surg.* 2005;130:1333-41). In an *in vitro* system, SDF-1 was expressed by human skeletal myoblast-derived cell sheets (*Eur J Heart Fail.* 2008;10:1065-72).

PMDA's view:
The submitted study data do not provide any direct evidence that physiologically active substances such as HGF, VEGF, and SDF-1 were produced from the transplanted skeletal myoblast-derived cell sheets and contributed to the promotion of angiogenesis and the suppression of fibrosis in the heart tissue. Thus much remains unknown about the detailed mechanism of the pharmacological effect of physiologically active substances produced from the proposed product. However, the efficacy study using the mini-pig model of heart failure yielded results suggesting improved cardiac function, such as increased FAC and reduced fibrosis, in the group transplanted with cell sheets prepared using porcine autologous skeletal myoblast-derived cell. This suggests that skeletal myoblast-derived cell sheets prepared from the proposed product may contribute to improved cardiac function.

6. Biodistribution of the Product

6.1. Summary of the submitted data

No study was conducted to evaluate the biodistribution of skeletal myoblast-derived cell sheets prepared from the proposed product. Based on the results of the "Systemic toxicity study using superimmunodeficient mice (NOG mice)" (Attached document 6-a) and the "Efficacy study using the mini-pig model of heart failure" (Attached document 4), the applicant explained the engraftment, subsequent changes, and distribution (engraftment, etc.) of the transplanted cells as follows:
In the systemic toxicity study, human skeletal myoblast-derived cell sheets, fitted to the heart size of each animal, were transplanted onto the heart of NOD/Shi-*scid*, IL-2Rγ^{null} mice (NOG mice), and a histopathological examination was performed on the heart at 1 month posttransplantation. Engraftment, etc. of the transplanted human skeletal myoblast-derived cell sheets were checked by immunostaining using anti-human [redacted] antibodies. Human [redacted]-positive cells were observed sporadically at the site of adhesion in the mildly or very mildly fibrotic pleura or within the thoracic cavity in the transplantation group, whereas no human [redacted]-positive cells were detected in the heart. Also, the study showed no evidence for the transfer or engraftment of skeletal myoblast sheet-derived cells to tissues other than the pleura or the thoracic cavity.

In the efficacy study using the mini-pig model of heart failure, a histopathological examination at 13 weeks after porcine cell sheet transplantation showed no porcine cell sheets remaining on the heart, the site of transplantation.

Based on these results, the applicant considered that skeletal myoblast-derived cell sheets are unlikely to engraft onto the surface of the heart, where they were transplanted, for an extended period or to affect tissues or organs other than the transplanted site in humans.

6.2. Outline of the review by PMDA

Human [redacted]-positive cells were sporadically observed at the adhesion site after the transplantation of human skeletal myoblast-derived cell sheets into NOG mice. PMDA asked the applicant to discuss the engraftment of cells after transplantation in humans, taking account of the difference in the transplantation procedure between the non-clinical toxicity study in NOG mice and the clinical practice.

The applicant's response:
In NOG mice, human [redacted]-positive cells were scattered sparsely and locally in the loose connective tissue at the adhesion site of the pleura or thoracic cavity. The pericardial membrane of mice, unlike that of humans, is fragile and cannot be sutured. Therefore, in the nonclinical toxicity study in NOG mice, the chest was closed without the closure of the pericardial membrane after the transplantation of skeletal myoblast-derived cell sheets. As a consequence, the skeletal myoblast-derived cell sheets were prone to move from the transplantation site on the surface of the heart, which could have resulted in the detection of human [redacted]-positive cells at the site of adhesion in the pleura or in the pleural cavity. In the efficacy study using the mini-pig model of heart failure, the chest was

closed after the closure of the pericardial membrane after the transplantation of porcine cell sheets, as is the case with the transplantation procedure in humans. This non-clinical study in mini pigs showed no engraftment of the cell sheets.

The non-clinical studies thus showed no evidence for prolonged engraftment of skeletal myoblast-derived cell sheets on the heart (the transplantation site). This suggests that engraftment is unlikely to be prolonged in humans. In the clinical studies conducted in Osaka University (Studies MP0604 and HM0801, Reference data 7-1, 7-2), biopsy performed at several months or years after skeletal myoblast-derived cell sheet transplantation showed no engraftment of the skeletal myoblast-derived cell sheets.

PMDA confirmed that the non-clinical studies showed no prolonged engraftment of skeletal myoblast-derived cell sheets at the transplantation site, and accepted the discussion of the applicant.

7. Nonclinical Safety Data

The applicant submitted non-clinical safety study data, namely the results of the systemic toxicity study and the tumorigenicity study in NOG mice, karyotype analysis, and a soft agar colony assay. The efficacy study in mini-pig model of heart failure evaluated the risk of ventricular arrhythmia after porcine skeletal myoblast-derived cell sheet transplantation.

7.1. Systemic toxicity study (Attached document 6-a)

A skeletal myoblast-derived cell sheet prepared from the proposed product (1.2×10^6 cells/animal [6×10^7 cells/kg]) was transplanted, in an amount corresponding to 10 times that of the clinical dose (6×10^6 cells/kg), onto the surface of the heart of male and female NOG mice. A sham-operation group not receiving skeletal myoblast-derived cell sheet transplantation and a no-treatment group were used as control groups. Skeletal myoblast-derived cell sheet transplantation had no effect on the systemic conditions immediately after transplantation. Necropsy showed adhesion between the heart and the thoracic wall and between the lung and the heart in females receiving a skeletal myoblast-derived cell sheet at 4 weeks posttransplantation. Histopathological examination showed increased frequency of fibrosis in the pleura and at the adhesion site, and human [redacted]-positive cells (human skeletal myoblasts) were observed at the adhesion site. No changes associated with skeletal myoblast-derived cell sheet transplantation were observed in other tissues or organs of the body.

7.2. Tumorigenicity studies

7.2.1. Tumorigenicity study in immunodeficient mice (Attached document 6-b-1)

[redacted][5] (3.0×10^7 cells/animal [1.5×10^9 cells/kg]), obtained by [redacted] from [redacted] prepared from over-cultured skeletal myoblasts,[6] was transplanted subcutaneously to male NOG mice. At 3 months posttransplantation, histopathological examination did not show tumor formation at the transplantation site, lung, liver, kidneys, or spleen.

7.2.2. Soft agar colony formation assay (Attached document 6-b-2)

[redacted][7] prepared from over-passaged[5] skeletal myoblasts was seeded onto soft agar plates and cultured for 3 weeks. Anchorage-independent growth were not observed in any colonies.

7.2.3. Karyotype analysis (Attached document 6-b-3)

[redacted][8] prepared from over-passaged[5] skeletal myoblasts was subjected to evaluation of the number of chromosomes, sex chromosomal constitution, and G band pattern. In [redacted] of 3 batches of the test substance (production No. [redacted]), no chromosomal aberration was observed during the early stage of culture (cell-doubling number, [redacted]), whereas trisomy of chromosome [redacted] was observed in 3 of 100 cells at the end of the over-culture.

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[5] Cells were over-passaged to a cell-doubling number of approximately [redacted] beyond the specified cell-doubling number ([redacted]).

[6] A total of [redacted] batches were used for the test. Of these, [redacted] prepared from the sample (batch No. [redacted]) that showed trisomy of chromosome [redacted] in karyotype analysis (Section 7.2.3).

[7] A total of [redacted] batches were used for the test. Of these, [redacted] prepared from the sample (batch No. [redacted]) that showed trisomy of chromosome [redacted] in karyotype analysis (Section 7.2.3).

[8] Production No. [redacted], [redacted], and [redacted]

7.3. Evaluation of effect on the heart in mini-pig model of heart failure (Attached document 4)

In mini-pig model of heart failure, porcine cell sheets were transplanted onto the heart of the pigs. The occurrences of premature ventricular contraction were investigated using the results of electrocardiography (ECG) at 1, 6, and 13 weeks posttransplantation. Premature ventricular contraction of Lown grade[9] ≥3 were not observed at any time point in the porcine cell sheet transplantation group. The histopathological examination of the heart at 13 weeks posttransplantation did not show any toxicological changes caused by cell sheet transplantation.

7.4. Outline of the review by PMDA

7.4.1. Effect on fibrosis of the pleura and the adhesion site

The systemic toxicity study in NOG mice showed an increased frequency of fibrosis in the pleura and the adhesion site, and skeletal myoblast-derived cell sheet-derived cells were observed in these sites. PMDA asked the applicant to explain the safety of skeletal myoblast-derived cell sheet transplantation in humans.

The applicant's response:
Since skeletal myoblast sheet-derived cells were observed at the adhesion site, a possible relationship between skeletal myoblast-derived cell sheets and the fibrosis of the adhesion site cannot be excluded. However, observation of general conditions did not reveal any effect on respiration in the systemic toxicity study. Also, the clinical trial of skeletal myoblast-derived cell sheets did not reveal any adhesion-induced adverse events of the cardiopulmonary function. In humans, skeletal myoblast-derived cell sheets are transplanted onto the surface of the heart without left lung ventilation, then the pericardial membrane is sutured. The heart and the lung are therefore unlikely to adhere to the thoracic cavity together with skeletal myoblast-derived cell sheets. Thus, the increased frequency of fibrosis at the adhesion site in the thoracic cavity observed in the toxicity study will not pose any safety concerns in humans.

PMDA accepted the response of the applicant.

7.4.2. Chromosomal aberrations observed upon extended passage

The applicant's explanation on the trisomy of chromosome [redacted] observed in the over-passaged skeletal myoblasts and the tumorigenic risk in humans:
There is no report that suggests the presence of cells with chromosomal aneuploidy in the skeletal muscle of healthy individuals. Tumorigenicity was not indicated in the soft agar colony assay in cell batches with chromosomal aberration or in the tumorigenicity study in NOG mice. Accordingly, humans have a low risk of tumorigenicity due to the observed chromosomal aberrations.

PMDA accepted the response of the applicant.

7.4.3. Effect of skeletal myoblast-derived cell sheet transplantation on the heart

The applicant's explanation on the safety of skeletal myoblast-derived cell sheet transplantation onto an infarcted site of the heart in humans:
The efficacy study in the mini-pig model of heart failure evaluated the effect of skeletal myoblast-derived cell sheets on the heart in pigs. Grade ≥3 premature ventricular contraction[9] with a high risk of ventricular fibrillation were not observed in the porcine cell sheet transplantation group. Transplantation of skeletal myoblast-derived cell sheets to the infarcted site is thus unlikely to cause any safety concerns of the heart.

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[9] Grade 0, no premature ventricular contraction; Grade 1, sporadic unifocal premature ventricular contraction (<30 premature ventricular contraction per hour); Grade 2, sporadic unifocal premature ventricular contraction (≥30 premature ventricular contraction per hour); Grade 3, polymorphic premature ventricular contraction; Grade 4a, coupled repetitive premature ventricular contraction; Grade 4b, repetitive triplets or more of premature ventricular contraction (≥3 premature ventricular contraction); Grade 5, R on T premature ventricular contraction

[10] Class I: Cardiac disease with no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or angina.

PMDA asked the applicant to explain the appropriateness of the observation period to evaluate the effect of porcine skeletal myoblast sheets on the heart in the mini-pig model of heart failure.

The applicant's explanation:
The ECG performed during the 13-week observation period after porcine skeletal myoblast sheet transplantation did not show increased risk of ventricular arrhythmia. The histopathological examination performed at 13 weeks posttransplantation did not reveal remaining porcine skeletal myoblast sheets or any changes of toxicological significance. The effect of transplantation of porcine skeletal myoblast sheets to the site of myocardial infarction was thus adequately evaluated during the 13-week observation period.

PMDA accepted the response of the applicant and concluded that skeletal myoblast-derived cell sheets had no particular problems in nonclinical safety.

8. Clinical Data

The applicant submitted efficacy and safety evaluation data (the results of a Japanese clinical trial) and reference data (the results of 2 Japanese clinical studies).

8.1. Japanese clinical trial (Attached document 7, Study M-51073-21 [[redacted] to [redacted]])

A multi-center, open-label, uncontrolled study was conducted in patients aged ≥20 years with severe heart failure due to ischemic heart disease (target sample size, 6) in 3 medical institutions in Japan. The purpose of the trial was to confirm the appropriateness of efficacy evaluation method, the safety of skeletal myoblast-derived cell sheets, and the feasibility of skeletal myoblast-derived cell sheet transplantation at multiple medical institutions.

The main inclusion criteria were as follows: 1) patients with chronic ischemic heart disease; 2) patients with NYHA class[10] III or IV heart failure; 3) patients who remained in heart failure status despite maximal drug therapy, including digitalis, diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), β-blockers, aldosterone antagonists, and oral inotropic agents; 4) patients aged ≥20 years; 5) patients at risk of worsening heart failure despite having received standard-of-care therapy ≥3 months earlier (e.g., CABG, mitral valvuloplasty, left ventricular restoration, CRT, and percutaneous coronary intervention [PCI]); and 6) patients with resting LVEF ≤35% by echocardiography. Patients who met all of these criteria were enrolled in the trial. Patients undergoing thyroid hormone therapy, patients who remained in shock because of worsening heart failure, and patients with severe pulmonary hypertension were excluded from the trial.

Table 8.1 shows the baseline characteristics of 7 subjects from whom skeletal muscle was harvested and who were transplanted with skeletal myoblast-derived cell sheets prepared from the proposed product.

Table 8.1. Patient characteristics

Subject ID	Age	Sex	Onset date of primary disease	Heart-related complications	Treatment history of heart disease	Drug therapy	NYHA class[a)]	LVEF (echocardiography)[a]
T01-01	35	Man	May 2007	-	PCI	• ACE inhibitor • β-Blocker • Diuretic	III	33
T01-03	61	Man	June 2008	-	• PCI • CABG	• ACE inhibitor • β-Blocker • Aldosterone antagonist • Vasodilator (isosorbide dinitrate)	III	31
T02-01	71	Man	June 1986	• Nonsustained ventricular tachycardia • Sleep apnea syndrome	• PCI • CABG • Implantable cardioverter defibrillator	• ACE inhibitor • β-Blocker • Diuretic • Aldosterone antagonist	III	22
T03-01	58	Man	1998	• Hypothyroidism • Supraventricular tachycardia	• PCI • CABG • Intraaortic balloon pumping	• ARB • β-Blocker • Inotropic agent • Anti-arrhythmic • Diuretic • Aldosterone antagonist	III	27.9
T01-04	71	Man	June 1992	• Chronic atrial fibrillation	• PCI • CABG	• ACE inhibitor • β-Blocker • Diuretic • Aldosterone antagonist	III	33
T02-02	53	Man	August 2000	• Nonsustained ventricular tachycardia • Sleep apnea syndrome	• PCI • CABG • Cardiac resynchronization therapy defibrillator (CRT-D)	• ACE inhibitor • β-Blocker • Inotropic agent • Anti-arrhythmic • Diuretic • Aldosterone antagonist • Vasodilator (isosorbide dinitrate)	III	31
T01-05	45	Man	July 2012	-	• CABG • Valvuloplasty	• β-Blocker • Anti-arrhythmic • Diuretic • Aldosterone antagonist	III	27

[a)] At eligibility screening

[10] Class I: Cardiac disease with no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or angina.
Class II: Cardiac disease with slight limitation of physical activity. Ordinary physical activity results in fatigue, palpitation, dyspnea, or angina.
Class III: Cardiac disease with marked limitation of physical activity. Less than ordinary activity causes fatigue, palpitation, dyspnea, or angina.
Class IV: Cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or angina may occur even at rest. If any physical activity is undertaken, discomfort increases.

Shown below are the methods for harvesting skeletal muscle and preparing skeletal myoblast-derived cell sheets, and the dosage and administration or method of use for the proposed product.

Harvest of skeletal muscle and preparation of skeletal myoblast-derived cell sheets
Skeletal muscle (approximately 2-5 g) is aseptically harvested from the quadriceps or brachium of the subject by the investigator or the subinvestigator at least 7 weeks before transplantation. The harvested skeletal muscle is made into cryopreserved cells by marketing authorization holder, and the cells are delivered to medical institutions to be prepared into skeletal myoblast-derived cell sheets.

Dosage and administration or method of use
Five skeletal myoblast-derived cell sheets (containing 3×10^8 cells) are transplanted onto the surface of the heart exposed by left thoracotomy. No other concomitant cardiac surgery should be performed. The skeletal myoblast-derived cell sheets should be used only once because skeletal myoblast-derived cell sheet transplantation involves a thoracotomy.

The primary efficacy endpoint was change in LVEF on cardiac pool scintigraphy from baseline to 26 weeks posttransplantation. Table 8.2 shows the results. According to the pre-defined criteria for improvement based on change in LVEF ("worsened," "unchanged," "improved," Table 8.3), 2 subjects were rated as "worsened," 5 subjects as "unchanged," and 0 subjects as "improved." Since the subjects in this trial had severe heart failure likely to worsen over time, responders were defined as those rated as "unchanged" or "improved." In total, 5 of 7 subjects were classified as responders.

Table 8.2. Change in LVEF from baseline to 26 weeks posttransplantation (cardiac pool scintigraphy)

Subject ID	T01-01	T01-03	T02-01	T03-01	T01-04	T02-02	T01-05
Baseline	24	47	16.5	24	29	34	33
26 weeks posttransplantation	26	37	20	28	25	31	35
Change (%)	2.0	−10.0	3.5	4.0	−4.0	−3.0	2.0
Judgment	Unchanged	Worsened	Unchanged	Unchanged	Worsened	Unchanged	Unchanged

Table 8.3. Criteria for improvement (LVEF measurement by cardiac pool scintigraphy)

	Worsened	Unchanged	Improved
LVEF (%)	<3 decrease	≥3 decrease and <5 increase	≥5 increase

The secondary efficacy endpoints included procedural success (success or failure of transplantation[11]), the contractile function of the entire left ventricle (LVEF) at 26 weeks posttransplantation (echocardiography, cardiac CT), severity of heart failure (NYHA classification, specific activity scale [SAS]), 6-minute walk distance, peak oxygen uptake (peak VO_2), and anaerobic threshold (AT).

Table 8.4 shows the results of LVEF determined by echocardiography and cardiac CT.

Table 8.4. Change in LVEF from baseline to 26 weeks posttransplantation (echocardiography, cardiac CT)

	Echocardiography			Cardiac CT		
Subject ID	Baseline	26 weeks posttransplantation	Change	Baseline	26 weeks posttransplantation	Change
T01-01	21	26	5.0	24.2	26.8	2.6
T01-03	33	42	9.0	33.9	33.6	−0.3
T02-01	22	32	10.0	20.5	22	1.5
T03-01	24	30	6.0	15.3	22.1	6.8
T01-04	27	31	4.0	13.1	-	-
T02-02	27	32	5.0	27.2	27.3	0.1
T01-05	28	39	11.0	20.7	21.7	1.0

Changes in exercise tolerance are shown in Table 8.5. All subjects were classified as the NYHA Class III at baseline. At 6 weeks posttransplantation, 2 subjects (29%) were classified as Class I, 4 subjects

[11] The outcome was determined as "success" if all 5 skeletal myoblast sheets (derived from the clinical trial batches) were successfully transplanted to the heart and, otherwise, as "failure."

(57%) as Class II, and 1 subject (14%) as Class III, showing improvement in 6 of 7 subjects. SAS score improved by ≥1 metabolic equivalent (MET) in 3 subjects (43%) and remained unchanged in 4 subjects (57%) from baseline to 26 weeks posttransplantation. In 2 subjects, six-minute walk distance increased by ≥45 m from baseline to 26 weeks posttransplantation. Peak VO_2 and AT improved in some subjects but worsened or could not be determined in others, precluding accurate evaluation.

Table 8.5. Changes in exercise tolerance between before and after skeletal myoblast-derived cell sheet transplantation

Subject ID	Evaluation time point	NYHA class	SAS (METs)	6-minute walk distance	Peak VO_2	AT
T01-01	Baseline	III	3-4	485	19.4	10.5
	13 weeks posttransplantation	II	3-4	-	-	-
	26 weeks posttransplantation	II	6-7	520	16.5	8.6
T01-03	Baseline	III	4-5	400	20.4	11.3
	13 weeks posttransplantation	I	6-7	-	-	-
	26 weeks posttransplantation	I	6-7	570	21.1	12
T02-01	Baseline	III	4	486	12.6	10.1
	13 weeks posttransplantation	II	5	-	-	-
	26 weeks posttransplantation	III	4	462	7.6	7.0
T03-01	Baseline	III	4	264	9.1	6.7
	13 weeks posttransplantation	II	4	-	-	-
	26 weeks posttransplantation	I	4	478	13.3	7.7
T01-04	Baseline	III	4-5	285	7.8	5.4
	13 weeks posttransplantation	II	4-5	-	-	-
	26 weeks posttransplantation	II	4-5	291	-	-
T02-02	Baseline	III	4	640	11	8.3
	13 weeks posttransplantation	II	6	-	-	-
	26 weeks posttransplantation	II	5	530	14.5	11
T01-05	Baseline	III	4-5	311	-	-
	13 weeks posttransplantation	I	4-5	-	-	-
	26 weeks posttransplantation	II	4-5	337	-	-

The safety analysis revealed that the incidence of adverse events in this trial was 100% (7 of 7 subjects). A total of 6 non-fatal arrhythmia occurred in 71.4% (5 of 7 subjects) and were considered due to the primary disease, the transplantation surgery, or concomitant drugs. A causal relationship to skeletal myoblast-derived cell sheets was ruled out for all events. Other frequent adverse events were wound complication (57.1%, 4 of 7 subjects), hypokalaemia (42.9%, 3 of 7 subjects), and postoperative fever (42.9%, 3 of 7 subjects) (Table 8.6).

Three serious adverse events occurred in 3 subjects (2 events of heart failure in 2 subjects, 1 event of colon cancer in 1 subject). A causal relationship to skeletal myoblast-derived cell sheets could not be ruled out for heart failure in 1 of the 2 subjects. The other heart failure was considered by the subject's attending physician to be unrelated to skeletal myoblast-derived cell sheets but due to effects from surgery, inappropriate drug administration, and daily habits of the subject. The subject experiencing colon cancer showed anemic symptoms at the visit for the prescribed test during the trial, and a subsequent endoscopic examination revealed suspected colon cancer. Histopathological examination identified it as a primary cancer, then a causal relationship of colon cancer to skeletal myoblast-derived cell sheets was ruled out. The subject underwent a right hemicolectomy.

Adverse events caused by skeletal muscle harvesting, namely wound complication and post-procedural swelling, occurred in 28.6% (2 of 7) of the subjects.

Table 8.6. Adverse events or product-related adverse events occurring in ≥2 subjects

Event	Adverse events (n = 7)	Product-related adverse events (n = 7)
Arrhythmia	5 (71.4%)	0
Wound complication	4 (57.1%)	0
Hypokalaemia	3 (42.9%)	0
Postoperative fever	3 (42.9%)	0
Urine abnormality	2 (28.6%)	0
Blood pressure decreased	2 (28.6%)	0
Anaemia	2 (28.6%)	0
Post procedural infection	2 (28.6%)	0
Neutrophil count increased	2 (28.6%)	0
Cardiac failure	2 (28.6%)	1 (14.3%)

Abnormal vital signs observed were noted. Blood pressure decreased occurred in 2 subjects (mild and moderate in 1 subject each). Pyrexia and pulse rate increased occurred after transplantation of skeletal myoblast-derived cell sheets.

8.2. Reference data

8.2.1. Japanese clinical study (Reference data 7-1, Study MP0604 [[redacted] to [redacted]])

A clinical study was conducted to evaluate the safety and cardiac function-improving effect of autologous skeletal myoblast-derived cell sheet transplantation in combination with LVAD in patients aged ≥15 and <70 years who had end-stage dilated cardiomyopathy with persistent severe heart failure unresponsive to maximal drug therapy or surgical treatment (target sample size, 6) at a single medical center in Japan. All 4 patients who underwent skeletal myoblast-derived cell sheet transplantation had NYHA class IV cardiac failure at enrollment.

A total of 169 adverse events occurred during the study, and a causal relationship to skeletal myoblast-derived cell sheets could not be ruled out for 68 of these events. Major adverse events included pain and local haemorrhage, most of which were considered attributable to femoral muscle harvesting or thoracotomy performed before skeletal myoblast-derived cell sheet transplantation. A total of 3 serious adverse events (acute appendicitis, cerebral infarction, and embolism [splenic, renal]) occurred in a single subject. A causal relationship to skeletal myoblast-derived cell sheets could not be ruled out for embolism (splenic, renal), but the subject had a favorable clinical course after LVAD exchange.

After the completion of the study, 2 of the 4 subjects were weaned from LVAD because they showed improved left ventricular systolic function and decreased left ventricular volume, thus meeting the weaning criteria. Subsequently, one of the 2 subjects received LVAD support again because of postoperative complication and eventually underwent heart transplantation. The other subject underwent heart transplantation after the completion of the follow-up period. NYHA classification remained unchanged throughout the follow-up period.

8.2.2. Japanese clinical study (Reference data 7-2, Study HM0801 [[redacted] to [redacted], [redacted] (date of database lock)])

A clinical study was conducted to evaluate the safety, efficacy, and feasibility of transplantation of autologous skeletal myoblast-derived cell sheets in patients aged ≥20 and <75 years with severe cardiomyopathy (target sample size; 8 patients with dilated cardiomyopathy, 8 patients with ischemic cardiomyopathy) in a single medical center in Japan. Of 15 patients transplanted with skeletal myoblast-derived cell sheets (7 patients with dilated cardiomyopathy, 8 patients with ischemic cardiomyopathy), only 1 patient with dilated cardiomyopathy was classified as NYHA class IV at enrollment and all other patients as class III.

A total of 1041 adverse events were reported during the study. Major adverse events were 333 hematologic events (blood biochemistry), 212 hematologic events (haematology test), 144 cardiorespiratory events, 113 hepatic events, and 100 urologic events. Most of the adverse events for which a causal relationship to skeletal myoblast-derived cell sheets could not be ruled out were due to thoracotomy for skeletal myoblast-derived cell sheet transplantation or muscle harvest from the quadriceps. Six serious adverse events occurred in 5 subjects: heart failure aggravated (3 events in 3 subjects), appendicitis (1 event in 1 subject), stenosis of the diagonal branch of the left anterior

descending artery (1 event in 1 subject), and nonpersistent ventricular tachycardia (1 event in 1 subject). A causal relationship to skeletal myoblast-derived cell sheets could not be ruled out for 4 events in 4 subjects, i.e., heart failure aggravated (2 events in 2 subjects), stenosis of the diagonal branch of the left anterior descending artery, and nonpersistent ventricular tachycardia. All of the 4 events resolved. Neither death nor fatal arrhythmia occurred. A total of 13 subjects[12] (6 subjects with dilated cardiomyopathy, 7 subjects with ischemic cardiomyopathy) survived (as of [redacted]) after skeletal myoblast-derived cell sheet transplantation, and none of them underwent heart transplantation or LVAD implantation. After the end of the study, 1 subject died due to a heart-related problem caused by excessive activity and another due to gastrointestinal haemorrhage, both at approximately 2 and a half years posttransplantation.

Some patients showed time-dependent changes in left ventricular wall motion, and improvement in LVEF, left ventricular internal dimension in diastole (LVDd), and left ventricular internal dimension in systole (LVDs). At the final observation (24 weeks posttransplantation), 11 subjects showed improvement in NYHA classification, and 7 subjects were classified as NYHA class I, 4 subjects as class II, 1 subject as class III, and 1 subject as class IV.

8.3. Outline of the review by PMDA

8.3.1. Clinical positioning of skeletal myoblast-derived cell sheets

The applicant's explanation on the indication and clinical positioning of skeletal myoblast-derived cell sheets:

The skeletal myoblast-derived cell sheets are intended for the use in patients with ischemic heart disease-induced severe chronic heart failure corresponding to Goodlin et al's classification (*J Am Coll Cardiol.* 2009;54:386-96) of phase 3 (functional status declines with variable slope; intermittent exacerbations of heart failure that respond to rescue efforts) who are at risk of worsening heart failure despite standard therapies such as drug therapy, PCI, CABG, mitral valvuloplasty, left ventriculoplasty, or CRT.

The standard therapies for chronic heart failure include symptomatic therapy with digitalis, diuretics, etc., and pharmacotherapy with ACE inhibitors, ARBs, β-blockers, and aldosterone antagonists to control disease progression (Guidelines for diagnosis and treatment of cardiovascular diseases established by the Japanese Circulation Society [2009 joint working group report]: Guidelines for the treatment of chronic heart failure [2010 revised edition], *Circulation*. 2009;119:e391-479). CRT and surgical treatment are attempted in patients with severe heart failure who do not respond to standard therapies; CRT has been shown to improve prognoses, quality of life (QOL), and exercise tolerance. For patients with advanced heart failure unresponsive to these therapies, the only radical therapeutic option is heart transplantation. In Japan, however, only a few patients receive heart transplantation after a long waiting time because of a limited number of donors. Most patients thus use an LVAD while waiting for heart transplantation (*Gen Thorac Cardiovasc Surg*. 2012;60:639-44), but LVAD has been recognized as a bridge to heart transplantation, not as an alternative therapy to heart transplantation. In this situation, skeletal myoblast-derived cell sheets are expected to control the progression of heart failure and to be a new therapeutic option that allows patients to avoid interventions such as LVAD implantation and heart transplantation.

PMDA's view:

Chronic heart failure is a progressive disease. In some patients, symptoms of heart failure are difficult to control despite the standard drug therapy, treatment for the underlying disease of heart failure (revascularization for ischemic heart disease, surgery for valvular disease, etc.), or medical device therapy such as CRT. For severe heart failure in this stage, heart transplantation is the only radical therapy available. According to the applicant, skeletal myoblast-derived cell sheets are an innovative regenerative medical product with a mechanism different from the conventional therapies, and can be used in patients with advanced severe heart failure to control disease progression and avoid LVAD implantation or heart transplantation. Although this explanation is reasonable, the use of skeletal myoblast-derived cell sheets involves a thoracotomy in patients at high risk of operative stress, and limited data are available for the efficacy and safety of skeletal myoblast-derived cell sheets. Thus

[12] Among subjects who received skeletal myoblast transplantation, 2 subjects were lost to follow-up during the study period and were therefore withdrawn from the study.

whether skeletal myoblast-derived cell sheets are suitable for individual patients should be carefully determined [for the indication or performance of the proposed product, see Section 8.3.4.].

8.3.2. Efficacy

8.3.2.1. Clinical data package

The applicant's explanation on the background leading to this application for approval based on the results of Study M-51073-21 (the Japanese clinical trial):
The Japanese clinical trial was planned based on the results of the preceding Japanese clinical studies on the treatment with skeletal myoblast-derived cell sheets. The purpose of the trial was to evaluate in an exploratory manner, the efficacy and safety of skeletal myoblast-derived cell sheets in patients with severe heart failure due to ischemic heart disease without concurrent use of LVAD. Given the seriousness and poor prognosis of severe heart failure and inevitable operative stress associated with the transplantation of skeletal myoblast-derived cell sheets, the use of a control group was difficult. Therefore, an open-label, uncontrolled trial was designed with strict eligibility criteria so that the trial would include only patients with persisting heart failure despite adequate treatment with conventional therapies. The trial aimed at confirming the appropriateness of the efficacy evaluation method. LVEF determined by cardiac pool scintigraphy was used as an index for improvement and was defined as the primary efficacy endpoint. Other efficacy endpoints included cardiac function evaluation (echocardiography and cardiac CT), exercise tolerance, etc., to evaluate the efficacy of skeletal myoblast-derived cell sheets from multiple aspects and to assess the clinical significance of the efficacy results obtained. Since the trial suggested the efficacy of skeletal myoblast-derived cell sheets, it is important to offer access to skeletal myoblast-derived cell sheets to patients with severe heart failure unresponsive to conventional therapies. The applicant therefore created a clinical data package including the results of Japanese clinical studies as reference data.

PMDA reviewed the efficacy of skeletal myoblast-derived cell sheets according to the following policy:
The Japanese clinical trial was initially designed for an exploratory purpose. The trial had no control group and enrolled only 7 patients. There was no established efficacy evaluation method. For these reasons, the trial had limited ability to evaluate efficacy. Nevertheless skeletal myoblast-derived cell sheets, an autologous regenerative medical product, have a potential therapeutic effect produced by a mechanism different from conventional drugs or surgical procedures. It is therefore important to make this new therapeutic option available for patients with advanced severe heart failure, as long as the clinical trial suggests at least some efficacy. PMDA therefore evaluated the efficacy of skeletal myoblast-derived cell sheets based on the currently available results of the clinical trial.

8.3.2.2. Efficacy endpoints in Japanese clinical trial and evaluation results

8.3.2.2.1. Evaluation of cardiac function

Table 8.7 shows the results of LVEF assessed by cardiac pool scintigraphy (the primary endpoint) and LVEF assessed by echocardiography and by cardiac CT (the secondary endpoints). In some patients, LVEF (assessed by cardiac pool scintigraphy or cardiac CT) decreased from baseline to posttransplantation. LVEF assessed by echocardiography increased from baseline to posttransplantation in all patients.

Table 8.7. LVEF (%) assessed by cardiac pool scintigraphy, echocardiography, and cardiac CT

	Cardiac pool scintigraphy				Echocardiography			Cardiac CT		
Subject ID	Baseline	26 weeks post-transplantation	Change	Judgment	Baseline	26 weeks post-transplantation	Change	Baseline	26 weeks post-transplantation	Change
T01-01	24	26	2.0	Unchanged	21	26	5.0	24.2	26.8	2.6
T01-03	47	37	−10.0	Worsened	33	42	9.0	33.9	33.6	−0.3
T02-01	16.5	20	3.5	Unchanged	22	32	10.0	20.5	22	1.5
T03-01	24	28	4.0	Unchanged	24	30	6.0	15.3	22.1	6.8
T01-04	29	25	−4.0	Worsened	27	31	4.0	13.1	-	-
T02-02	34	31	−3.0	Unchanged	27	32	5.0	27.2	27.3	0.1
T01-05	33	35	2.0	Unchanged	28	39	11.0	20.7	21.7	1.0

LVEF results showed discrepancies among different testing methods. Since evaluation of cardiac function by echocardiography may be biased, PMDA asked the applicant to request an independent party to validate the analysis results of echocardiography.

The applicant requested independent physicians to validate the appropriateness of the analytical results of cardiac function assessed by echocardiography. Details are presented below.

Echocardiography images obtained at baseline and 1, 4, 13, and 26 weeks posttransplantation in the Japanese clinical trial were validated. The applicant provided the images to 2 evaluators (a cardiologist and a board certified fellow of the Japan Society of Ultrasonics in Medicine) at an independent organization, who were blinded to data on when and where (the names of study sites) the images were taken. The evaluators confirmed the appropriateness of the images (e.g., quality of images) used for the analysis and the appropriateness of the analytical method employed at individual study sites, based on the image data and basic information of the study sites. Table 8.8 shows the results of validation.

Table 8.8. Validation of echocardiography images by independent evaluators

	Time of imaging	Validity of images used		Validity of analytical method	
		Evaluator 1	Evaluator 2	Evaluator 1	Evaluator 2
T01-01	Baseline	Acceptable	Acceptable	Appropriate	Appropriate
	1 week posttransplantation	-[a)]	-[a)]	-[a)]	-[a)]
	4 weeks	Acceptable	Acceptable	Appropriate	Appropriate
	13 weeks	Acceptable	Acceptable	Appropriate	Appropriate
	26 weeks	Acceptable	Acceptable	Appropriate	Appropriate
T01-03	Baseline	-[a)]	-[a)]	-[a)]	-[a)]
	1 week posttransplantation	Acceptable	Acceptable	-[b)]	-[b)]
	4 weeks	Acceptable	Acceptable	Appropriate	Appropriate
	13 weeks	Acceptable	Acceptable	-[b)]	-[b)]
	26 weeks	Acceptable	Acceptable	-[b)]	-[b)]
T02-01	Baseline	Acceptable	Anterior wall is not clear enough in the 2-chamber image.	-[b)]	-[b)]
	1 week posttransplantation	-[a)]	-[a)]	-[a)]	-[a)]
	4 weeks	Acceptable	Acceptable	-[b)]	-[b)]
	13 weeks	Acceptable	Acceptable	-[b)]	-[b)]
	26 weeks	Acceptable	Acceptable	-[b)]	-[b)]
T03-01	Baseline	Acceptable	Acceptable	-[b)]	-[b)]
	1 week posttransplantation	Acceptable	Left ventricular lateral wall is not clear enough.	Appropriate	Tracing is inappropriate (poor quality)
	4 weeks	Acceptable	Acceptable	-[b)]	-[b)]
	13 weeks	Acceptable	Acceptable	Appropriate	Appropriate
	26 weeks	Acceptable	Acceptable	Appropriate	Appropriate
T01-04	Baseline	-[a)]	-[a)]	-[a)]	-[a)]
	1 week posttransplantation	Acceptable	Acceptable	-[b)]	-[b)]
	4 weeks	Acceptable	Acceptable	-[b)]	-[b)]
	13 weeks	-[a)]	-[a)]	-[a)]	-[a)]
	26 weeks	-[a)]	-[a)]	-[a)]	-[a)]
T02-02	Baseline	Acceptable	Acceptable	-[b)]	-[b)]
	1 week posttransplantation	Lateral wall is not clear enough in the 4-chamber image.	Poor apical 2-chamber image	-[b)]	-[b)]
	4 weeks	Lateral wall is not clear enough in the 4-chamber image.	Poor apical 2-chamber image	-[b)]	-[b)]
	13 weeks	Acceptable	Acceptable	-[b)]	-[b)]
	26 weeks	Acceptable	Acceptable	-[b)]	-[b)]
T01-05	Baseline	Acceptable	Acceptable	-[b)]	-[b)]
	1 week posttransplantation	Acceptable	Apical cross-section image was not submitted	Appropriate	Inappropriate cross section
	4 weeks	Acceptable	Acceptable	Appropriate	Appropriate
	13 weeks	Acceptable	Acceptable	-[b)]	-[b)]
	26 weeks	Acceptable	Acceptable	-[b)]	-[b)]

[a)] No images were provided for validation, [b)] Still image (traced image) not available

PMDA confirmed that the independent validation of cardiac function assessment did not reveal any problems affecting data reliability, although some images and analytical method were deemed inappropriate by the evaluators and there was a lack of still images (traced images).

8.3.2.2.2. Evaluation of exercise tolerance

The applicant's explanation:

The relationship between exercise tolerance and the severity of heart failure was evaluated according to the criteria shown in Table 8.9.

Table 8.9. Evaluation criteria for exercise tolerance

Parameters	Clinically significant change		
NYHA class	NYHA classification is widely used in and outside Japan. Change by 1 level reflects a change in the amount of activity and the extent of restriction, clearly indicating clinically significant improvement or aggravation. Change in NYHA class by 1 level is thus considered a clinically significant change.		
SAS	SAS, widely used in Japan, relates basic daily activities to oxygen consumption. Given measurement errors in SAS (*New Aspects in the Treatment of Failing Heart.* 1992;113-7) and the results of Japanese clinical studies on oral inotropic agents and on oxygen therapy (*Circ J.* 2002;66:149-57, *Circ J.* 2009;73:1255-62), change by ≥1 MET is considered clinically significant.		
6-minute walk distance	A change of 40 to 45 m in 6-minute walk distance is accompanied by at least moderate improvement in Peak VO_2 or QOL index (*Cardiopulm Phys Ther J.* 2012;23:5-15). A change of 45 m is therefore considered clinically significant.		
Peak VO_2	Based on the classification by Wever et al (*Am J Cardiol.* 1985;55:21A-31A), a change by 1 level is considered clinically significant.	Extent of decrease	Measurements
		Normal to mild	>20
		Mild to moderate	16-20
		Moderate to severe	10-16
		Severe	6-10
		Markedly severe	<6
AT	Based on the classification by Wever et al (*Am J Cardiol.* 1985;55:21A-31A), a change by 1 level is considered clinically significant.	Extent of decrease	Measurements
		Normal to mild	>14
		Mild to moderate	11-14
		Moderate to severe	8-11
		Severe	5-8
		Markedly severe	<5

PMDA's view:
In the Japanese clinical trial, some subjects showed a worsening in 6-minute walk distance, Peak VO_2, and AT despite improvement in NYHA classification and SAS. This suggests inconsistency in the evaluation of exercise tolerance between parameters. In addition, since NYHA classification and SAS are based on patient-reported symptoms, these endpoints have limitations in efficacy evaluation.

The above discussion on the relationship between the evaluation of cardiac function and exercise tolerance suggests inconsistency in the results of cardiac function tests and efficacy endpoints. Further, the test results may have been biased by evaluators or the subjects' self-evaluation. At the same time, according to the PMDA's review policy mentioned in Section 8.3.2.1., the efficacy of skeletal myoblast-derived cell sheets should be evaluated based not only on the above parameters but also on the overall condition of the individual subjects and on the clinical course of Japanese patients with similar disease condition. PMDA thus instructed the applicant to perform additional efficacy evaluation.

8.3.2.2.3. Additional evaluations

The applicant explained the results of additional efficacy evaluation, as shown below.

8.3.2.2.3.1. Overall evaluation of each subject by an independent committee

The applicant presented the clinical condition of each subject and the applicant's opinion to an independent committee consisting of 3 specialists (1 cardiac surgeon, 2 cardiologists). The committee discussed individual cases of the trial subjects. Table 8.10 shows the evaluation of each subject by the committee.

Table 8.10. Evaluation by the independent committee

Subject ID	Case summary	Main comments raised by the independent committee
T01-01	Cardiac function showed a trend toward improvement. Exercise tolerance also improved in NYHA classification and SAS. The patient's drug regimen was changed for postoperative management and for the treatment of adverse events. After transplantation, the patient has not been hospitalized for the treatment of heart failure-related events, or undergone any change in drug regimen due to worsened heart failure. The frequency of other heart failure-related events did not increase.	• The operative stress is controlled appropriately. • The doses of drugs administered to this subject pretransplantation were less than those used in other subjects, suggesting that the doses were insufficient. • Blood pressure remained stable, while echocardiography showed increased LVEF, decreased left ventricular end-systolic volume index (LVESVI), and decreased left ventricular end-diastolic volume index (LVEDVI). This suggest improved left ventricular systolic function. • These results, along with the clinical symptoms and the echocardiography findings, suggest that the patient's condition has improved.
T01-03	Cardiac pool scintigraphy and echocardiography showed a discrepancy in cardiac function evaluation. NYHA classification and SAS showed improvement. The patient has been making good progress without re-admission for 1year after transplantation. The patient's drug regimen was changed for postoperative management and for the treatment of adverse events. The patient has not been hospitalized for the treatment of heart failure-related events posttransplantation, or undergone any change in drug regimen due to worsened heart failure pre-or posttransplantation. The frequency of heart failure-related events did not increase.	• Pleural effusion, one of operation-related adverse events, improved 84 days after the onset. • Adverse events occurring immediately after the perioperative period are likely to have been affected by the perioperative management. • Blood pressure remained stable, and echocardiography showed increased LVEF and decreased size of the cardiac chamber, suggesting a favorable clinical course. • The echocardiography findings at 4 weeks posttransplantation appear too good.
T02-01	Cardiac function showed a trend toward improvement. Exercise tolerance remained unchanged in NYHA classification and SAS. The frequency of heart failure-related events did not increase posttransplantation. For 2 years after transplantation, the patient has not been hospitalized for the treatment of heart failure-related events, or undergone any change in drug regimen due to worsened heart failure pre- or posttransplantation, or experienced any other heart failure-related events.	• It is noteworthy that the patient well tolerated 2 surgeries for colon cancer and lung cancer. • The subject is unlikely to have anemia because Hb level remains at around 12 g/dL. • Shortness of breath on exertion and staggering at Week 26 are not consistent with the NYHA classification.
T03-01	Cardiac function showed a trend toward improvement. Exercise tolerance also improved in NYHA classification and SAS. The patient was hospitalized 4 times until 26 weeks posttransplantation and twice thereafter due to worsened heart failure. Tolvaptan was added to the regimen because of the worsened heart failure.	• The operative wound became pus-filled 2 months after the operation, which required incision and drainage. • The patient remained hospitalized even after 26 weeks posttransplantation. It is unusual that the patient in the NYHA class I was hospitalized for heart failure. • The severity of mitral regurgitation (MR) fluctuated. MR should have been treated before performing the transplantation.

Table 8.10. Evaluation by the independent committee

Subject ID	Case summary	Main comments raised by the independent committee
T01-04	Cardiac pool scintigraphy and echocardiography showed a discrepancy in the cardiac function evaluation. Because of prolonged heart failure supposedly due to decreased renal function and postoperative infection, the patient was not discharged at 4 weeks posttransplantation as per protocol. Instead, the patient remained hospitalized for treatment until 141 days posttransplantation and continued to receive drugs including diuretics and intravenous injection of inotropic agents such as dobutamine. At 159 days posttransplantation, the patient was re-hospitalized because of worsened heart failure and treated with adaptive servo-ventilation (ASV) and Tanadopa. Even after the second hospitalization, the patient was hospitalized again because of worsened heart failure and treated with additional and increased oral drugs as well as CRT-D. Heart failure-related events are persisting.	• If the patient had complete left bundle branch block, CRT should have been performed in advance. • The patient's condition should have been checked more carefully at enrollment. • A significant change is unlikely to be detected by echocardiography alone at 13 weeks posttransplantation. The measurements and the test methods should be reviewed. • Complications such as wound infection led to an extremely prolonged admission. • Increased eosinophil count requires attention.
T02-02	Cardiac pool scintigraphy and echocardiography showed a discrepancy in cardiac function evaluation. NYHA classification and SAS showed improvement. The patient's drug regimen was changed for postoperative management and for the treatment of adverse events. The patient has not been hospitalized for the treatment of heart failure-related events posttransplantation, or undergone any change in drug regimen due to worsened heart failure pre- or posttransplantation. The frequency of other heart failure-related events did not increase.	• Sleep apnea syndrome was treated with continuous positive airway pressure (CPAP), a therapy that significantly affects symptoms. • The relationship between the improved clinical symptoms and the efficacy of skeletal myoblast-derived cell sheets is unclear because body weight and sleep apnea syndrome may also have affected the symptoms. Although symptoms improved posttransplantation, it is difficult to determine whether the change is attributable to the effect of skeletal myoblast-derived cell sheets alone. • Clinical symptoms remained unchanged despite increased body weight; this suggests that skeletal myoblast-derived cell sheets were rather effective. However, other contributing factors should be considered.
T01-05	There was a trend toward improvement in cardiac function. As for exercise tolerance, NYHA classification improved by 1 class, but SAS remained unchanged. The patient has not been hospitalized for the treatment of heart failure-related events, or undergone any change in drug regimen due to worsened heart failure pre- or posttransplantation. The frequency of other heart failure-related events did not increase.	• The operative stress is controlled appropriately. • At 13 weeks posttransplantation, brain natriuretic peptide (BNP) level increased and LVEF on echocardiography decreased transiently. Except for these changes, the clinical course was generally favorable. • The increased eosinophil count requires the investigation of possible allergic reaction to skeletal myoblast-derived cell sheets.

The applicant comprehensively evaluated individual subjects based on the discussion by the independent committee (Table 8.11).

Table 8.11. Overall evaluation by the applicant of individual subjects based on the discussion by the independent committee

Subject ID	Judgment
T01-01	Effective
T01-03	Effective
T02-01	Effective
T03-01	Not evaluable
T01-04	Not evaluable
T02-02	Effective
T01-05	Effective

8.3.2.2.3.2. Clinical course of patients with disease characteristics similar to those of the Japanese clinical trial participants, and comparison of the efficacy and safety of skeletal myoblast-derived cell sheets

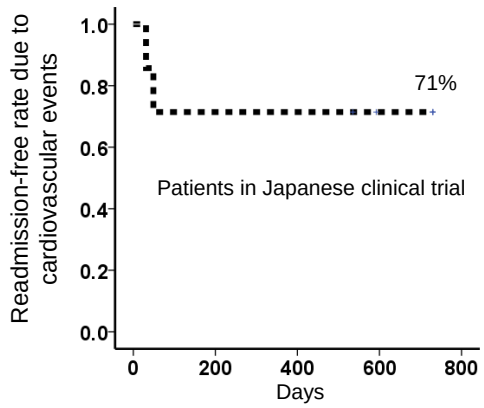
Information on the clinical course of patients with disease characteristics similar to those of patients enrolled in the Japanese clinical trial was analyzed based on a database consisting of data from 112 patients who received CRT between 2007 and 2014 and were followed up at the University of Tokyo Hospital.

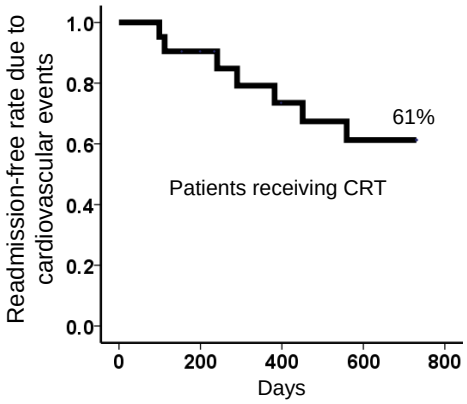
The propensity scores of 112 patients receiving CRT and 7 patients who participated in the Japanese clinical trial were calculated using 14 characteristics including age, anemic condition, nutritional state, renal function, and cardiac function, which were the characteristics considered to medically affect the patient's prognosis significantly. A total of 21 patients receiving CRT who had similar scores to those of the Japanese clinical trial participants were selected. Table 8.12 shows the characteristics of the trial participants and the matched patients. The percentage of patients with ischemic heart disease (as the primary disease), one of the 14 characteristics used for the matching, was 100% in the trial participants while it was only 9.5%, significantly low, in the patients receiving CRT. Nevertheless, despite different underlying diseases, all patients had severe heart failure such as decreased left ventricular wall motion, decreased exercise tolerance, and fatal arrhythmia; the trial participants and the matched patients are assumed to have received similar treatment and had similar clinical courses. The prognostic data in the patients receiving CRT who were matched for 14 characteristics would thus be useful to some extent.

Table 8.12. Comparison of patient characteristics (between the trial participants and matched patients)

Patient characteristics	Patients in Japanese clinical trial (n = 7)	Patients receiving CRT (n = 21)	*P* value
Age	56 ± 13	56 ± 13	0.993
Body surface area (m^2)	1.8 ± 0.1	1.7 ± 0.2	0.229
Male (N [%])	7 (100)	21 (100)	-
Ischemic heart disease (N [%])	7 (100)	2 (9.5)	<0.001
Heart rate	68 ± 7	73 ± 17	0.229
NYHA class			
I	0 (0)	0 (0)	-
II	0 (0)	0 (0)	-
III	7 (100)	21 (100)	-
IV	0 (0)	0 (0)	-
Hemoglobin (g/dL)	12.9 ± 1.9	12.9 ± 2.2	0.959
Platelet count ($\times 10^3/\mu L$)	19 ± 6	18 ± 5	0.839
Serum albumin (mg/dL)	4.4 ± 0.3	4.1 ± 0.4	0.126
Serum sodium (mEq/L)	139 ± 2	139 ± 2	0.808
Serum potassium (mg/dL)	4.2 ± 0.4	4.3 ± 0.4	0.831
Serum creatinine (mg/dL)	1.1 ± 0.2	1.2 ± 0.4	0.646
BNP (pg/mL)	226 ± 139	377 ± 249	0.142
LVDd (mm)	67 ± 6	71 ± 13	0.418
LVEF (%)	26 ± 4	26 ± 10	0.500

The prognostic factors were found to be almost identical in the clinical trial participants and the patients receiving CRT. Then, the true endpoints, i.e., admission rate due to cardiovascular events and cardiac death rate, were compared based on data obtained up to 2 years after transplantation or CRT implantation (data as of March 20, 2015). Among the trial participants, 2 experienced cardiovascular events (heart failure) immediately after the transplantation, resulting in readmission in one patient and prolonged admission in the other; but the remaining 5 patients had no cardiovascular event requiring readmission. At 2 years after transplantation of skeletal myoblast-derived cell sheets or CRT implantation, the readmission-free rate was 61% in the CRT-treated patients and 71% in the trial participants. No cardiac death occurred in the trial participants, and the cardiac death-free survival was 67% in the CRT-treated patients (Figures 8.1 and 8.2).

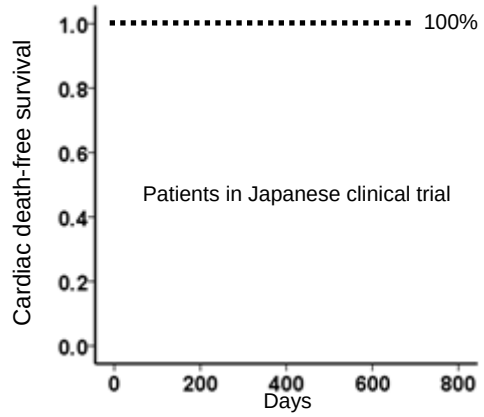


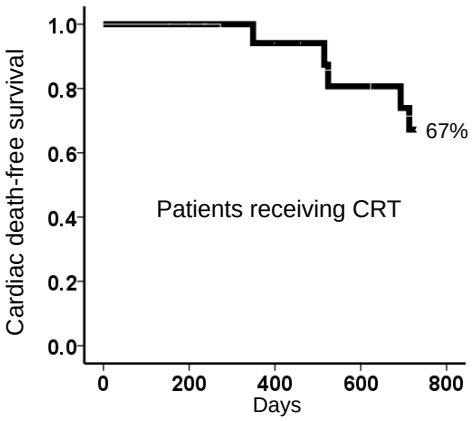


	0 months	12 months	18 months	24 months
Cumulative readmission events	0	2	2	2
n. at risk	7	5	4	3

	0 months	12 months	18 months	24 months
Cumulative readmission events	0	4	6	7
n. at risk	21	14	11	9

Figure 8.1. Readmission-free rate due to cardiovascular events





	0 months	12 months	18 months	24 months
Cumulative cardiac death events	0	0	0	0
n. at risk	7	7	6	5

	0 months	12 months	18 months	24 months
Cumulative cardiac death events	0	1	3	5
n. at risk	21	16	12	9

Figure 8.2. Cardiac death-free survival

8.3.2.3. Efficacy of skeletal myoblast-derived cell sheets in the Japanese clinical trial

PMDA's view on the efficacy evaluation of skeletal myoblast-derived cell sheets in the Japanese clinical trial:

The true endpoint for the treatment of chronic heart failure is improvement in prognosis, but a clinical trial on a scale that allows evaluation of the effect of skeletal myoblast-derived cell sheets on patients' prognosis is infeasible. The skeletal myoblast-derived cell sheets must be shown, at least, to clinically significantly improve cardiac function or delay cardiac functional decline before they become available in clinical practice. Based on these viewpoints, PMDA evaluated the efficacy of skeletal myoblast-derived cell sheets, focusing on the change in LVEF in the Japanese clinical trial.

In the Japanese clinical trial, the primary endpoint was left ventricular systolic performance at 26 weeks posttransplantation, namely LVEF determined by cardiac pool scintigraphy, a highly objective radioisotope examination. No patients achieved ≥5% improvement in LVEF, which corresponded to

"improved" according to the predetermined assessment criteria, and 5 of 7 patients were rated as "unchanged" (change in LVEF: ≥3% decrease and <5% increase), and 2 patients were rated as "worsened" (change in LVEF <3% decrease).

The clinical significance of the criteria for "improved," "unchanged," and "worsened" used by the applicant is not entirely clear. However, given that the Japanese clinical trial participants had severe heart failure at risk of a progressive cardiac functional decline, the "unchanged" LVEF in 5 of 7 patients may suggest the clinical usefulness of skeletal myoblast-derived cell sheets. Evaluation of LVEF by echocardiography (which showed a trend toward improvement) may have been biased by evaluators; NYHA classification and SAS are based on symptoms reported by patients. Echocardiography, NYHA, and SAS thus have some limitations. Nevertheless, the overall evaluation of individual patients by the independent committee member experts supported the trend toward improvement in some patients. This suggests that some patients achieved clinical improvement after the transplantation of skeletal myoblast-derived cell sheets. Meanwhile, Subject T02-02, who was considered by the applicant to be a responder, was also treated with continuous positive airway pressure (CPAP) because of concomitant sleep apnea syndrome. Some committee evaluators considered that skeletal myoblast-derived cell sheets exhibited benefits that outweighed the surgical risk in Subject T02-02. However the independent committee commented that (i) the relationship between the improved clinical symptoms and the effect of skeletal myoblast-derived cell sheets is difficult to identify and that (ii) the improvement cannot be attributed to skeletal myoblast-derived cell sheets alone. Hence, the efficacy of skeletal myoblast-derived cell sheets is not evaluable in Subject T02-02.

To investigate the prognosis expected for patients with severe heart failure with disease characteristics similar to those of the trial participants, the applicant compared the outcome between the trial participants and the 21 patients (matched for propensity scores comprising 14 characteristics supposed to affect clinical prognosis) extracted from the database of 112 patients receiving CRT. The comparison showed no significant difference in the admission rate due to cardiovascular event (up to 2 years posttransplantation) between the 2 patient groups. No cardiac death occurred in the trial participants while 33% of the CRT-treated patients died of cardiac events during the 2 years posttransplantation. These results indicate that the prognosis of the trial participants was at least non-inferior to that of CRT-indicated patients with severe heart failure, suggesting the clinical usefulness of skeletal myoblast-derived cell sheets. However, these results should be handled as reference information because the comparison was made between trial participants and matched patients from a database, not between patient groups in a single study.

PMDA's view based on the above results:
Since the Japanese clinical trial enrolled only 7 patients without control subjects, it has limitations in the efficacy evaluation of skeletal myoblast-derived cell sheets. Nevertheless, the overall assessment of individual patients suggests that skeletal myoblast-derived cell sheets have a certain level of efficacy for severe heart failure unresponsive to standard drug therapies. Because of the limited information available on the efficacy and safety of skeletal myoblast-derived cell sheets, the applicant should further collect efficacy data, including the long-term prognosis such as improvement of prognosis, the rate of re-admission-free due to a cardiovascular event, and cardiac death-free survival.

The above conclusion of PMDA will be discussed at the Expert Discussion.

8.3.3. Safety

PMDA asked the applicant to explain safety issues possibly associated with the transplantation of skeletal myoblast-derived cell sheets.

8.3.3.1. Risks associated with skeletal myoblast-derived cell sheet transplantation or thoracotomy

The applicant's explanation:
Adverse events related to skeletal myoblast-derived cell sheet transplantation or thoracotomy are shown in Table 8.13. All transplantations were completed without problems. These events commonly occur after heart surgeries, thus showing no risk unique to the transplantation of skeletal myoblast-derived cell sheets.

Table 8.13. Adverse events related to skeletal myoblast-derived cell sheet transplantation or thoracotomy[13]

Subject ID	Event (preferred term)	Days from transplantation to event (or to the date the event was detected)	Days to recovery or outcome confirmation	Severity	Seriousness	Outcome
T01-01	Peripheral coldness	0	0	Moderate	Non-serious	Recovered or improving
	Wound complication	0	4	Moderate	Non-serious	Recovered or improving
	Procedural nausea	1	2	Mild	Non-serious	Recovered or improving
	Oropharyngeal pain	2	1	Mild	Non-serious	Recovered or improving
	Hypokalaemia	7	5	Moderate	Non-serious	Recovered or improving
T01-03	Oliguria	1	4	Moderate	Non-serious	Recovered or improving
	Wound complication	1	1	Mild	Non-serious	Recovered or improving
	Postoperative fever	1	6	Moderate	Non-serious	Recovered or improving
	Hypokalaemia	2	10	Moderate	Non-serious	Recovered or improving
	Pleural effusion	7	84	Mild	Non-serious	Recovered or improving
	Wound complication	22	1	Moderate	Non-serious	Recovered or improving
	Erythema	25	3	Moderate	Non-serious	Recovered or improving
T01-04	Cardiac failure	31	141	Severe	Serious	Recovered or improving
	Renal impairment	1	193	Moderate	Non-serious	Not recovered
	Right atrial pressure increased	0	3	Moderate	Non-serious	Recovered or improving
	Pulmonary arterial pressure increased	2	1	Moderate	Non-serious	Recovered or improving
	PO_2 decreased	0	0	Moderate	Non-serious	Recovered or improving
	Wound complication	0	66	Moderate	Non-serious	Recovered or improving
	Mental disorder due to a general medical condition	2	0	Moderate	Non-serious	Recovered or improving
	Postoperative fever	3	4	Moderate	Non-serious	Recovered or improving
	Post procedural infection	24	49	Moderate	Non-serious	Recovered or improving
	Phlebitis	36	9	Moderate	Non-serious	Recovered or improving
	Hepatic congestion	36	4	Mild	Non-serious	Recovered or improving
	Urethral pain	36	86	Moderate	Non-serious	Recovered or improving
	Urine abnormality	55	1	Moderate	Non-serious	Recovered or improving
	Urethritis	122	4	Moderate	Non-serious	Recovered or improving
	Catheter site related reaction	3	2	Moderate	Non-serious	Recovered or improving
	Purpura	0	4	Mild	Non-serious	Recovered or improving
	Skin exfoliation	1	140	Mild	Non-serious	Recovered or improving
	Pruritus generalised	36	158	Moderate	Non-serious	Recovered or improving
T01-05	Premature ventricular contraction	1	84	Moderate	Non-serious	Recovered or improving
	Oropharyngeal discomfort	1	1	Moderate	Non-serious	Recovered or improving
	Diarrhoea	7	21	Moderate	Non-serious	Recovered or improving
	Post procedural infection	10	11	Moderate	Non-serious	Recovered or improving

Table 8.13. Adverse events related to skeletal myoblast-derived cell sheet transplantation or thoracotomy[13]

Subject ID	Event (preferred term)	Days from transplantation to event (or to the date the event was detected)	Days to recovery or outcome confirmation	Severity	Seriousness	Outcome
T02-02	Atrial flutter	4	7	Mild	Non-serious	Recovered or improving
	Atrial fibrillation	5	7	Mild	Non-serious	Recovered or improving
	Pneumonia	2	6	Moderate	Non-serious	Recovered or improving
T03-01	Wound complication	0	3	Moderate	Non-serious	Recovered or improving
	Blood pressure decreased	2	17	Moderate	Non-serious	Recovered or improving
	Atelectasis	2	191	Mild	Non-serious	Recovered or improving
	Postoperative fever	1	3	Mild	Non-serious	Recovered or improving
	Postoperative wound infection	56	15	Moderate	Non-serious	Recovered or improving

Not applicable in T02-01
MedDRA/J ver. 17.0

PMDA's view:
Since transplantation of skeletal myoblast-derived cell sheets requires a thoracotomy, attention should be paid to 1 case of worsened heart failure occurring after thoracotomy, given the small number of patients enrolled in the trial. The possibility of thoracotomy-associated complications should be adequately considered because skeletal myoblast-derived cell sheets are indicated for patients with severe heart failure, a population at high risk for anesthesia and operative stress. Whether the invasive thoracotomy is tolerable for individual patients should be carefully examined in advance based on their systemic condition. The Japanese clinical trial showed no adverse event obviously associated with skeletal myoblast-derived cell sheet transplantation. However, transplantation of skeletal myoblast-derived cell sheets onto the surface of the heart has risks such as localized inflammation, localized infection, and associated pericardial effusions. On a long term basis, skeletal myoblast-derived cell sheet transplantation also poses a risk of adhesion of the sheets to tissues around the transplantation site. Information on these events should be collected after the market launch.

8.3.3.2. Arrhythmia

The applicant's explanation:
Arrhythmia-related adverse events observed in the Japanese clinical trial are shown in Table 8.14. No fatal arrhythmia occurred. All events were considered due to the transplantation procedure or the primary disease, and a causal relationship to skeletal myoblast-derived cell sheets was ruled out.

[13] Adverse events for which a causal relationship to the proposed product was ruled out but were assessed to be related to the surgical treatment

Table 8.14. Arrhythmia-related adverse evens[14]

Subject ID	Event (preferred term)	Days from transplantation to event (or to the date the event was detected)	Days to recovery or outcome confirmation	Severity	Seriousness	Outcome
T01-01	Ventricular tachycardia	6	0	Moderate	Non-serious	Recovered or improving
T03-01	Atrial fibrillation	2	2	Moderate	Non-serious	Recovered or improving
T01-04	Ventricular tachycardia	4	191	Mild	Non-serious	Not recovered
T02-02	Atrial flutter	4	7	Mild	Non-serious	Recovered or improving
	Atrial fibrillation	5	7	Mild	Non-serious	Recovered or improving
T01-05	Premature ventricular contraction	1	84	Moderate	Non-serious	Recovered or improving

MedDRA/J ver. 17.0

Table 8.15 shows time-dependent changes in cardiac condition monitored by 24-hour Holter ECG. No specific tendency was observed in the time-dependent changes in individual patients. The frequency of the premature ventricular contraction showed no significant changes over time.

Table 8.15. Time-dependent changes in cardiac conditions monitored by 24-hour Holter ECG

Subject ID	Lown grade (Grade)						
	Baseline (first/second)	1 week post-transplantation (first/second)	2 weeks post-transplantation (first/second)	3 weeks post-transplantation (first/second)	4 weeks post-transplantation (first/second)	13 weeks post-transplantation	26 weeks post-transplantation
T01-01	3/4b	3/3	4a/4b	4a/3	4a/3	4a	4a
T01-03	3/4a	3/4a	3/4a	4a/3	3/3	3	4b
T02-01	1/1	1/4a	4a/4a	1/1	4a/1	4a	1
T03-01	3/4a	3/1	4a/3	3/4a	3/3	4a	3
T01-04	4b/4a	4b/4b	4b/4b	4b/4b	4b/4b	4b	4b
T02-02	4a/4a	1/4a	1/4a	1/1	1/1	4a	1
T01-05	2/2	4a/4a	4a/4a	4a/4a	4a/2	2	3

No serious ventricular arrhythmia occurred in the Japanese clinical trial, nor was fatal arrhythmia reported in the clinical studies (Studies MP0604 and HM0801). This suggests that, although skeletal myoblast-derived cell sheets have a potential risk of causing arrhythmia, the risk can be reduced by normal perioperative management and subsequent continuous monitoring commonly performed after cardiac operations.

PMDA's view:
No fatal arrhythmia occurred either in the Japanese clinical trial or the clinical studies, but transient atrial fibrillation (2 patients) and ventricular tachycardia (2 patients) occurred after skeletal myoblast-derived cell sheet transplantation in the Japanese clinical trial, which enrolled a limited number of patients. The skeletal myoblast-derived cell sheets are intended for patients at high risk of fatal arrhythmia due to the primary disease. Therefore, after the market launch, healthcare professionals should be informed of the risk of arrhythmia that may occur following skeletal myoblast-derived cell sheet transplantation, including the risk of transient arrhythmia associated with a thoracotomy. Further, relevant information should be collected.

8.3.3.3. Worsening of heart failure

The applicant's explanation:
Serious adverse events of heart failure occurred in 2 patients in the Japanese clinical trial (Table 8.16). Subject T01-04 underwent, after skeletal myoblast-derived cell sheet transplantation, bilevel positive airway pressure (BiPAP) for the treatment of decreased arterial partial pressure of oxygen (1 day posttransplantation) and re-intubation to treat ICU syndrome (2 days posttransplantation). In addition to the adverse events associated with skeletal myoblast-derived cell sheet transplantation or thoracotomy

[14] Adverse events classified as "arrhythmia" in the high level group term of MedDRA/J ver. 17.0

(Section 8.3.3.1.), the subject experienced mild blood pressure decreased (11 days posttransplantation) and moderate hyponatraemia and moderate hypokalaemia (17 days posttransplantation) (all of them resolved or were improving). Worsened heart failure occurred at 31 days posttransplantation. Moderate anaemia occurred at 38 days posttransplantation, and the outcome was "not recovered." Subject T03-01 showed moderate oedema at 27 days posttransplantation and worsened heart failure at 49 days posttransplantation. A causal relationship to skeletal myoblast-derived cell sheets could not be ruled out, but the events were considered likely due to changes in the living condition after discharge and the insufficient dose of diuretics.

Table 8.16. Adverse events related to heart failure[15]

	Event (preferred term)	Days from transplantation to event (or to the date the event was detected)	Days to recovery or outcome confirmation	Severity	Seriousness	Outcome
T01-04	Cardiac failure	31	141	Severe	Serious	Recovered or improving
T03-01	Cardiac failure	49	145	Severe	Serious	Recovered or improving

Heart failure did not occur in T01-01, T01-03, T01-05, T02-01, or T02-02.
MedDRA/J ver. 17.0

PMDA's view:
The skeletal myoblast-derived cell sheets are intended for patients at a high risk of worsening of heart failure because of the nature of their primary cardiac disorders. Considering this situation, PMDA has the following view on the serious worsening of heart failure observed in 2 patients after skeletal myoblast-derived cell sheet transplantation.

Subject T01-04 received BiPAP and underwent re-intubation after skeletal myoblast-derived cell sheet transplantation, which suggests that the invasive thoracotomy for skeletal myoblast-derived cell sheet transplantation may have contributed to the worsened clinical symptoms. Subject T03-01 experienced atrial fibrillation 2 days after the operation. As explained by the applicant, the event may have been caused by change in the living condition after discharge [see Section 8.3.3.2.]. However, the event suggests that the invasive thoracotomy for skeletal myoblast-derived cell sheet transplantation may have caused the subsequent worsening of heart failure. Healthcare professionals should be fully informed of the risk of worsening of heart failure, along with the advice that (i) whether a patient is eligible for treatment with skeletal myoblast-derived cell sheets must be determined considering the risk of worsening of systemic condition associated with the stress of thoracotomy and that (ii) appropriate measures should be taken in perioperative management, including treatment against the risk of worsening of heart failure, and in life management after discharge.

8.3.3.4. Secondary malignant tumors

The applicant's explanation:
In the Japanese clinical trial, 1 patient had colon cancer (ascending colon cancer) and lung cancer. Both events were found to be primary cancers by histopathological examination and considered unrelated to skeletal myoblast-derived cell sheets. The tumorigenicity study in immunodeficiency mice (NOG mice) has shown that cells in skeletal myoblast-derived cell sheets have a low risk of growing into malignant tumors [see Section 7.2.1.].

PMDA's view:
It is unclear whether skeletal myoblast-derived cell sheets induced or aggravated the malignant tumors (colon and lung cancers) found after skeletal myoblast-derived cell sheet transplantation. The short follow-up period in the clinical trial precludes the evaluation of a long-term risk of the growth of malignant tumors. After the market launch, information on the growth of malignant tumors after skeletal myoblast-derived cell sheet transplantation should be further collected.

[15] Adverse events classified as "heart failure" in the high level group term of MedDRA/J ver. 17.0

8.3.3.5. Complications associated with skeletal muscle tissue harvest

The applicant's explanation:
In the Japanese clinical trial, 2 of 7 subjects (28.6%) experienced adverse events caused by skeletal muscle tissue harvest, namely 2 events of wound complication (wound pain) and 1 event of post-procedural swelling. All events resolved after appropriate treatment.

PMDA's view:
Although harvesting of skeletal muscle tissue is inevitable for skeletal myoblast-derived cell sheet preparation, the procedure involves a certain risk of complications such as hemorrhage. After market launch, healthcare professionals should be appropriately trained in the harvesting procedure so that they can safely harvest skeletal muscle tissues.

8.3.3.6. Effect of skeletal myoblast-derived cell sheet transplantation on the subsequent treatment options for heart failure

Patients transplanted with skeletal myoblast-derived cell sheets may later need to undergo LVAD implantation or heart transplantation. The applicant explained the possibility that skeletal myoblast-derived cell sheet transplantation may limit subsequent therapeutic options. The applicant's explanation is as follows:
Skeletal myoblast-derived cell sheet transplantation may cause strong tissue adhesion at the operative site and around the heart, and this may hinder the conduct of subsequent LVAD implantation or heart transplantation. Thus, skeletal myoblast-derived cell sheet transplantation may limit subsequent treatment options (e.g., LVAD implantation, heart transplantation). However, this risk is not unique to skeletal myoblast-derived cell sheet transplantation but also common to CABG and valvuloplasty. So far, none of the patients in the Japanese clinical trial have subsequently undergone LVAD implantation or heart transplantation. In the clinical study (Study MP0604) conducted in Osaka University, 2 patients received heart transplantation following the completion of the follow-up period.

PMDA's view:
Only 2 patients in Study MP0604 underwent LVAD implantation or heart transplantation after the skeletal myoblast-derived cell sheet transplantation, and how skeletal myoblast-derived cell sheet transplantation affects subsequent treatment is yet to be determined. Since treatment options such as LVAD implantation and heart transplantation are extremely important to maintain the cardiac function and the lives of patients with severe heart failure, patients receiving skeletal myoblast-derived cell sheet transplantation should continue to be followed up after the market launch, to collect further information on the effect of skeletal myoblast-derived cell sheet transplantation on subsequent treatment or possible treatment options.

8.3.3.7. Other adverse events

PMDA asked the applicant to explain the following risks possibly associated with skeletal myoblast-derived cell sheet transplantation: Local infection, diastolic dysfunction due to pericarditis constrictive, coronary artery stenosis, and localized inflammation.

The applicant's response:
In the Japanese clinical trial, 1 patient experienced postprocedural wound site infection (a local infection) and underwent incision and drainage. The risk of local infection can be avoided by appropriate perioperative management of heart surgery, including the prevention of surgical site infection (SSI), along with strict control over the handling of skeletal myoblast-derived cell sheets for the prevention of microbial contamination.

Diastolic dysfunction caused by pericarditis constrictive after heart surgery may occur at an early stage after the operation or after a long interval. No case of suspected pericarditis constrictive has been reported from the Japanese clinical trial. Pericarditis constrictive can be identified by imaging such as CT and echocardiography, and can be controlled at an early stage by normal perioperative management of heart surgery and subsequent monitoring. The risk of pericarditis constrictive can thus be reduced.

The possibility of coronary artery restenosis following skeletal myoblast-derived cell sheet transplantation cannot be excluded completely. The transplantation procedure, however, is very unlikely

to affect the coronary arteries, because skeletal myoblast-derived cell sheets are transplanted directly onto the surface of the heart through thoracotomy. Some patients may have undergone revascularization such as PCI and CABG before skeletal myoblast-derived cell sheet transplantation. The risk of coronary artery restenosis in these patients can be reduced by constant monitoring and treatment with oral antiplatelet drugs after skeletal myoblast-derived cell sheet transplantation.

In the Japanese clinical trial, no patient experienced local inflammation suspected to be due to skeletal myoblast-derived cell sheets. Local inflammation is not a clinical concern associated with skeletal myoblast-derived cell sheets.

PMDA's view:
Skeletal myoblast-derived cell sheets are transplanted onto the surface of the heart through thoracotomy, and the procedure may cause adverse events such as diastolic dysfunction due to pericarditis constrictive, effects on the coronary arteries, and local infection. In the use of skeletal myoblast-derived cell sheets, perioperative management by imaging (e.g., cardiac CT and echocardiography) should be conducted as with other heart surgeries, and post-operative monitoring should be continued. Given the extremely limited number of patients receiving skeletal myoblast-derived cell sheets in the Japanese clinical trial, further information on these events should be collected after the market launch.

8.3.3.8. Long-term safety

The applicant's explanation on the long-term safety of skeletal myoblast-derived cell sheets:
A total of 7 patients underwent skeletal myoblast-derived cell sheet transplantation in the Japanese clinical trial. As of October 30, 2014, 6 of the 7 patients were surviving for ≥1 year after transplantation. In the 2 clinical studies (Studies MP0604 and HM0801), 19 patients underwent a skeletal myoblast-derived cell sheet transplantation. Follow-up after the study period revealed that 2 patients died approximately 2 and a half years posttransplantation, but a causal relationship between the deaths and skeletal myoblast-derived cell sheets was ruled out for both patients. At present, 14 of 17 surviving patients have been alive for >2 years after transplantation, with the longest survival of 7 years.

PMDA's view on the long-term safety of skeletal myoblast-derived cell sheets:
In the efficacy study using the mini-pig model of heart failure, porcine skeletal myoblasts were undetectable at 13 weeks posttransplantation [see Section 6.1.]. Also, in the clinical studies (Studies MP0604 and HM0801), engrafted skeletal myoblast-derived cell sheets were not found by biopsy at several months or years posttransplantation. These results indicate that skeletal myoblast-derived cell sheets do not remain engrafted for a long time. Thus, skeletal myoblast-derived cell sheets are unlikely to pose safety problems over a long-term posttransplantation period. At the same time, during the follow-up after the clinical studies, 2 deaths were reported approximately 2 and a half years posttransplantation, although a causal relationship of the deaths to skeletal myoblast-derived cell sheets was ruled out. Since 1 of the 2 patients had heart failure symptoms aggravated by hyperactivity, the applicant should inform healthcare professionals of the necessity of long-term adequate management of heart failure after skeletal myoblast-derived cell sheet transplantation. No fatal arrhythmia was reported either in the Japanese clinical trial or the clinical studies. Nevertheless, patients eligible for skeletal myoblast-derived cell sheet transplantation are at high risk of fatal arrhythmia because of their primary disease. In Study HM0801, a serious adverse event of non-sustained ventricular tachycardia developed in 1 patient a half year posttransplantation, and a relationship to the transplantation could not be ruled out. Therefore, information on the risk of arrhythmia occurring long after skeletal myoblast-derived cell sheet transplantation should be further collected. Whether skeletal myoblast-derived cell sheets have some effect on the occurrence or worsening of malignant tumor is unknown. Since colon and lung cancers occurred in 1 patient after skeletal myoblast-derived cell sheet transplantation, information on the risk of malignant tumors should also be further collected.

Only a small number of patients participated in the Japanese clinical trial and the clinical studies (Studies MP0604 and HM0801), and they were followed up for a relatively short period after transplantation. This requires the applicant to further collect long-term safety data, including the risk of the events described above.

8.3.4. Indication or performance

The applicant's explanation on the rationale for the proposed indication or performance:
The Japanese clinical trial suggested that skeletal myoblast-derived cell sheets are effective in patients with severe heart failure due to ischemic heart disease [see Section 8.3.2.1.]. "Indication or performance" and "Precautions for indication or performance" should be defined as shown below, based on the inclusion/exclusion criteria in the clinical trial.

[Proposed indication or performance]
Maintenance or improvement of the condition of patients with severe heart failure due to ischemic heart disease

Precautions for indication or performance
Patients with impaired left ventricular systolic function due to ischemic heart disease who remain in heart failure status despite maximal drug therapy, including digitalis, diuretics, ACE inhibitors, ARBs, β-blockers, aldosterone antagonists, and oral inotropic agents, who are at risk of worsening heart failure despite standard-of-care therapy, and who meet all of the following criteria.
(1) Patients who have chronic ischemic heart disease
(2) Patients with NYHA class III or IV heart failure
(3) Patients who remain in heart failure status despite maximal oral drug therapy
(4) Patients who are at risk of worsening heart failure despite ≥3 months of standard-of-care therapy
(5) Patients who have resting LVEF ≤35%

PMDA's view:
The main inclusion criteria in the Japanese clinical trial were (1) patients who had chronic ischemic heart disease; (2) patients with NYHA class III or IV heart failure; (3) patients who remained in heart failure status despite maximal drug therapy, including digitalis, diuretics, ACE inhibitors, ARBs, β-blockers, aldosterone antagonists, and oral inotropic agents; (4) patients aged ≥20 years; (5) patients who were at risk of worsening heart failure despite ≥3 months of standard-of-care therapy (e.g., CABG, mitral valvuloplasty, left ventricular restoration, CRT, and PCI); and (6) patients who had LVEF ≤35% on resting echocardiography. Patients were required to meet all of these criteria. Therefore, eligibility of patients for treatment with skeletal myoblast-derived cell sheets should be determined based on these criteria in the Japanese clinical trial.

Despite the criterion "patients with NYHA class III or IV heart failure," all patients enrolled in the clinical trial had class III heart failure. PMDA asked the applicant to discuss the efficacy and safety of skeletal myoblast-derived cell sheets in patients with NYHA class IV.

The applicant's explanation:
In the 2 clinical studies, 5 patients with NYHA class IV (4 patients in Study MP0604, 1 patient in Study MH0801) underwent a skeletal myoblast-derived cell sheet transplantation. Of these, 4 patients were supported with an extracorporeal artificial heart, which did not pose any safety problems. One patient in Study HM0801 felt an improvement in symptoms although NYHA class remained IV at 26 weeks posttransplantation. To date, only a limited number of patients with NYHA class IV have undergone the transplantation of skeletal myoblast-derived cell sheets. Skeletal myoblast-derived cell sheet transplantation involves a left thoracotomy. Therefore concurrent diseases that may increase the risks associated with thoracotomy (e.g., pulmonary hypertension, mitral valve insufficiency, and renal dysfunction) should be considered before the use of skeletal myoblast-derived cell sheets. However, skeletal myoblast-derived cell sheets can be used safely in patients with NYHA class IV who are eligible for a heart surgery, if their symptoms are mild enough to allow outpatient care. The efficacy of skeletal myoblast-derived cell sheets should be further evaluated.

PMDA's view on the severity of heart failure and the primary disease in patients eligible for treatment with skeletal myoblast-derived cell sheets:
(a) Severity of heart failure
Given the inclusion criteria in the Japanese clinical trial, skeletal myoblast-derived cell sheets should be provided to patients with severe heart failure who are at risk of worsening heart failure despite maximum drug therapy. In other words, patients with milder heart failure of NYHA class I or II are ineligible for

treatment with skeletal myoblast-derived cell sheets. The demarcation between NYHA class III and class IV is not always clear, and in some patients NYHA classification may change from IV to III. The use of skeletal myoblast-derived cell sheets should thus not be restricted only to patients with NYHA class III, just because patients with NYHA class III alone underwent skeletal myoblast-derived cell sheet transplantation in the Japanese clinical trial. Accordingly, skeletal myoblast-derived cell sheets should be indicated for patients with severe heart failure of NYHA class III to IV who have not sufficiently responded to existing therapies.

However, patients with NYHA class IV tend to have more advanced heart failure than patients with NYHA class III, and are therefore likely to have fewer remaining cardiac muscles and poorer cardiac function and systemic condition. No patients with NYHA class IV underwent skeletal myoblast-derived cell sheet transplantation in the Japanese clinical trial, and they are considered to have a higher risk associated with thoracotomy, an inevitable procedure for transplantation. Extreme caution is therefore required when determining whether a patient with NYHA class IV is eligible for skeletal myoblast-derived cell sheet transplantation. In order to raise the awareness of healthcare professionals, the applicant should inform healthcare professionals that skeletal myoblast-derived cell sheets were not transplanted into patients with NYHA class IV in the Japanese clinical trial and that patients with class IV may have an increased risk associated with thoracotomy. At the same time, adequate efficacy and safety data of skeletal myoblast-derived cell sheets should be collected after the market launch.

(b) Underlying disease of heart failure
Because the Japanese clinical trial enrolled only patients with severe heart failure who had underlying ischemic heart disease, the efficacy of skeletal myoblast-derived cell sheets for heart failure due to other underlying diseases (e.g., dilated cardiomyopathy) has not been evaluated. In the clinical studies (Studies MP0604 and MH0801, the reference data), skeletal myoblast-derived cell sheets were transplanted into 11 patients with dilated cardiomyopathy, but the experience is not enough to provide sufficient information to evaluate the effect of various underlying diseases on the safety of skeletal myoblast-derived cell sheets. Thus skeletal myoblast-derived cell sheets should be indicated only for the treatment of heart failure due to underlying ischemic heart disease, as proposed by the applicant.

PMDA has the following view on the indication or performance of skeletal myoblast-derived cell sheets, based on the inclusion criteria of the Japanese clinical trial and the discussion described in (a) and (b) above:
The subjects enrolled in the Japanese clinical trial were patients with heart failure who were at risk of worsening of heart failure despite maximum drug therapy or standard-of-care therapy (e.g., CABG, mitral valvuloplasty, left ventricular restoration, CRT, PCI). Transplantation of skeletal myoblast-derived cell sheets involves invasive thoracotomy. According to these facts and available efficacy data, skeletal myoblast-derived cell sheets should be indicated only for "patients with severe heart failure who have not sufficiently responded to standard drug therapies or surgical treatment" among "patients with severe heart failure due to ischemic heart disease." Overall clinical significance of skeletal myoblast-derived cell sheets was discussed based on the following observations: (i) no patient was assessed as having "improved" LVEF on cardiac pool scintigraphy in the Japanese clinical trial; (ii) the overall evaluation, albeit post hoc, of individual patients by the independent committee supported a trend toward improvement in some patients; and (iii) the comparison between the Japanese clinical trial participants and matched patients selected from the database of patients receiving CRT suggested that prognosis of the trial participants was at least not inferior to the matched patients in the rates of admission due to cardiovascular events and cardiac death-free survival after 2 years. Considering these facts, the indication or performance should not focus on the disease condition, i.e., retention or improvement in the left ventricular systolic function. Accordingly, the indication or performance of skeletal myoblast-derived cell sheets should be defined as shown below.

[Indication or performance]
The product is used for the treatment of patients with severe heart failure due to ischemic heart disease unresponsive to standard drug therapies or surgical treatments who meet all of the following criteria.

Eligibility criteria:
- NYHA class III or IV heart failure; and

- Resting left ventricular ejection fraction ≤35%

The above conclusion of PMDA will be discussed at the Expert Discussion.

8.3.5. Dosage and administration or method of use

The applicant explained the methods for harvesting skeletal muscle tissue for the preparation of skeletal myoblast-derived cell sheets and the methods for transplanting skeletal myoblast-derived cell sheets. Details are presented below.

8.3.5.1. Harvesting skeletal muscle

The standard procedure for muscle biopsy was used in the Japanese clinical trial; persons in charge of harvesting in all studies sites were trained on how to harvest skeletal muscle cells so that they could follow the procedure correctly. At least 7 weeks before transplantation, a required amount of skeletal muscle for skeletal myoblast-derived cell sheet preparation (approximately 2-5 g) was harvested. The muscle tissue was obtained from the quadriceps, due to its less impact on the patient's QOL, or any other appropriate site of the body depending on the patient's condition. In the Japanese clinical trial, adverse events associated with skeletal muscle harvest occurred in 2 patients [see Section 8.3.3.5.], but the harvesting procedure was considered to have no safety or tolerability problems.

8.3.5.2. Method for skeletal myoblast-derived cell sheet transplantation

Table 8.17 shows the infarcted area and the transplantation site in each subject participating in the Japanese clinical trial. The infarcted area was identified based on the findings on single photon emission computed tomography (SPECT) performed when patients were screened for eligibility. No test was performed to determine the site of skeletal myoblast-derived cell sheet transplantation before patients received skeletal myoblast-derived cell sheets. It was decided to apply 5 pieces of skeletal myoblast-derived cell sheets on the surface of the left ventricle by left thoracotomy as a general rule, and to use a skeletal myoblast-derived cell sheet only once.

Table 8.17. Infarcted area and transplantation sites in subjects in the Japanese clinical trial

	Infarcted area[a)]	Site of transplantation
T01-01	Wide area extending from anteroseptum to apex	5 pieces over left ventricular anterior wall to lateral wall
T01-03	Lateral wall, wide area extending from posterior to lower wall, anteroseptum close to the apex	5 pieces over left ventricular anterior wall to lateral wall
T02-01	Left anterior descending artery region, right coronary artery region	4 pieces to left side of left anterior descending artery, 1 piece over right internal thoracic artery
T03-01	Upper to lower anterior wall, apex, upper to lower part of posterior lateral wall to posterior lower wall	5 pieces over left ventricular anterior wall to lateral wall
T01-04	Anteroseptum to apex, lower wall	5 pieces over left ventricular anterior wall to lateral wall
T02-02	Anterior wall to apex to lower wall	5 pieces over left ventricular lateral wall to posterior wall
T01-05	Anteroseptum to apex, posterior lateral wall	5 pieces over left ventricular anterior wall to lateral wall

[a)] Initial SPECT findings at eligibility screening

PMDA asked the applicant to elaborate how to evaluate the transplantability of skeletal myoblast-derived cell sheets prior to transplantation, sites of transplantation, the transplantation method, and the number of sheets to be transplanted.

The applicant's explanation:
Most patients eligible for skeletal myoblast-derived cell sheet transplantation are expected to have already undergone a heart surgery such as CABG. Therefore before skeletal myoblast-derived cell sheet transplantation, cardiac CT or other examinations should be used to identify the bypass graft site and the locations of ribs, etc., in order to find out how to approach the transplantation site. Skeletal myoblast-derived cell sheets should not be applied over preexisting structures, such as a bypass graft and adhesion sites. If cardiac CT or other examinations has revealed that the approach to the surface of the heart may injure bypass graft, the patient should not undergo skeletal myoblast-derived cell sheet transplantation.

Most patients eligible for skeletal myoblast-derived cell sheet transplantation probably have decreased contractility in a large portion of the entire left ventricle. Skeletal myoblast-derived cell sheets should

be applied over an area as large as possible rather than only over non-contractile fibrotic infarct area, because skeletal myoblast-derived cell sheets are expected to act on viable tissue. Left thoracotomy allows surgeons to apply skeletal myoblast-derived cell sheets only to the area from the anterior to lateral wall of the left ventricle, which is directly visible. Regardless of the extent of the infarct area, 5 pieces of skeletal myoblast-derived cell sheets (round sheets of approximately 4 cm in diameter, each containing 3×10^8 cells) should be sequentially applied on the surface of the heart, to cover almost the entire transplantable surface of the heart, including the surrounding normal tissue. In the Japanese clinical trial, skeletal myoblast-derived cell sheets were apt to slide down the steep lateral wall. To prevent this from happening, each skeletal myoblast-derived cell sheet was sewed on the surface of the heart with 1 stitch. In the Japanese clinical trial, skeletal myoblast-derived cell sheet transplantation was performed according to this procedure, without the occurrence of adverse events that pose tolerability or feasibility problems [see Section 8.3.3.1.].

PMDA's view on the method of skeletal myoblast-derived cell sheet transplantation:
Transplantation of skeletal myoblast-derived cell sheet is indicated for patients with severe heart failure resulting from advanced chronic heart failure caused by old myocardial infarction, despite the standard drug therapies or surgical treatment. Most of these patients are considered to have extensive anteroseptal infarction resulting from a lesion in the proximal left anterior descending artery or ischemic cardiomyopathy caused by diffuse multivessel disease. Because of extremely limited experience in skeletal myoblast-derived cell sheet transplantation in the Japanese clinical trial, the optimal transplantation method that would maximize the efficacy of skeletal myoblast-derived cell sheets is not entirely clear. In all 7 patients in the trial, left thoracotomy was performed to secure a sufficient operative field; thus the left ventricle was located under direct vision and all 5 skeletal myoblast-derived cell sheets were transplanted over a largest possible area covering the anterior to lateral wall of the left ventricle and the surrounding area. Because all of the 7 trial subjects had the main lesion on the left anterior descending artery (Table 8.17), this method is reasonable. Thus the proposed method for skeletal myoblast-derived cell sheet transplantation (determined based on the method used in the Japanese clinical trial) is considered acceptable. Given the risk associated with a thoracotomy, healthcare professionals should be advised to carefully discuss how to approach the surface of the heart after locating a bypass graft with cardiac CT or other examinations, and to carefully examine patients' heart condition to assess their eligibility for skeletal myoblast-derived cell sheet transplantation. Generally, patients with high posterior myocardial infarction alone do not progress into severe heart failure, and are therefore unlikely to receive skeletal myoblast-derived cell sheets. However, healthcare professionals should be informed that skeletal myoblast-derived cell sheets have never been transplanted to patients with infarction in an area not under direct vision through a left thoracotomy, including high lateral infarction.

It is unknown whether difference in procedures of skeletal myoblast-derived cell sheet transplantation may affect the efficacy and safety of skeletal myoblast-derived cell sheets. In order to minimize gaps in procedures among medical institutions, the procedures used in the Japanese clinical trial should be defined as the standard procedures. Educational materials and training should be provided to healthcare professionals to ensure that skeletal myoblast-derived cell sheets are appropriately transplanted by the standardized procedure [see Section 8.3.6.].

8.3.6. Qualifications of medical institutions, physicians, and surgeons who use the product

Since skeletal myoblast-derived cell sheets are derived from autologous skeletal muscle tissue, the applicant defined qualifications necessary for medical institutions, physicians, and surgeons to use the proposed product after marketing approval (see below). The qualifications will ensure the proper use and quality of the proposed product in clinical practice.

(a) Skeletal myoblast-derived cell sheets should be used by physicians and surgeons who completed a training course, provided by the marketing authorization holder, on the proposed product.

(b) Skeletal muscle and blood of patients, raw materials of skeletal myoblast-derived cell sheets, should be harvested at a facility that has necessary manpower and equipment for hygienic control.

(c) Skeletal myoblast-derived cell sheets should be prepared at a facility with an appropriate, well-equipped building including a contamination control area and aseptic processing area. Each medical institution should conclude an agreement or contract with the marketing authorization holder, as necessary, on the maintenance of the facility.

(d) After skeletal myoblast-derived cell sheet transplantation, the patient should be carefully monitored and examined. If any sign or symptom of infection is noted, appropriate measures, such as antibiotic therapy, should be taken.

PMDA's view:
PMDA generally agrees to the proposed qualifications for medical institutions, physicians, and surgeons, to ensure the proper use and quality of the proposed product in clinical practice. However, the following topics should be included in a training course planned by the applicant: (i) the requirement that skeletal myoblast-derived cell sheet preparation facility should maintain a certain level of equipment and its control, (ii) appropriate procedures for harvesting skeletal muscle, the raw material of skeletal myoblast-derived cell sheets, (iii) how to apply skeletal myoblast-derived cell sheets to the infarct area, and (iv) patient management after thoracotomy [see Sections 8.3.3. and 8.3.5.]. Further, available efficacy and safety data are limited because the only clinical study that evaluated skeletal myoblast-derived cell sheets was an exploratory clinical trial in a few patients. In addition, patients undergoing transplantation have health risks associated with thoracotomy. It is therefore critical to select patients who can gain the benefits that outweigh the risks of skeletal myoblast-derived cell sheets. For this reason, eligibility of patients should be determined by a heart team comprising cardiac surgeons and cardiologists; this should be communicated to healthcare professionals through the package insert and the training course.

Accordingly, the applicant should establish the qualification for medical institutions, physicians, and surgeons who use skeletal myoblast-derived cell sheets in collaboration with concerned academic societies, to restrict the use of the proposed product to qualified institutions and physicians and surgeons that meet the above requirements. A collaborative system should be established for prompt information collection and analysis on the efficacy and safety of the proposed product. Educational materials etc. should be used to inform physicians and surgeons who will use skeletal myoblast-derived cell sheets of the criteria for eligible patients, properties of skeletal myoblast-derived cell sheets, transplantation method, treatment pre- and post-transplantation, and precautionary advice for the use of skeletal myoblast-derived cell sheets.

9. Risk Analysis

The applicant plans a post-marketing surveillance (i.e., a use-results survey). Details are described below.

The Japanese clinical trial revealed only limited data on the efficacy and safety of skeletal myoblast-derived cell sheets. The applicant therefore plans to conduct a use-results survey in all patients undergoing skeletal muscle harvest for the preparation of skeletal myoblast-derived cell sheets, to further evaluate the efficacy and safety of skeletal myoblast-derived cell sheets in clinical use after the market launch.

The survey will investigate "survival rate after skeletal myoblast-derived cell sheet transplantation," "treatment events due to worsened heart failure (e.g., admission)," and other investigation items, to evaluate the long-term efficacy. Ideally, patients should be followed for ≥2 years to evaluate the effect of skeletal myoblast-derived cell sheet transplantation on their life prognosis, in view of (i) the survival rates of patients who underwent skeletal myoblast-derived cell sheet transplantation in the Japanese clinical trial and the clinical study (Study HM0801), and (ii) the predicted survival rate in a patient population with similar characteristics to those of patients eligible for skeletal myoblast-derived cell sheet transplantation (i.e., the survival rate predicted from the database of patients who received CRT and were followed up at the University of Tokyo Hospital [see Section 8.3.2.2.3.2.] and from the Seattle heart failure model [*Circulation.* 2006;113:1424-33, *Am J Cardiol.* 2011;108:391-6]). However, prolonged follow-up may affect patient characteristics (e.g., advanced age, complications) and may increase a risk of loss to follow-up leading to dropout. To avoid such risks, the primary endpoint was defined as survival rate at 2 years posttransplantation. Since the survival rate at 3 years

posttransplantation remained high in the clinical study (Study HM0801), secondary endpoints were determined as the survival rate at 3 years posttransplantation, time-dependent changes in the survival rate, and the admission rate due to treatment event caused by worsened heart failure. The patients will be followed-up thereafter for safety evaluation.

The applicant will also consider a survey on the clinical course of patients eligible for skeletal myoblast-derived cell sheet transplantation. However, since the feasibility of the survey is currently unclear, the applicant plans to evaluate the efficacy of skeletal myoblast-derived cell sheets based on a comparison with the prognosis data of patients who received CRT and were followed up in the University of Tokyo Hospital and with data for patients with chronic heart failure in a database under construction at Osaka University.

Furthermore, the following parameters were selected to evaluate the short-term efficacy: NYHA class at baseline, discharge (or 1 month posttransplantation), and 6 and 12 months posttransplantation; cardiac functions by echocardiography (LVEF, left ventricular end-diastolic volume [LVEDV], left ventricular end-systolic volume [LVESV], LVDd, LVDs), exercise tolerance (6-minute walk distance), etc.

Safety investigation items include product-related adverse events/adverse reactions. Risks associated with skeletal myoblast-derived cell sheets include heart failure, infection caused by human-derived or animal-derived materials, hypersensitivity to antibiotics and animal-derived materials, and serious ventricular arrhythmia.

PMDA's view on the plan for the post-marketing use-results survey:
Because of the limited efficacy data from the Japanese clinical trial, information relevant to efficacy evaluation should be collected through the post-marketing use-results survey. The proposed investigation items will allow efficacy evaluation to a certain extent. However, the follow-up period for individual patients should be determined so that the applicant can evaluate whether skeletal myoblast-derived cell sheets reduce, to a certain extent, cardiac deaths, cardiovascular events, and other events in patients with severe heart failure. Based on the survival rate and the admission rate due to cardiovascular events suggested by Figures 8.1 and 8.2 in Section 8.3.2.2.3.2., a ≥2-year follow-up is required to evaluate the efficacy. In the clinical studies (Studies MP0604 and HM0801), deaths occurred 2.5 years posttransplantation. Accordingly, patients should be followed-up for approximately 3 years. In the future, the survival rate and the admission rate due to cardiovascular events should be obtained through the patient registration system for regenerative medical products, which is currently under development, and each patient should continue to be evaluated after the third year as well utilizing the system data. Cardiac function and exercise tolerance are defined as short-term efficacy investigation items, and only limited data are available for cardiac function and exercise tolerance at 3 years posttransplantation. Therefore, data on these parameters should be collected throughout the follow-up period of the use-results survey if possible, as with cardiac deaths and the admission rate due to cardiovascular events. Data on these parameters (cardiac function and exercise tolerance) can be constantly collected because they are commonly used in routine clinical practice.

The efficacy data to be obtained from the use-results survey should be evaluated by comparing the clinical course of patients treated and not treated with skeletal myoblast-derived cell sheets; therefore it is important to collect data on the clinical course of patients not treated with skeletal myoblast-derived cell sheets who have severe heart failure with similar disease characteristics and to evaluate the appropriateness of such data. However, clinical practice and disease characteristics of patients with severe heart failure may differ according to the medical institution. In addition, past clinical studies and retrospective studies may provide inappropriate data, namely data for patients treated with old therapeutic system for serious heart failure, which may be different from the current or future therapeutic system. For these reasons, data collected from different institutions or past studies may not necessarily provide appropriate information. Therefore, during the survey period, the applicant should prospectively collect data on the clinical course of patients with severe heart failure who are potentially eligible for skeletal myoblast-derived cell sheet transplantation from medical institutions that will actually transplant skeletal myoblast-derived cell sheets into patients and other equivalent institutions, as long as possible. Obtained data should be compared with the results of patients treated with skeletal myoblast-derived cell sheets.

Safety investigation items should include worsening of heart failure, arrhythmia, local infection, the events reported by the Japanese clinical trial, and other risks associated with thoracotomy or the transplantation procedures (e.g., diastolic dysfunction due to pericarditis constrictive, localized inflammation and associated pericardial effusion, effect on coronary arteries, adhesion), risks of tumor formation and secondary malignant tumor, and events caused by skeletal muscle harvest (e.g., wound complication, post-procedural swelling). Because of the limited data available on the safety of skeletal myoblast-derived cell sheets, the applicant should provide safety data obtained through the post-marketing use-results survey to healthcare professionals, and take prompt and appropriate actions, including additional safety measures, as necessary.

Details of the post-marketing use-results survey will be finalized based on the Expert Discussion on the evaluation of the efficacy and safety of skeletal myoblast-derived cell sheets.

10. Results of Compliance Assessment Concerning the Data Submitted in the New Regenerative Medical Product Application and Conclusion by PMDA

10.1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

The review is currently ongoing. The results and PMDA's conclusion will be reported in the Review Report (2).

10.2. PMDA's conclusion on the results of GCP on-site inspection

The review is currently ongoing. The results and PMDA's conclusion will be reported in the Review Report (2).

11. Overall evaluation at the time of preparation of the Review Report (1)

Based on the submitted data, PMDA concluded that treatment with skeletal myoblast-derived cell sheets is effective to a certain extent in patients with severe heart failure due to ischemic heart disease and that the safety of skeletal myoblast-derived cell sheets is clinically acceptable. Although available efficacy and safety data are limited, skeletal myoblast-derived cell sheets have clinical significance and are worth providing to clinical practice as a therapeutic option for patients with advanced, severe heart failure who have no other options available.

PMDA considers that the skeletal myoblast-derived cell sheets may be approved if the above conclusion is determined to be acceptable based on comments from the Expert Discussion, with conditions and a time limit defined according to Article 23-26 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. The conditions require the applicant to further evaluate the efficacy of skeletal myoblast-derived cell sheets and collect safety data until a certain time limit after market launch. The time limit should be 5 years according to the Article, taking account of the post-marketing surveillance plan submitted by the applicant (e.g., preparation time for marketing, follow-up period of individual patients, preparation time for reapplication for approval), but it will be finalized upon receiving comments from the Expert Discussion.

Review Report (2)

August 10, 2015

1. Product Submitted for Registration

[Brand name] HeartSheet

[Non-proprietary name] Human (autologous) skeletal myoblast-derived cell sheet

[Applicant] Terumo Corporation

[Date of application] October 30, 2014

2. Content of the Review

The comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations, etc., concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

The brand name of the product was changed from [redacted] to HeartSheet because of a potential trademark conflict.

2.1. Efficacy

PMDA's view:

The Japanese clinical trial (M-51073-21) allowed only limited evaluation of the efficacy of HeartSheet on severe heart failure due to ischemic heart disease. Nevertheless, a comprehensive assessment of individual patients demonstrated a certain level of efficacy of HeartSheet in patients with severe heart failure unresponsive to standard drug therapies or surgical treatment. However, due to the limited information available, the efficacy should be further evaluated after marketing approval to confirm the effectiveness of HeartSheet [see Section 8.3.2. of the Review Report (1)].

At the Expert Discussion, expert advisors discussed issues arising from the Japanese clinical trial (see below). The expert advisors generally supported the PMDA's conclusion that the efficacy of HeartSheet should continue to be evaluated after marketing approval because of limited information currently available. The expert advisors also commented that the efficacy should be prospectively evaluated after marketing approval based on predefined strict criteria.

- Change in LVEF on cardiac pool scintigraphy, the primary endpoint of the Japanese clinical trial, was "unchanged" (≥3% decrease and <5% increase) in 5 of 7 subjects and "worsened" (<3% decrease) in 2 subjects, whereas no subjects showed "improvement" (≥5% increase).

- In the clinical trial, efficacy criteria were not defined in advance. Instead, efficacy was evaluated in an exploratory manner based on the data obtained from the clinical trial.

- The prognosis of the trial participants was evaluated retrospectively by a comparison with the prognosis of patients with similar disease characteristics selected from a database of patients with severe heart failure receiving CRT.

Taking account of the above issues raised by the expert advisors, PMDA concluded that efficacy data should be collected and evaluated according to predetermined objectives and criteria for the evaluation, as a part of the approval condition-based post-marketing evaluation [for the plan for the approval condition-based post-marketing evaluation (draft), see Section 2.5.].

2.2. Safety

PMDA considers that the use of HeartSheet requires attention for worsening of heart failure, arrhythmia, local infection, other risks associated with thoracotomy or transplantation procedures (including diastolic dysfunction due to pericarditis constrictive, localized inflammation and associated pericardial effusion, effect on coronary arteries, adhesion), risks of tumor formation and the development of secondary malignant tumors, and events caused by skeletal muscle harvest (e.g., wound complication, post procedural swelling). Therefore, HeartSheet should be used at medical institutions with capacity for emergency responses, under the supervision of a physician or surgeon with adequate knowledge and experience in severe heart failure and thoracotomy who have completed a training course on the use of HeartSheet provided by the applicant, and under a system ensuring appropriate perioperative management. PMDA concluded that the safety of HeartSheet is acceptable as long as such appropriate measures are taken [see Section 8.3.3. of the Review Report (1)].

The above conclusions of PMDA were supported by the expert advisors at the Expert Discussion. PMDA instructed the applicant to appropriately address the above requirements and the applicant agreed. PMDA accepted the applicant's response.

2.3. Indication or performance

PMDA considered that the "Indication or performance" of HeartSheet should be defined as shown below, based on the characteristics of patients treated in the Japanese clinical trial [see Section 8.3.4. of the Review Report (1)].

[Indication or performance]
The product is used for the treatment of patients with severe heart failure due to ischemic heart disease unresponsive to standard drug therapies or surgical treatments who meet all of the following criteria.

Eligibility criteria:
- NYHA class III or IV heart failure; and
- Resting left ventricular ejection fraction ≤35%

The above conclusions of PMDA were supported by the expert advisors at the Expert Discussion. The expert advisors also made the following comments:
(i) Taking account of the clinical positioning of HeartSheet, the wording "the standard drug therapies and surgical treatments" in Indication or performance should be modified to include other existing drugs, in order to specify that HeartSheet therapy should be considered only in patients who have already received common treatments for severe heart failure due to ischemic heart failure in clinical practice.
(ii) The relationship between HeartSheet and LVAD is unclear.

Considering the above comments of the expert advisors and the clinical positioning of HeartSheet, PMDA concluded that "Indication or performance" should be defined as shown below, and that the package insert should emphasize that HeartSheet is not a substitute for LVAD.

[Indication or performance]
Treatment of patients with severe heart failure due to ischemic heart disease unresponsive to standard treatments including drug and invasive therapies who meet all of the following criteria.

Eligibility criteria:
- NYHA class III or IV heart failure; and
- Resting left ventricular ejection fraction ≤35%

PMDA instructed the applicant to define the indication or performance as above, and the applicant agreed. PMDA accepted the applicant's response.

2.4. Dosage and administration or method of use

PMDA's view:

The left anterior descending artery was affected in most patients in the Japanese clinical trial. Although HeartSheet was used in an extremely small number of patients in the trial, the following transplantation procedure was considered reasonable: (1) perform a left thoracotomy to secure a sufficient operative field, (2) locate the left ventricle under direct vision, and (3) apply all 5 pieces of skeletal myoblast-derived cell sheets over a largest possible area of the surface of the heart covering the anterior to lateral wall of the left ventricle. PMDA thus considers that the dosage and administration or method of use of HeartSheet may be defined based on the experiences in the Japanese clinical trial, provided that the applicant appropriately advises healthcare professionals to use HeartSheet properly [see Section 8.3.5. of the Review Report (1)].

The above conclusions of PMDA were supported by the expert advisors at the Expert Discussion. PMDA concluded that the dosage and administration or method of use should be modified as shown below based on the experiences in the Japanese clinical trial.

[Dosage and administration or method of use]
The product is used according to the following method.

Prior to the production of skeletal myoblast-derived cell sheets

1) Skeletal muscle is harvested from the patient. As a general procedure, the harvesting is performed according to the procedure for diagnostic muscle biopsy for neuromuscular diseases. Skeletal muscle should be harvested from the quadriceps, in principle, but may be harvested from any other appropriate site of the body depending on the patient's condition. The harvested skeletal muscle is delivered in a dedicated container to the facility designated by the marketing authorization holder.

2) Blood is collected from the patient to separate serum. The separated serum is delivered in a container to the facility designated by the marketing authorization holder.

Preparation and transplantation of skeletal myoblast-derived cell sheets

1) Using the secondary constituent parts, 5 skeletal myoblast-derived cell sheets (preferably 6 sheets including 1 spare) are prepared from the cryopreserved cells. Each step is taken aseptically.

2) Each of 5 skeletal myoblast-derived cell sheets is sequentially transplanted onto the surface of the heart. The transplantation procedure is usually performed through left thoracotomy.

2.5. Approval condition-based post-marketing evaluation (draft)

Because of the limited efficacy and safety data on HeartSheet, PMDA concluded that an approval condition-based post-marketing evaluation (hereafter "the post-marketing evaluation") should be conducted in all patients to confirm the efficacy and safety of HeartSheet after marketing approval, in accordance with the following requirements [see Section 9. of the Review Report (1)].

- The post-marketing evaluation should be conducted in all patients who have undergone skeletal muscle harvest for the treatment with HeartSheet during the period between the conditional and time-limited approval and the reapplication for marketing approval.

- The Japanese clinical trial has shown limited efficacy data of HeartSheet, and HeartSheet therapy involves invasive procedures. In order to assess the risk-benefit balance of HeartSheet, the post-marketing evaluation should focus on whether HeartSheet has reduced events including cardiac deaths, cardiovascular events to a certain degree in patients with severe heart failure. The efficacy should also be evaluated based on cardiac function, exercise tolerance, etc. At the same time, the applicant should collect data on the clinical course, prospectively and concurrently, from patients with severe heart failure who are potentially eligible for HeartSheet therapy but are receiving other treatment at medical institutions providing HeartSheet therapy and other equivalent institutions. Data of these control patients should be compared with those in patients receiving HeartSheet.

- Because of limited safety data of HeartSheet, new information obtained during the post-marketing evaluation should be provided to healthcare professionals and added to information materials for patients as appropriate.

The above conclusions of PMDA were generally supported by the expert advisors at the Expert Discussion. The expert advisors commented that the following matters should be considered before conducting the post-marketing evaluation.

- The applicant should define efficacy criteria (i.e., what results support the efficacy of HeartSheet) in advance.

- The applicant should prospectively and concurrently collect data on the clinical course of the control group, namely patients with severe heart failure who are potentially eligible for HeartSheet therapy but are not treated with the therapy.

Based on the above deliberation at the Expert Discussion, PMDA instructed the applicant to reconsider the plan for the post-marketing evaluation. The applicant then submitted the outline of the evaluation plan as shown in Tables 9.1 and 9.2, and responded that it would conduct the post-marketing evaluation under the following conditions.

- The applicant will collect data on the long-term clinical course of patients receiving HeartSheet (the HeartSheet group) in clinical practice and prospectively collect data on the prognosis of patients eligible for HeartSheet therapy but not receiving the therapy (the external control group). Matching factors that are likely to affect prognosis will be defined in advance, then propensity scores on patient characteristics will be defined. The ratio of the number of patients receiving HeartSheet and the number of control patients matched for baseline characteristics will be 1:1. If the survival time analysis shows significant improvement in the HeartSheet group as compared with the control group, HeartSheet should be considered effective.

- "Time to admission due to a cardiovascular event" and "the percentage of patients who showed ≥5% improvement in LVEF at 6 months after transplantation of HeartSheet" should be defined as important secondary endpoints. These secondary endpoints should be compared between the HeartSheet and control groups.

- The term "admission due to a cardiovascular event" should be clearly defined in advance. A central assessment committee should be formed to evaluate the effect of concomitant therapy, etc. on cardiovascular events to minimize bias in the evaluation of individual events.

- LVEF is a prognostic factor that reduces the mortality rate (*Circulation.* 1993;87(6Suppl):VI17-23). Patients who show ≥5% improvement in LVEF after transplantation of HeartSheet are therefore considered as having an improvement in cardiac function. Echocardiography will be used to measure LVEF since many patients eligible for HeartSheet therapy cannot undergo MRI or CT because of an implanted pacemaker, cardioverter defibrillator, or CRT, or impaired renal function. Because most of these patients are not eligible for MRI or CT, LVEF should be evaluated by echocardiography. LVEF measurements will be analyzed centrally at the core laboratory. In patients eligible for MRI or CT, LVEF should be measured by these methods. The results of MRI or CT should be compared with echocardiogram to confirm the similarity in the overall LVEF behavior.

Table 9.1. Outline of the approval condition-based post-marketing evaluation (draft)

Objective	To evaluate the efficacy and safety of HeartSheet
Survey method	All-case surveillance
Population	All patients who underwent skeletal muscle harvest to receive HeartSheet
Follow-up period	Between the conditional and time-limited approval and reapplication for approval
Efficacy evaluation	The primary and secondary endpoints (except for "success or failure of transplantation") will be compared between the HeartSheet and control groups. • Primary endpoint - Time to cardiac death • Secondary endpoints - Time to admission due to a cardiovascular event - Percentage of patients achieving ≥5% improvement in LVEF at 6 months after transplantation of HeartSheet - Success or failure of transplantation
Safety evaluation	• Incidence of cardiac death • Incidence of serious aggravation of heart failure resulting in artificial heart implantation or heart transplantation • Incidence of product-related adverse events, etc. • Priority investigation items - Aggravation of heart failure - Arrhythmia - Local infection - Events associated with thoracotomy and transplantation procedures (including diastolic dysfunction due to pericarditis constrictive, localized inflammation and associated pericardial effusion, effect on coronary arteries, and adhesion) - Onset and relapse of tumors - Events caused by skeletal muscle harvest (e.g., wound complication, post procedural swelling).
Target sample size	Since the evaluation involves all patients undergoing skeletal muscle harvest for preparing skeletal myoblast-derived cell sheets, the target sample size will not be defined. At ≥2 years posttransplantation, the prognosis of the first 60 patients enrolled in the survey will be compared with the prognosis of control patients, to evaluate the efficacy of HeartSheet (the primary endpoint). [Rationale] The 2-year survival rate according to the Seattle heart failure model was predicted to be approximately [redacted]% in the patient population with disease characteristics eligible for HeartSheet therapy, and the 2-year survival rate in patients not treated with HeartSheet (the control group) was assumed to be [redacted]%. On the other hand, the 2-year survival rate in patients treated with HeartSheet (the HeartSheet group) was assumed to be [redacted]% based on the results of the Japanese clinical trial and a clinical study (Study HM0801) of HeartSheet. When survival time was analyzed by [redacted] test by assuming the ratio of HeartSheet-treated patients/control patients to be 1:1, two-sided significance level [redacted]%, statistical power [redacted]%, and the follow-up period ≥2 years after transplantation or enrollment, then the number of patients required in the HeartSheet group and in the control group was [redacted] and [redacted], respectively. Therefore, the target sample size to be analyzed in the HeartSheet group was determined as 60 to allow possible drop-outs of approximately [redacted]%.

Table 9.2. Outline of the plan for collecting information on the clinical course of patients not treated with HeartSheet (the control group) (draft)

Objective	To survey the characteristics, prognosis, and disease progression in patients with severe heart failure eligible for HeartSheet but not requiring heart transplantation or implantation of a left ventricular assist device.
Survey method	A multi-center, prospective clinical study
Population	Patients with severe heart failure due to ischemic heart disease who have already received optimal drug therapy or other treatments and meet the following inclusion criteria. [Inclusion criteria] • Patients with chronic ischemic heart disease • LVEF ≤35% • NYHA III or IV • Patients who have been treated with optimal drug therapy using anti-heart failure drugs such as ACE inhibitor, ARB, β-blocker, and diuretics for ≥3 months • Patients who have received the standard treatment including invasive treatment (e.g., CABG, mitral valve replacement, left ventricular restoration, CRT, PCI) ≥3 months before
Survey period	Between the first day of enrollment and reapplication for approval (reapplication is submitted after the conditional and time-limited approval.)
Follow-up period	≥2 years after enrollment
Investigation items	• Patient characteristics Age (at baseline), sex, height, body weight, body surface area, primary disease, medical history, concurrent illness, treatment history of heart disease, drug therapy, NYHA class, cardiac function assessed by echocardiography (LVEF, LVEDV, LVESV, LVDd, LVDs), heart rate, hematology (BNP, hemoglobin, platelet count, albumin, sodium, potassium, creatinine) • Evaluation of events Time to heart disease-related death, time to admission due to a cardiovascular event • Evaluation of cardiac function (echocardiography) • NYHA class • Laboratory test (BNP)
Target sample size	120 (patients who have been followed up for ≥2 years) [Rationale] The number of patients to be surveyed should be twice the number of patients in the skeletal myoblast-derived cell sheet transplantation group (60 patients) to select characteristic-matched patients for evaluating the efficacy of HeartSheet.

PMDA's view:
The plan for the post-marketing evaluation has been designed to evaluate and compare the prognosis of patients undergoing the transplantation of HeartSheet, obtained from the use-results survey, with the prognosis of external control patients eligible for but not treated with HeartSheet. If time to cardiac death, the primary endpoint, in the HeartSheet group is statistically significantly prolonged as compared with the control group, HeartSheet is considered effective. Ideally, the prognosis of patients treated with HeartSheet should be compared with the prognosis of patients randomly assigned to a concurrent control group. However, a randomized comparative study of HeartSheet will become unfeasible once HeartSheet is made available for clinical use; the applicant therefore has no choice but to use external control patients. Another option for the control group is to enroll patients receiving similar treatment other than HeartSheet at medical institutions providing HeartSheet therapy. However, such patients may tend to receive HeartSheet therapy because they are eligible; this may result in a situation where most control patients are ineligible for HeartSheet therapy (i.e., patient selection bias). Therefore selecting control patients from other medical institutions is acceptable. HeartSheet therapy is restricted to qualified physicians, surgeons, and medical institutions that are able to prepare skeletal myoblast-derived cell sheets and provide appropriate perioperative management [see Section 8.3.6. of the Review Report (1)]. Control patients may therefore be selected from unqualified medical institutions that treat patients with severe heart failure who have disease characteristics similar to those of patients receiving treatment in qualified medical institutions. In this situation, attention should be paid to differences in patient characteristics among medical institutions. To adjust the differences, patients treated with HeartSheet and control patients should be matched for baseline characteristics to minimize the bias in efficacy evaluation.

Time to cardiac death, the primary endpoint, can be evaluated in routine clinical practice. It is a hard endpoint unlikely to be biased by a subjective view of patients or evaluators or differences among medical institutions. This suggests that the use-results survey, although not a blinded post-marketing

clinical study, can evaluate the efficacy and safety of HeartSheet with a certain level of accuracy. Time to admission due to a cardiovascular event, a secondary endpoint, is considered to provide clinically significant information as does time to cardiac death. It is thus important to evaluate the efficacy of HeartSheet based on a statistical comparison between the HeartSheet and control groups. Meanwhile, the endpoints of "cardiac death" and "admission due to a cardiovascular event" may not allow accurate efficacy evaluation if such events occur in a small number of patients. Therefore the percentage of patients achieving ≥5% improvement in LVEF at 6 months after transplantation of HeartSheet, another important secondary endpoint for evaluating the cardiac function, should also be statistically compared between the HeartSheet and control groups, to evaluate the efficacy of HeartSheet. The applicant proposed an efficacy endpoint, "the percentage of patients achieving ≥5% improvement in LVEF at 6 months after transplantation of HeartSheet." PMDA considers that "patients with ≥5% improvement in LVEF" can be interpreted as "patients with a certain level of improvement in cardiac function," based on the applicant's explanation for the endpoint and a report on the accuracy of LVEF measurements by echocardiography (*Eur J Echocardiography.* 2006;7:373-8). According to the report, LVEF by echocardiography in 25 patients varied in a range of 0.350% ± 2.012% (95% CI, –3.594% to 4.293%) when measured by a single person (intra-measurer variability), and in a range of –0.906% ± 2.701% (95% CI, –6.201% to 4.388%) when measured by different persons (inter-measurer variability); an approximately ±5% difference in LVEF measurements is considered a measurement error. However, as discussed in the Review Report (1), measurements of LVEF by echocardiography may be biased by measurers. To avoid this, evaluation should be centralized to the core laboratory. The measuring procedures including imaging, tracing, etc. should be determined in advance. In addition to echocardiography, more objective testing methods (e.g., MRI, CT, and cardiac pool scintigraphy) should be used to measure LVEF whenever feasible to confirm that echocardiography results do not differ from the results of other testing methods. This will increase the robustness of echocardiography-based evaluation.

Accordingly, the secondary endpoints (time to admission due to a cardiovascular event, the percentage of patients achieving ≥5% improvement in LVEF at 6 months after transplantation of HeartSheet) are regarded as important parameters in efficacy evaluation. These endpoints should be statistically compared between the HeartSheet and control groups. The applicant should develop a concrete, appropriate statistical analysis plan before analyzing the results of the endpoints, taking into consideration of multiplicity.

2.6. Safety evaluation of adventitious agents in collagenase [see Section 3.1.2.2. of the Review Report (1)]

Collagenase is a material of biological origin used in the manufacturing process of HeartSheet. The collagenase used in the clinical trial will be changed to another collagenase that meets the Standards for Biological Ingredients; the new collagenase will be used in the manufacturing process after approval (Table 3.3 in Section 3.1.2.2. of the Review Report [1]). The applicant explained the conformity of the new collagenase to the Standards for Biological Ingredients, as follows:

The new collagenase preparation is an enzyme cocktail containing both collagenase and thermolysin, and thermolysin is produced by bacteria cultivated using a culture medium containing bovine milk-derived casein (Table 9.3). The casein-containing medium has been heat-processed (121°C for 20 minutes) and thus is shown to meet the Standards for Biological Ingredients.

Table 9.3. Biological material contained in the new collagenase

Use	Material or raw material thereof		Animal	Site of use	Country of origin
[redacted]	Collagenase	Casein	Bovine	Milk	[redacted]

PMDA accepted the explanation of the applicant.

2.7. Stability of skeletal muscle container (filled with tissue transport solution) [see Section 4.3. of the Review Report (1)]

The applicant's explanation:

The applicant evaluated the antibacterial activity of antibiotics added to the tissue transport solution to be filled in the skeletal muscle container. The antibiotics did not show the desired antibacterial activity under the condition of skeletal muscle transportation (2°C-8°C). The applicant therefore decided not to add antibiotics to the tissue transport solution. The stability of the skeletal muscle container (filled with the tissue transport solution) not containing antibiotics can be evaluated from the past stability data [see Table 4.3 in Section 4.3. of the Review Report (1)]. Based on the data, the shelf life of the skeletal muscle container (a secondary constituent part) should be 12 weeks, when stored at 2°C to 8°C.

PMDA accepted the explanation of the applicant.

2.8. Verification [see Section 3.3.1. of the Review Report (1)]

In response to the advice given by PMDA during the review for approval, the applicant proposed changing the scope and names of some manufacturing processes of the primary constituent part (cryopreserved cells) so that the names and description of the processes accurately reflect the actual procedures. The applicant also proposed modifying the manufacturing process parameters and in-process control tests (Table 3.7 in Section 3.3.1. of the Review Report [1]). Also, [redacted] was added to the in-process control tests in the Step 1 ([redacted]), and sterility test and mycoplasma testing [redacted], using the supernatant of the discarded wash fluid, were added to the in-process control tests in the Step 13 ([redacted]) in order to control the risk of microbial contamination.

Pursuant to [redacted], [redacted] was removed from the specifications of the secondary constituent part (Table 3.10 in Section 3.3.1. of the Review Report [1]) and, instead, [redacted] against [redacted] was added as an acceptance test for skeletal muscle at the manufacturing site.

As for the quality control by verification, the manufacturing process parameters and in-process control tests for the primary constituent part (cryopreserved cells) and the specifications of a secondary constituent part (skeletal muscle container filled with tissue transfer solution) were changed from those shown in Tables 3.7 and 3.10 in the Review Report (1) to those shown in Tables 9.4 and 9.5 below.

Table 9.4. Manufacturing process parameters and in-process control tests for the primary constituent part (cryopreserved cells)

Process	Control items	
[Step 1] Preparation of [redacted]	In-process control tests	[redacted] [redacted] Endotoxin Sterility [redacted]
[Step 2] Preparation of [redacted]	Control of raw materials	[redacted]
	In-process control tests	[redacted] [redacted] Sterility
[Step 3] Preparation of [redacted]	In-process control tests	[redacted] [redacted] Endotoxin Sterility
[Step 4] Preparation of [redacted]	In-process control tests	[redacted] [redacted] Endotoxin Sterility
[Step 5] Preparation of [redacted]	In-process control tests	[redacted] [redacted] Endotoxin Sterility
[Step 6] Preparation of [redacted]	Control of raw materials	Endotoxin Sterility
[Step 7] Receipt of skeletal muscle	Control of raw materials	[redacted] [redacted] Virus testing (HBV, HCV, HIV, and HTLV-1) [redacted]
[Step 8] [redacted]	Parameters	[redacted]
	In-process control tests	[redacted]
[Step 9] [redacted]	Parameters	[redacted]
	In-process control tests	[redacted]
[Step 10] [redacted]	Parameters	[redacted]
	In-process control tests	[redacted] Sterility [redacted]
[Step 11] [redacted]	Parameters	[redacted]

Table 9.4. Manufacturing process parameters and in-process control tests for the primary constituent part (cryopreserved cells)

Process	Control items	
[redacted]	[redacted]	
	In-process control tests	[redacted]
[Step 12] [redacted]	Parameters	[redacted]
	In-process control tests	[redacted]
[Step 13] [redacted]	Parameters	[redacted]
	In-process control tests	[redacted]
		Sterility
		Mycoplasma testing [redacted]
[Step 14] Labeling, filling, and capping	Parameters	[redacted]
	In-process control tests	Container integrity
[Step 15] Cryopreservation	Parameter	[redacted]
	In-process control tests	[redacted]

Table 9.5. Specifications for skeletal muscle container filled with tissue transport solution (a secondary constituent part)

Secondary constituent part	Tests	Method
Skeletal muscle container (filled with tissue transport solution)	Appearance	Visual inspection
	Description	Visual inspection
	pH	Japanese Pharmacopoeia
	Osmolarity	Japanese Pharmacopoeia
	Bacterial endotoxins	Japanese Pharmacopoeia
	Sterility	Japanese Pharmacopoeia

PMDA concluded that the proposed changes and modifications do not substantially change the manufacturing process and the added parameters and control items are aimed at reducing the risk of microbial contamination. PMDA therefore accepted the changes and modifications proposed by the applicant, concluding that they do not affect the conclusion of the Review Report (1).

2.9. Results of Compliance Assessment Concerning the Data Submitted in the New Regenerative Medical Product Application and Conclusion by PMDA

2.9.1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection and data integrity assessment were conducted in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. The inspection and assessment revealed no particular problems. PMDA thus concluded that there should be no problem with conducting a regulatory review based on the submitted product application documents.

2.9.2. PMDA's conclusion on the results of GCP on-site inspection

A GCP[16] on-site inspection was conducted in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics for the data submitted in the new regenerative medical product application (Attached document 7). PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted product application documents.

2.10. Necessity of the designation of regenerative medical product

PMDA's conclusion:

As regards the designation of biological products, specified biological products, and designated regenerative medical products (PFSB/ELD Notification No.1105-1 and No.1105-2, dated November 5, 2014), HeartSheet is a regenerative medical product manufactured from human and animal-derived raw materials, and it is difficult to inactivate or remove pathogens during the manufacturing process. However, HeartSheet need not be classified as a designated regenerative medical product, for the following reasons.

- The raw material is skeletal muscle derived from the patient himself/herself.
- All biological materials are manufactured through processes capable of inactivating or removing pathogens.
- Among the biological materials, human serum albumin is a drug approved in Japan. HeartSheet contains an extremely small amount of human serum albumin, compared with the standard dose or a cumulative dose of human serum albumin administered as a drug.

3. Overall Evaluation

As a result of the above review, PMDA concludes that HeartSheet may be approved after modifying the indication or performance and dosage and administration or method of use as shown below, with the following conditions for approval, provided that the package insert includes precautionary advice and healthcare professionals are informed of how to use HeartSheet properly after the market launch. The approval should be conditional and time-limited in accordance with Article 23-26 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals and Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. The time limit should be 5 years according to the Article. HeartSheet need not be classified as a designated regenerative medical product.

[16] The application of HeartSheet was submitted as a regenerative medical product. However, the clinical trial was conducted under the Ministerial Ordinance on GCP for drugs because it was before the enforcement of the Ministerial Ordinance on GCP for regenerative medical products.

[Indication or performance]
Treatment of patients with severe heart failure due to ischemic heart disease unresponsive to standard treatments including drug and invasive therapies who meet all of the following criteria.

Eligibility criteria:
- NYHA class III or IV heart failure; and
- Resting left ventricular ejection fraction ≤35%

[Dosage and administration or method of use]
The product is used according to the following method.

Prior to the production of skeletal myoblast-derived cell sheets

1) Skeletal muscle is harvested from the patient. As a general procedure, the harvesting is performed according to the procedure for diagnostic muscle biopsy for neuromuscular diseases. Skeletal muscle should be harvested from the quadriceps, in principle, but may be harvested from any other appropriate site of the body depending on the patient's condition. The harvested skeletal muscle is delivered in a dedicated container to the facility designated by the marketing authorization holder.

2) Blood is collected from the patient to separate serum. The separated serum is delivered in a container to the facility designated by the marketing authorization holder.

Preparation and transplantation of skeletal myoblast-derived cell sheets

1) Using the secondary constituent parts, 5 skeletal myoblast-derived cell sheets (preferably 6 sheets including 1 spare) are prepared from the cryopreserved cells. Each step is taken aseptically.

2) Each of 5 skeletal myoblast-derived cell sheets is sequentially transplanted onto the surface of the heart. The transplantation procedure is usually performed through left thoracotomy.

[Conditions for approval]

1. The applicant is required to ensure that the product is used by physicians and surgeons with adequate knowledge and experience in severe heart failure and thoracotomy at medical institutions with capacity for emergency response under a system that ensures appropriate patient control through laboratory tests, etc.
2. The applicant is required to conduct an approval condition-based post-marketing evaluation in all patients transplanted with the product during the period between the conditional and time-limited approval and reapplication for marketing approval.