Report on the Deliberation Results

June 22, 2012

Office of Medical Devices Evaluation
Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

Category	Instrument & Apparatus 7, Organ function replacement device
Term Name	Human autologous tissue for transplantation
Brand Name	JACC
Applicant	Japan Tissue Engineering Co., Ltd. (J-TEC)
Date of Application	August 24, 2009 (Application for marketing approval)

Results of Deliberation

The results of deliberation of the Committee on Medical Devices and *In-vitro* Diagnostics on June 22, 2012, are as described below. The committee concluded that this result should be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product may be approved with a re-examination period of 7 years under the following conditions. The product is classified as a biological product, but is not classified as a specified biological product.

Conditions of Approval

1. The applicant is required to take appropriate measures to ensure that the product is used in eligible patients by surgeons with a full understanding of its efficacy and safety and sufficient knowledge and experience in the treatment of traumatic cartilage defect and osteochondritis dissecans of the knee at medical institutions with facilities that enable such surgeons to perform relevant procedures.
2. The applicant is required to conduct use-results surveys involving all patients treated with this product for a certain period in the post-market stage to collect data on the efficacy and safety of the product, and take appropriate measures as necessary.

Review Report

June 5, 2012
Pharmaceuticals and Medical Devices Agency

The following are the results of the review of the following medical device submitted for marketing approval conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Category	Instrument & Apparatus 7, Organ function replacement device
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Reviewing Office	Office of Biologics II

Review Results

June 5, 2012

Classification	Instrument & Apparatus 7 Organ function replacement device
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Results of Review

JACC is a combination product of autologous cultured chondrocytes and collagen gel, and the product is created by culturing autologous chondrocytes in a three-dimensional environment using atelocollagen gel. After transplantation of JACC to a full-thickness cartilage defect in the knee, cartilage matrix produced by autologous cultured chondrocytes contained in the product forms a cartilage-like tissue that fills and repairs the defect, resulting in reduction of pain and improvement of joint function. JACC is the first cellular and tissue-based product developed for this intended use in the field of orthopedics in Japan.

Despite the proposed intended use of JACC, the nonclinical and clinical data submitted has not demonstrated that JACC fills the cartilage defect and produces cartilage matrix, thereby repairing the cartilage tissue.

The clinical study included different patient populations having different diagnoses and defect sites that should be separately evaluated for efficacy and therefore did not appropriately evaluate the efficacy according to the difference in the characteristics of the target diseases. For this reason, PMDA has concluded that the efficacy of JACC cannot be evaluated on the basis of the clinical study data submitted. However, when the efficacy of JACC was individually evaluated in each patient from the clinical point of view, the clinical symptoms of traumatic cartilage defect or osteochondritis dissecans tended to improve at 12 months post-transplantation although the extent of the contribution of JACC remains unclear.

The safety of JACC can be assured up to 12 months after transplantation although it has risks for infection, etc. because of its nature as a biological product.

In conclusion, as far as JACC is used to improve the clinical symptoms of traumatic cartilage defect or osteochondritis dissecans in patients with a cartilage defect of ≥ 4 cm^2 for which no standard surgical treatment is available, JACC has clinical significance because it can offer a new treatment option to the patient populations.

To market JACC, the applicant should take necessary measures in order to ensure that the product is properly used based on the clinical position of JACC. Because only very limited data are available from the clinical study, the applicant should conduct a use-results survey involving all patients treated with JACC for a certain period in the post-market stage in order to collect information on the safety and efficacy of JACC.

As a result of its review, PMDA has concluded that JACC may be approved for the following intended use and indication, with the conditions below, and that the application should be presented to the Committee on Medical Devices and *In-vitro* Diagnostics for further deliberation.

Intended Use or Indication

Alleviation of clinical symptoms of traumatic cartilage defect or osteochondritis dissecans (excluding gonarthrosis) of the knee only when no other treatment options are available,[1] and it is used at a cartilage defect with a defect size of $\geq$4 cm^2

Conditions of Approval

1. The applicant is required to take appropriate measures to ensure that the product is used in eligible patients by surgeons with a full understanding of its efficacy and safety and sufficient knowledge and experience in the treatment of traumatic cartilage defect and osteochondritis dissecans of the knee at medical institutions where the surgeons can treat such conditions.
2. The applicant is required to conduct a use-results survey involving all patients treated with the product for a certain period in the post-marketing stage to collect data on the efficacy and safety of the product, and take appropriate measures as necessary.

[1] A minor change was made to the Japanese text (with no change to the English translation) after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics.

Review Report

June 5, 2012

I. Product Submitted for Approval

Category	Instrument & Apparatus 7, Organ function replacement device
Term Name	Human autologous tissue for transplantation
Brand Name	JACC
Applicant	Japan Tissue Engineering Co., Ltd. (J-TEC)
Date of Application	August 24, 2009
Proposed Intended Use	1. Intended patient population and diagnosis Patients with full-thickness cartilage defect in the knee 2. Expected situations of use Patients having the above condition caused by transient external force such as accidents or repeated external force such as sports In the cases that alignment abnormality, subchondral bone defect or necrosis, or any complication, including fracture, ligament injury, and meniscus injury, is present around the recipient site, patients must be treated for such concurrent condition before the product is transplanted. 3. Expected results The atelocollagen gel containing chondrocytes fill a defect site. Chondrocytes contained in the product and cartilage matrix produced by chondrocytes repair the cartilage defect. 4. Indications Filling/repair of cartilage defect of the knee and improvement in joint function
Items Warranting Special Mention	None
Reviewing Office	Office of Biologics II

II. Product Overview

JACC is an autologous cultured cartilage created by culturing autologous chondrocytes from the patient's cartilage tissue embedded in atelocollagen gel. JACC is intended to fill and repair a full-thickness cartilage defect in the knee with produced cartilage matrix, as well as to improve joint function.

At the implantation of JACC, a periosteal patch is harvested from the anteromedial surface of the proximal tibia and fixed over JACC with sutures, and then the wound is closed.

III. Summary of the Data Submitted and the Outline of Review Conducted by PMDA

The data submitted by the applicant in the application and the applicant's responses to the inquiries from PMDA, and the outline of the reviews on the applicant's responses are outlined below.

This submission has the major problems described below.

The applicant's originally proposed intended use was "Chondrocytes contained in JACC and cartilage matrix produced by chondrocytes repair cartilage tissue" and "Filling/repair of cartilage defect of the knee and improvement in joint function." PMDA, however, concluded that none of the quality, nonclinical, and clinical studies provided data supporting these claims [see Sections "2. Physicochemical properties and specifications," "5. Performance," and "6. Clinical data"]. Since no evidence is available that supports the applicant's claim that JACC repairs cartilage defect, JACC cannot be positioned as a regenerative medicine for cartilage tissue.

There were problems with the clinical study design, including selection of the target diseases and evaluation method of efficacy. It was difficult to assess the efficacy and clinical position of JACC for the proposed indication ("patients with full-thickness cartilage defect of the knee") [see Section "6. Clinical data"].

However, the proposed target diseases of JACC include those that cannot be fully treated by conventional therapies or not be studied in a clinical study because of a limited number of patients. PMDA assessed the efficacy of JACC in individual patients from the clinical point of view by making best use of the results of the clinical study in order to evaluate the potential of JACC as a new option for the treatment of rare diseases that cannot be fully treated by conventional therapies.

The details of the above major problems in the application and the results of efficacy evaluation conducted by PMDA are presented below.

1. Origin or history of discovery, use in foreign countries, and other information

1.A Summary of the submitted data

1.A.(1) Origin or history of discovery or development

Histologically, the articular cartilage is a hyaline cartilage consisting of extracellular matrix, including chondrocytes, collagen, proteoglycan, and water. This cartilage has a structure and function that can withstand static and dynamic loads on the joint. Spontaneous repair of cartilage defects such as traumatic cartilage defect and osteochondritis dissecans caused by excessive joint loading is unlikely to occur because of the low self-repair capacity of articular cartilage. A cartilage defect is accompanied by clinical symptoms, including pain, joint swelling, and a limited range of motion, eventually leading to secondary osteoarthritis because of changes in the characteristics of chondrocytes, damage to the subchondral bone, etc.

A cartilage defect of the knee is conservatively treated by heat therapy or cooling therapy, with or without drug therapy. These therapies are, however, symptomatic and have limited effects. The following surgical options are also available: (a) joint debridement, (b) microfracture, and (c) autologous osteochondral mosaicplasty.

(a) Arthroscopic joint debridement is performed to smoothen out the cartilage surface surrounding the defect in order to protect the cartilage of the opposite side, but the technique does not repair the cartilage defect itself. (b) Microfracture is a surgical technique that promotes the leakage of blood and bone marrow from drill holes made in the subchondral bone so that cells in the blood and bone marrow can repair cartilage tissue. However, the tissue formed by such cells is fragile fibrocartilage and would wear out in the long term. This technique is indicated only for relatively small defects, approximately 1 cm^2 in size. (c) Autologous osteochondral mosaicplasty uses a normal osteochondral graft containing normal hyaline cartilage harvested from a non-weight-bearing area of the patient, which has the advantage of repairing the defect with the hyaline cartilage. However, the size of the recipient site is limited because osteochondral tissue to be harvested should be the same size as the defect.

Recently, published literature has reported a new treatment method that can address problems, including a poor long-term outcome resulting from repair with fragile fibrocartilage formed after microfracture surgery (Gudas R, et al. *Knee Surg. Sports Traumatol. Arthrosc.* 2006;14:834-842) and the infeasibility of autologous osteochondral mosaicplasty because of a shortage of donor sites. First, a small amount of cartilage tissue is harvested from a non-weight-bearing area of the joint of the patient's knee. Chondrocytes are isolated from this tissue and cultured in monolayer to prepare a cell suspension, which is then transplanted to a defect site (Takaoka K. *Standard Orthopedics 10th Edition:* Igaku-Shoin Ltd.; 2008:56-59, Brittberg M, et al. *N. Engl. J. Med.* 1994;331:889-895). However, this surgical technique has the following shortcomings: the dedifferentiation of chondrocytes in monolayer culture and the possible leakage of a cell suspension from the recipient site because of early postoperative weight loading on the knee. In order to solve these problems, Ochi et al. started the development of an autologous cultured cartilage in 1997. Their technique involved autologous chondrocytes seeded on atelocollagen gel and cultured in a three-dimensional environment (Ochi M, et al. *J. Bone Joint Surg. Br.* 2002;84:571-578).

JACC is an autologous cultured cartilage developed by the applicant based on the technology transferred from Ochi et al. An application for JACC was filed in Japan based on the results of a clinical study in patients with traumatic cartilage defect (including cases associated with ligament injury), osteochondritis dissecans, and gonarthrosis. The clinical study was initiated in 2004.

1.A.(2) Usage conditions in foreign countries and Japan

As of June 2012, JACC is not approved in any country or region. No autologous cultured chondrocyte product has been commercialized to date in Japan. Outside of Japan, no autologous cultured chondrocyte product that is manufactured using atelocollagen or other matrices in a similar manner to JACC is approved. On the other hand, some autologous cultured chondrocyte products produced in monolayer culture are available overseas. Carticel (Genzyme Tissue Repair, US) was approved in 1997 as a biologic

in the US and has been reportedly used in ≥14,000 patients. In South Korea, Chondron™ (Cellontech) was approved in 2001 as a bio-pharmaceutical product. In Europe, ChondroCelect™ (TiGenix, Belgium) obtained the "Positive Opinion" from the European Medicines Agency in June 2009 and approved as an Advanced Therapy Medicinal Product in October 2009 (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Summary_for_the_public/human/000878/WC500026033.pdf). This product has been reportedly used in approximately 700 patients so far.

Findings at submission of application for confirmation

Since JACC falls under the category of cellular and tissue-based products, submission of an application for confirmation of the safety and quality of this investigational device (hereinafter referred to as "confirmation application") prior to clinical study notification was necessary in accordance with the "Quality and Safety Assurance of Cell/Tissue-derived Medical Devices and Pharmaceuticals" (PMSB Notification No. 906 dated July 30, 1999, issued by Director of the Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare). Confirmation application for JACC was submitted to the Minister of Health, Labour and Welfare on September 7, 2001. At a meeting on April 25, 2003, the Committee on Pharmaceutical Affairs and Biotechnology of the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) decided the continued deliberation of the confirmation application for JACC (http://www.mhlw.go.jp/shingi/2003/04/txt/s0425-2.txt). The applicant's responses to issues pointed out in this meeting were deliberated by the Committee on Biotechnology of the PAFSC at a meeting on February 6, 2004 and accepted on February 19, 2004. The applicant was requested to discuss the following issues (http://www.mhlw.go.jp/shingi/2004/02/txt/s0206-1.txt):

- Culture methods using serum-free and autologous serum media
- Acceptance criterion for the amount of residual bovine serum albumin in the final product
- Verification of the usefulness of JACC in an animal study(ies) in dogs (beagle dogs) and other appropriate animal species
- Conduct of the clinical study meeting scientific and ethical standards, including development of clear criteria for selection of eligible patients, and inclusion of the relevant information in the informed consent form

2. Physicochemical properties and specifications

2.A Summary of the submitted data

2.A.(1) Raw materials

2.A.(1).1) Collection, transportation, and receipt of cartilage tissue

Patient's cartilage tissue to be used as a raw material is harvested by an attending surgeon from the tapetum, and superficial, middle, and deep layers of the articular cartilage in a non-weight-bearing area (proximal femur or femoral intercondylar area) where the joint function is unlikely to be affected by tissue collection. The amount of tissue to be collected is 0.2 or 0.4 g depending on the number of product units to be manufactured and the effective diameter of each product unit. Tissue collection from the calcified layer, subchondral bone, and trabecular bone should be avoided because these tissues contain osteocytes, osteoblasts, bone marrow cells and other cells. A loose body in the joint (free cartilage piece) must not be used as a material source. Cartilage tissue harvested is immersed in Dulbecco's phosphate

buffered saline (DPBS), placed in a tube for tissue transportation, and transported to the manufacturing site in a special insulated transport container.

Upon receipt of the cartilage tissue at the manufacturing site, acceptance tests are performed to confirm that the insulated transport container is sealed; that the tissue was received within 84 hours after shipping; that there is no damage to or leakage from the tissue transport tube; and that the cartilage tissue is immersed in the transport media (Table 1). The cartilage tissue is stored at 2°C to 20°C as necessary. The manufacture using the tissue should be initiated within [redacted] hours after collection. Since autologous chondrocytes are derived from the patient's own cartilage tissue, donor screening is not performed. To assess success in manufacturing the product and treatment outcome, and to determine the cause of infection, if any, in patients, etc., blood samples collected are stored at or below −[redacted]°C for a certain period of time, i.e., the shelf life of the product plus 10 years. This period was determined based on the storage period for biological materials used for manufacturing specified biological medical devices, etc.[2] defined in the Ordinance of the Ministry of Health, Labour and Welfare (MHLW) No. 169 dated December 17, 2004.

Table 1. Acceptance tests of cartilage tissue

	Specimen	Test method, etc.
Transportation condition of tissue	A tissue transport tube placed in an insulated transport container	Confirm that (a) the insulated transport container is sealed and (b) the insulated transport container containing the collected tissue (shipped from the medical institution) is received (by the manufacturer) within 84 hours after the insulated transport container has been shipped to the medical institution (from the manufacturer). If either (a) or (b) is not met, the cartilage tissue specimen must be disposed of.
Appearance of cartilage tissue	Cartilage tissue contained in the tissue transport tube	Confirm that (a) there is no damage to or leakage from the tissue transport tube and (b) the cartilage tissue is immersed in DPBS. If either (a) or (b) is not met, whether to continue the manufacture should be discussed with the medical institution.

2.A.(1).2) Biological materials other than cartilage tissue

Atelocollagen derived from bovine dermis ([redacted]) is used for manufacturing JACC. This atelocollagen is the same as [redacted] that was subjected to "washing, rinsing with water, enzyme treatment, and ion-exchange treatment" and "alkali treatment" to inactivate and remove viruses. Fetal bovine serum (FBS) derived from healthy cattle from Australia or New Zealand is subjected to a virus free test in accordance with EMEA/CPMP/BWP/1793/02 or FDA 9CFR113.53 and γ-irradiation before being used in manufacture. Trypsin derived from healthy swine pancreas is subjected to a virus free test in accordance with FDA 9CFR113.53 and γ-irradiation before being used in manufacture. This trypsin contains lactose derived from milk from cattle from the US as an excipient. Amphotericin B for Injection is a pharmaceutical drug (brand name, Fungizone for infusion 50 mg, Approval No. 22000AMX00242000). Sodium desoxycholate contained in this pharmaceutical drug is derived from bile from healthy cattle ([redacted]) and sheep ([redacted]). [redacted]

[2] Amended after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics (before amendment, "specified biological medical device")

[redacted]

To assess success in manufacturing a product(s) and treatment outcome, and to determine the cause of infection, if any, in patients, etc., approximately [redacted] mL each of the FBS and [redacted] trypsin [redacted] solution per batch are stored at or below −[redacted]°C for a certain period of time, i.e., the shelf life of the last product produced using this batch plus 10 years. This period was determined based on the storage period for biological materials used for manufacturing specified biological medical devices, etc.[3] defined in the Ordinance of MHLW No. 169 dated December 17, 2004.

2.A.(2) Manufacturing and shipping of JACC

2.A.(2).1) Manufacturing process

The tissue transport media (DPBS) is removed from the tissue transport tube and washed with [redacted] diluent and then with [redacted] times. The cartilage tissue is shaken in [redacted] solution at [redacted]°C ± [redacted]°C for [redacted] hours and then in [redacted] solution ([redacted] vol% [redacted], [redacted] vol% [redacted], [redacted] vol% [redacted] solution, [redacted] vol% [redacted], [redacted] vol% [redacted], and [redacted] units/mL) at [redacted]°C ± [redacted]°C for [redacted] hours. After [redacted] of [redacted] and [redacted] μm, chondrocytes collected by centrifugation are washed with a medium for preparation of cell suspension ([redacted] vol% [redacted], [redacted] vol% [redacted], [redacted] vol% [redacted] solution, [redacted] vol% [redacted], and [redacted] vol% [redacted]) and suspended in the same medium to make a viable cell concentration of [redacted] to [redacted] cells/mL. The cell suspension and atelocollagen (collagen concentration, [redacted]% to [redacted]%) are mixed at a ratio of [redacted] and seeded in a disk shape on a culture vessel using [redacted] with an inner diameter of [redacted], [redacted], [redacted], or [redacted] mm so that a density of [redacted] to [redacted] cells/cm^3 is achieved. After removal of [redacted], the culture vessel is allowed to stand at [redacted]°C ± [redacted]°C for [redacted] to [redacted] minutes to gelatinize the atelocollagen. To this, [redacted] mL of a cartilage culture medium per vessel ([redacted] vol% [redacted], [redacted] vol% FBS, [redacted] vol% [redacted] solution, [redacted] vol% Amphotericin B solution, [redacted] vol% gentamicin sulfate injection, and [redacted] vol% [redacted]) is added and incubated at [redacted]°C ± [redacted]°C in [redacted]% [redacted] for [redacted] days. The medium is changed every [redacted] to [redacted] days.

The cartilage culture medium is removed, the vessel is washed with [redacted] times, [redacted], and then washed with shaking in a washing container with [redacted] at [redacted]°C ± [redacted]°C for [redacted] to [redacted] hours.

In-process tests and release tests to be conducted in the manufacturing process are presented in Tables 2 and 3.

[3] Amended after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics (before amendment, "specified biological medical device")

Table 2. In-process tests

<table>
<tr><th></th><th>Specimen</th><th>Test method, etc.</th></tr>
<tr><td>[redacted] (at start of separation of chondrocytes)</td><td>[redacted]</td><td>[redacted] (in case of non-conformity, the cartilage tissue should be disposed of)</td></tr>
<tr><td>Morphological observation of cells in culture vessel ([redacted] and [redacted])</td><td>[redacted] of culture vessel</td><td>1) Visual inspection
(a) [redacted],
(b) [redacted], (c) [redacted], (d) [redacted], (e) [redacted], (f) [redacted]
(in case of non-conformity to (a), (b), (c), or (f), the culture vessel should be disposed of; in case of non-conformity to (d), the culture vessel should be disposed of if [redacted]; in case of non-conformity to (e), the culture vessel should be disposed of if [redacted])
2) [redacted] (≥[redacted] fold)
(a) [redacted], (b) [redacted] (in case of non-conformity to (a), the culture vessel should be disposed of; and in case of non-conformity to (b), all should be disposed of)</td></tr>
<tr><td>Morphology maintenance of cultured cartilage ([redacted])</td><td>[redacted] of cultured cartilage</td><td>[redacted] using [redacted] (in case of non-conformity, the cultured cartilage should be disposed of)</td></tr>
<tr><td>Appearance of cultured cartilage ([redacted])</td><td>[redacted] of cultured cartilage</td><td>(a) [redacted]
(b) The diameter is within the acceptance range.
<table>
<tr><th>Effective diameter (mm)</th><th>Acceptance range (mm)</th></tr>
<tr><td>10</td><td>10-13</td></tr>
<tr><td>15</td><td>15-18</td></tr>
<tr><td>20</td><td>20-23</td></tr>
<tr><td>25</td><td>25-28</td></tr>
</table>
(c) [redacted] (in case of non-conformity, the cultured cartilage should be disposed of)</td></tr>
</table>

Table 3. Specifications (release tests)

	Specimen	Test method, etc.
Viable cell count ( )	Mixture of collected from	The specimen is tested as conforming to Microbial Enumeration Tests (membrane filtration method) specified under the section of Microbial Limit Test of General Tests of the Japanese Pharmacopoeia; no colonies are formed (in case of non-conformity, all products should be disposed of).
Mycoplasma testing ( )	Mixture of collected from	PCR detects no mycoplasma (in case of non-conformity, all products should be disposed of).
( )	of the final product	(a) , (b) , (c) (in case of non-conformity to (a), the cultured cartilage should be re-packed in a new transport container; in case of non-conformity to (b), the product should be disposed of if ; in case of non-conformity to (c), the product should be disposed of).
Viable cell density of cultured cartilage ( )	Specimen for quality control	≥ cells/cm^3 (in case of non-conformity, all products should be disposed of)
Percentage of viable cells of cultured cartilage ( )	Specimen for quality control	The percentage of viable cells should be ≥ % (in case of non-conformity, all products should be disposed of).
of cultured cartilage ( )	Specimen for quality control	should be ≥ of the number of cells seeded (in case of non-conformity, all products should be disposed of).
concentration of cultured cartilage ( )	used in confirmation of the viable cell density of cultured cartilage	The concentration measured by should be μg/cm^3 (in case of non-conformity, all products should be disposed of).
Confirmation of of cultured cartilage* ( )	used in confirmation of the viable cell density of cultured cartilage	detected using should be % (in case of non-conformity, all products should be disposed of).
Confirmation of of cultured cartilage ( )	Specimen for quality control	measured by should be to mm (in case of non-conformity, all products should be disposed of).
Amount of residual bovine serum albumin (BSA) in cultured cartilage ( )	Specimen for quality control	The amount of residual BSA measured using should be ≤ μg/cm^3 (in case of non-conformity, all products should be disposed of).
Bacterial endotoxin ( )	Specimen for quality control	The specimen is tested by the gel-clot technique specified under the section of Bacterial Endotoxins Test of General Tests of the Japanese Pharmacopoeia; the endotoxin level should be EU/cm^3 (in case of non-conformity, all products should be disposed of).

* The acceptance criterion of of the cultured cartilage was calculated not from the observed data in the clinical study, but from the results of characterization of the knee cartilage derived from patients with gonarthrosis [see Section "2.A.(3).1) Characterization of knee cartilage derived from patients with gonarthrosis"].

2.A.(2).2) Packaging, labeling, and shipping

The cultured cartilage is immersed in the transport medium for cultured cartilage (vol% , vol% solution, and vol% solution) in the transport container. After the cap of the container is closed, a label carrying "Brand name," "Manufacturing No.," and "Shelf life" is attached to the transport container. The final product that conforms to (Table 3) is placed in the insulated transport container capable of maintaining the temperature between °C and °C for ≥ hours at an ambient temperature of °C or −°C and stored at °C ± °C before shipping. Once its conformity to the release tests (Table 3) is confirmed, the product is shipped. To assess success in manufacturing a product(s) and treatment outcome, and to determine the cause of

infection, if any, in patients, etc., remaining chondrocytes after the release tests are stored at or below −[redacted]°C for a certain period of time, i.e., the shelf life of the product plus 10 years. This period was determined based on the storage period for biological materials used for manufacturing specified biological medical devices, etc.[4] defined in the Ordinance of MHLW No. 169 dated December 17, 2004.

Upon receipt of the product at a medical institution, the insulated transport container is checked for sealing, tissue code in the manufacturing No., product size, and the number of products written on the label. The cultured cartilage transport container is visually checked for cracks, chips, and leakage. The transport medium for cultured cartilage is also visually checked for turbidity and foreign matters. Then, the product is stored at 8°C to 25°C until immediately before use.

2.A.(2).3) Measures to prevent mix-up in manufacturing process

The following measures are taken to prevent mix-up of patient's cartilage tissue and chondrocytes: Identification using tissue codes, such as [redacted], prohibition of handling cells derived from more than one patient in the same working area, follow-up investigation by operational records, and training of healthcare professionals.

Since JACC is a product manufactured from autologous chondrocytes, no donor screening for infection etc. or acceptance tests of collected cartilage tissue are performed. In addition, to prevent mix-up and reduce the risk for contamination of cultured chondrocytes and contamination of the environment, such as the manufacturing facilities, by cultured chondrocytes (cross contamination), cell and tissue specimens from only 1 patient are allowed to be handled in each working area.

The applicant has explained that they will obtain information regarding the results of screening for infection in patients from medical institutions in advance, if possible, and take appropriate measures to prevent cross contaminations via facilities, equipment, tools, and healthcare professionals.

2.A.(3) Physicochemical properties

The applicant submitted the following 2 results of characterization.

2.A.(3).1) Characterization of knee cartilage derived from patients with gonarthrosis

Suspension cells obtained by enzyme treatment of knee cartilage tissue specimens from 3 patients with gonarthrosis, intermediate product ([redacted] days of manufacture), final product ([redacted] days of manufacture), and long-term cultured product ([redacted] days of manufacture) were fixed and subjected to [redacted] immunostaining (chondrocytes), [redacted] immunostaining (chondrocytes), [redacted] immunostaining ([redacted] cells and [redacted] cells), [redacted] immunostaining ([redacted] cells and [redacted] cells), [redacted] staining ([redacted] cells), and [redacted] staining (osteoblasts) (Table 4). As the incubation was prolonged, the number of [redacted]-positive cells remained unchanged or increased. The number of [redacted]- and [redacted]-positive cells substantially increased, while that of [redacted]-positive cells profoundly decreased. No [redacted] stain-positive cells were observed during the incubation period. [redacted] stain-

[4] Amended after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics (before amendment, "specified biological medical device")

positive cells were detected in the final and long-term cultured products manufactured from [redacted] of 3 specimens tested.

Table 4. Percentage of stain-positive cells of specimens from 3 patients

Patient ID	[redacted]				[redacted]				[redacted]			
Percentage (%) of stain-positive cells of specimens for each staining	Cartilage tissue	Intermediate product	Final product	Long-term cultured product	Cartilage tissue	Intermediate product	Final product	Long-term cultured product	Cartilage tissue	Intermediate product	Final product	Long-term cultured product
[redacted] immunostaining	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] immunostaining	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] immunostaining	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] immunostaining	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] staining	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] staining	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Tissue specimens made from the product at each stage were subjected to [redacted] immunostaining, [redacted] immunostaining, [redacted] staining ([redacted]), [redacted] staining ([redacted]), and [redacted] staining. [redacted] staining of the final and long-term cultured products revealed the substantial proliferation of [redacted] or [redacted] on the surface layer, and [redacted] cells in the middle and deep layers. In [redacted] staining, almost no cells on the surface layer of each product were stained, while cells in the middle and deep layers were stained. [redacted], [redacted], and [redacted] staining was positive for all products, suggesting the presence of [redacted].

Based on the above, the middle and deep layer of JACC contain chondrocytes expressing both [redacted] and [redacted], while the surface layer contains cells expressing [redacted], [redacted], and [redacted] in a condition different from dedifferentiation.

In addition, the product at each stage was subjected to “Morphological observation of cells in the culture vessel,” “[redacted] of cultured cartilage,” and “Appearance test of cultured cartilage” in the in-process tests (Table 2). All of the intermediate and final products conformed to the acceptance criteria. However, the long-term cultured product made from [redacted] of the 3 specimens failed to conform to the acceptance criteria for the “Appearance of cultured cartilage” because of [redacted]. The non-conformity is considered attributable to the contamination of the cultured cartilage by [redacted] cells.

2.A.(3).2) Characterization of specimens from the clinical study

Table 5 shows the results of characterization of the extracellular matrix of the product (32 specimens) derived from 32 patients used in the clinical study. The applicant’s consideration on the results is shown below.

Table 5. Characterization of extracellular matrix of specimens obtained in the clinical study (number of subjects with positive result)

		Gonarthrosis (N = 6)	Osteochondritis dissecans (N = 6)	Traumatic cartilage defect (N = 20)	Total (N = 32)
Tissue staining	[redacted] staining				
	[redacted] immunostaining				
RT-PCR	[redacted]				
	[redacted]				
	[redacted]				
	[redacted]				

Of the 32 specimens, [redacted] and [redacted] were positive for [redacted] staining and [redacted] staining, respectively. RT-PCR using the same specimens confirmed the expression of mRNA of [redacted], [redacted], [redacted], and [redacted] in [redacted], [redacted], [redacted], and [redacted] of the 32 specimens, respectively.

[redacted] are expressed in [redacted] cells or [redacted] cells. In this test, however, the mRNA was expressed in [redacted] specimens. This finding indicates that the presence of [redacted] cells in JACC created by culturing chondrocytes embedded in atelocollagen gel. [redacted] is known to be expressed in [redacted] chondrocytes. In this test, however, the mRNA was expressed in [redacted] of the 32 specimens. Although the significance of the expression of [redacted] in cultured cartilage is unclear, it may be involved in [redacted] after transplantation because [redacted] was observed in the subchondral bone after transplantation of cultured cartilage in a transplantation study using an animal model.

2.A.(4) Quality control of specifications

The quality of the manufacturing process is controlled through confirmation tests (Table 6), in addition to acceptance tests (Table 1), in-process tests (Table 2), and release tests (Table 3).

Table 6. Confirmation tests

	Specimen	Test method, etc.
Mycoplasma testing	A mixture of [redacted] collected from [redacted] and [redacted] collected from [redacted]	DNA staining using indicator cell
Sterility test	A mixture of [redacted] collected from [redacted] and [redacted] collected from [redacted]	Membrane filtration method, Sterility Test, General Tests, Japanese Pharmacopoeia

"Gene expression of extracellular matrix" and "Hardness of cultured cartilage" were also tested. However, the applicant explained that it is not appropriate to include them in the specification for the reasons later discussed in Section "2.B.(2).1) Justification for the proposed tests and acceptance criteria in the specification" and Section "2.B.(3) Responses to the issues pointed out by the committee of the PAFSC at submission of confirmation application."

2.B Outline of the review conducted by PMDA

2.B.(1) Characterization

2.B.(1).1) Justification for specimens

PMDA considers that the quality attributes of cartilage tissue as a raw material and a cultured chondrocyte product may differ according to the patient's disease. Characterization of the product created using the cartilage tissue derived from patients with gonarthrosis requiring knee joint replacement (Table 4) detected [redacted] stain-positive cells (osteoblasts) in the product. Such cells are usually not present in the cartilage. On the basis of this finding, PMDA asked the applicant to explain the justification for claiming that the results of characterization of the joint cartilage tissue derived from patients with gonarthrosis and their resulting product can be extrapolated to the data for joint cartilage tissue derived from patients with different pathologies (traumatic cartilage defect and osteochondritis dissecans) and their resulting product.

The applicant's response:

The quality of the product used in the clinical study did not differ according to the patient's disease. This indicates that the cartilage at the donor site was well maintained in each disease. Although there is no clear evidence, the quality or characteristics of collected cartilage specimens are unlikely to profoundly differ between the diseases. Although characterization of the product revealed the presence of [redacted] stain-positive cells (osteoblasts) in the product created using the cartilage tissue from patients with gonarthrosis, this finding can be explained by the fact that these patients had a pathological condition that had been advanced to a level requiring joint prosthesis replacement. On the other hand, it is very difficult to collect the cartilage from healthy individuals. The use of cartilage tissue derived from patients with gonarthrosis, which is relatively available and most similar to the healthy cartilage, is therefore reasonable. Based on the above, the cartilage tissue derived from a patient with gonarthrosis is considered similar to the normal cartilage tissue provided that the product conforms to the specification. Although it does not necessarily have the same characteristics of the cartilage derived from a patient with traumatic cartilage defect or osteochondritis dissecans, the above characterization results can, therefore, be extrapolated to the data for joint cartilage tissues derived from patients with these diseases and their resulting product.

PMDA's view:

As explained by the applicant, it is difficult to collect normal cartilage. However, it is not acceptable to draw a conclusion on the potential for extrapolation without comparing quality attributes to a possible extent.

PMDA compared the data of the following key parameters to understand the quality attributes of JACC among the final product manufactured using [redacted] specimens from patients with gonarthrosis used in the characterization, the final product used in the clinical study ([redacted] specimens derived from individuals with normal cartilage and [redacted] specimens derived from patients with gonarthrosis, hereinafter "clinical study specimens"), and the final product manufactured using [redacted] specimens derived from patients with gonarthrosis used for validation of the manufacturing process:

(a) Viable cell density (cells/cm^3)
(b) Percentage of viable cells (%)

(c) [redacted] of cultured cartilage
(d) [redacted] concentration (μg/cm^3)
(e) Confirmation of [redacted] (number of positive specimens)
(f) Confirmation of [redacted] (number of positive specimens)

The test methods and conditions, etc. presented by the applicant differ between specimens. To confirm that the product characteristics do not depend on the patient's disease, it is necessary to compare the data on the parameters (a) to (f) determined using the same test methods and conditions, and to further collect and compare information regarding [redacted] using [redacted], included in the specification, the content of [redacted] and [redacted] double-positive cells, which are claimed to be important by the applicant as target cells, the content of each cartilage matrix, and the type and content of non-target cells. Although the comparative discussion by PMDA has limitations, the data obtained showed no profound difference in the product quality depending on the donor's disease.

In conclusion, although only very limited information is available regarding the product characteristics, considering PMDA's discussion on the important parameters to understand the characteristics of JACC and the infeasibility of obtaining additional specimens, it is inevitable to use, as a reference, the information from the specimens created using the cartilage derived from patients with gonarthrosis. However, the applicant should continue investigation/comparison on characterization of the product derived from the non-weight-bearing area of the cartilage using specimens for release, etc. of the post-marketing products with consent from patients, together with the description in Section "2.B.(1).2) Characteristics of cells comprising JACC" below, and reflect the results appropriately in quality control as required.

2.B.(1).2) Characteristics of cells comprising JACC

PMDA asked the applicant to explain the following issues about the target and non-target cells of JACC.

JACC is characterized by the presence of [redacted]- and [redacted]-producing cells. On the other hand, JACC also contains cells that are positive for [redacted] but do not produce [redacted] (Table 4). Considering the applicant's explanation about (a) the quality attributes only possessed by chondrocytes that are required for JACC to exert and maintain its function and the applicant's discussion that (b) contamination by [redacted] cells may have contributed to the failure to meet the specifications due to [redacted] in the long-term cultured product, PMDA asked the applicant to explain whether it is necessary to identify the type of non-target cells contained in JACC and to control and specify non-target cells.

The applicant's response:

For JACC to exert and maintain its function, cells need to be positive for [redacted]. Since this indicates that JACC needs to maintain at least the characteristics possessed by chondrocytes, the expression of [redacted] and the production of [redacted] are confirmed in release tests. The staining tests showed the presence of [redacted] and [redacted] double-negative cells despite the fact that the cells in the product used in the characterization were the cells that originally comprised the cartilage. Although this finding is considered attributable to contamination by non-target

cells, the following hypothesis can explain the negative test results of chondrocytes: [redacted] required for tests may have changed the staining property of [redacted] and [redacted]. While no [redacted]-positive cells are present on the surface layer of JACC, cells in the middle and lower layers are [redacted] and [redacted] double-positive cells. The presence of these cells and their matrices appear to contribute to the efficacy of JACC.

In the characterization, the presence of "[redacted] cells," "[redacted] cells," and "osteoblasts" was investigated as representative non-target cells that was potentially mixed in JACC because there were no established methods for identifying all cells contained in JACC. Not only [redacted] cells, [redacted] cells, and osteoblasts, but also other cells including blood cells can be mixed in JACC. The possibility of such contamination is, however, small and even if it occurs, these cells are very unlikely to specifically proliferate in the course of manufacture. In addition, the contamination by [redacted] cells is indirectly checked by confirmation of [redacted] in the final product. Furthermore, taking into account the fact that no adverse event attributable to contaminant cells occurred in the clinical study and considering the feasibility of tests in terms of the quantity of cells required for tests, it is unnecessary to control or specify contamination by non-target cells.

PMDA's view:
At present, only very limited information is available regarding the target cells, cartilage matrix, and non-target cells. The applicant's above discussion is only speculative.

As for the target cells, the content of [redacted] and [redacted] double-positive cells, which are considered important for JACC to exert its function, and a change in the content in the manufacturing process need to be confirmed to obtain information on product characteristics. Based on this information, the manufacturing process and quality control strategy should also be validated.

To investigate the cartilage matrix, [redacted] in the product has been quantified using the specimens for the release tests in the clinical study. However, no detailed information is available regarding the contents of [redacted] and [redacted], which also comprise the cartilage matrix and possibly contribute to the efficacy of JACC. Since the cartilage matrix consists of a variety of molecules, including proteoglycans and hyaluronic acids, there is room to examine cartilage matrix that is possibly involved in the mechanism of JACC.

According to the applicant's explanation, contamination by [redacted] cells may have contributed to the failure to meet the specification due to [redacted] in the long-term cultured product used in the characterization and contamination by these cells can be indirectly investigated by measuring [redacted] of JACC in the "appearance of cultured cartilage" (Table 2). If the contamination may cause the failure to meet the specification, the establishment of an acceptable range for such contamination should be highly necessary. However, the applicant has not discussed it yet. In fact, it is evident from the "percentage of staining-positive cells" (Table 4) that [redacted] of the target cells and non-target cells in the product are subjected to change during the culture step. Information on the types and contents of the target and non-target cells should be collected proactively. On the basis of such information, a review of the

manufacturing process and the necessity of additional quality control parameters should be considered continuously.

2.B.(2) Quality control and manufacturing conditions

2.B.(2).1) Justification for the proposed tests and acceptance criteria in the specification

PMDA asked the applicant to explain the justification of acceptance criteria for the confirmation of the [redacted] concentration of the cultured cartilage and the amount of residual BSA.

The applicant's response:

The proposed acceptance criterion has been established for the confirmation of [redacted] concentration based on the lower limit of detection of the validated commercial detection kit. The acceptance criterion can be justified because the important characteristic of JACC is the presence of cells that can produce the cartilage matrix and because quantification is unnecessary as far as the production of the cartilage matrix is confirmed.

The acceptance criterion for the amount of residual BSA is ≤[redacted] μg/cm^3 as determined based on the mean + 3SD ([redacted] μg/cm^3) of the observed values ([redacted] μg/cm^3) in [redacted] product samples with [redacted] mm because it is difficult to establish an acceptance criterion for [redacted] of the product due to a large variation in the observed data, though [redacted] is observed between the amount of residual BSA and [redacted] of cultured cartilage. To reduce residual BSA, the manufacturing process already incorporates a washing process. At present, addition of further reduction measures is technically infeasible. The safety with regard to residual BSA is discussed below. In the clinical study, the acceptance criterion for the amount of residual BSA was ≤[redacted] μg/cm^3. None of the 32 patients experienced any adverse event probably attributable to BSA or tested positive for beef allergy at [redacted] and [redacted] months after transplantation of JACC. The amount of BSA possibly taken up by the body after the transplantation of JACC is sufficiently lower than that of BSA in Apligraf, a similar allogeneic cultured dermis product. The incidence of allergy-related adverse events due to BSA remaining in JACC appear to be low. The above discussion justifies the acceptance criterion for the amount of residual BSA of ≤[redacted] μg/cm^3.

PMDA's view:

PMDA agreed on the applicant's justification for the acceptance criterion for the confirmation of [redacted] concentration.

The applicant's justification for the acceptance criterion for the amount of residual BSA is understandable. While the provisional acceptance criterion of ≤[redacted] μg/cm^3 was used in the clinical study, the maximum observed value was [redacted] μg/cm^3, which does not meet the acceptance criterion of ≤[redacted] μg/cm^3 specified by the applicant. On the basis of the applicant's explanations and countermeasures later described in Section "4.A.(2).1) Safety with regard to the amount of residual BSA," however, the proposed acceptance criterion of ≤[redacted] μg/cm^3 is acceptable.

Nevertheless, it is desirable to proactively collect data on quality and clinical experience and continue discussion on the quality control tests and acceptance criteria for the confirmation of

[redacted] concentration and the amount of residual BSA, thereby further improving the quality of JACC.

2.B.(2).2) Biological materials used in the manufacturing process of JACC

PMDA asked the applicant to list the raw materials used in the manufacturing process of JACC that must comply with the Standards for Biological Ingredients and clarify their compliance with the standards.

The applicant's response:

The biological materials used in the manufacturing process that are described in the submitted data have been shown to comply with the Standards for Biological Ingredients. The collagenase used for treatment of patient-derived cartilage tissue in the manufacturing process of JACC contains a biological material, which is not described in the submitted data. Some material that does not comply with the Standards for Biological Ingredients was used in the manufacturing process of this collagenase. Collagenase from a different manufacturer will be used in future production. The manufacture of this new collagenase uses casein derived from cow milk from [redacted]. This collagenase is autoclaved at 121°C for 20 minutes as a component of media and complies with the Standards for Biological Ingredients. The treatment conditions (e.g., composition of collagenase solution, enzyme activity, and treatment duration) for manufacturing JACC using the new collagenase were investigated using [redacted] cartilage because an investigation using human cartilage takes time. Although only limited information is available regarding differences in composition, etc. between the [redacted] cartilage and human cartilage, the [redacted] cartilage is likely to be more susceptible to enzyme digestion than the human cartilage based on the thickness of cartilage tissue. The range determined from the results of investigation, etc. using the [redacted] cartilage plans to be used as a condition for collagenase treatment of JACC. The new treatment condition is as follows: [redacted]°C ± [redacted]°C for ≤[redacted] hours in a collagenase solution ([redacted] vol% [redacted], [redacted] vol% [redacted], [redacted] vol% [redacted] solution, [redacted] vol% [redacted], and collagenase [redacted] to [redacted]U/mL). The concentration of [redacted] in the collagenase solution increases from [redacted] vol% to [redacted] vol%. This increase, however, appears not to affect [redacted] remaining in the final product because the chondrocytes are washed in a medium for cell suspension preparation after collagenase treatment.

PMDA's view:

The condition for the collagenase treatment in the manufacturing process newly proposed after switching of collagenase must be validated. However, the new treatment condition has not been validated yet. A validation needs to be conducted at the earliest possible time to justify the condition for collagenase treatment using the [redacted] cartilage. The manufacture of JACC should not be started until the treatment condition is verified.

2.B.(2).3) Summary of quality control and manufacturing conditions

PMDA's view:

For the quality and manufacturing control of JACC, tests for adventitious agents, tests for chondrocytes and cartilage matrix, test for the amount of residual BSA in the final product, etc. are appropriately

included in the in-process tests and release tests. These tests appear to assure the quality of JACC to a certain extent.

Basically, however, the quality and manufacturing control of a product is intended to ensure that the product with an intended quality has been obtained based on basic data regarding the product collected by analyzing the characteristics/properties of the product through characterization, and to select important indexes for quality assurance of the product as quality control items. Characterization of a product makes it possible to prove the consistency of the manufacturing process, specify a more appropriate manufacturing condition, and establish quality control methods, including the in-process control tests.

The applicant explained that obtaining normal cartilage tissue to collect data regarding the manufacturing and quality of JACC is difficult. The applicant's explanation is understandable. Therefore, data regarding the important product characteristics to validate the quality control methods and manufacturing process of JACC should have been collected using the specimens from the clinical study in order to improve the quality control methods, in parallel to the clinical study. However, only partial data are currently available regarding the product characteristics [aforementioned in Section "2.B.(1).1) Justification for specimens"]. The applicant has a very poor attitude although the developer of JACC should deepen the understanding on its nature and characteristics.

Nevertheless, the quality of JACC can be further improved by establishing more appropriate manufacturing conditions and quality control methods in the future. The applicant should continue characterization of JACC, including collection of information listed below, after taking appropriate procedures, such as informed consent, to use specimens derived from the normal cartilage. In addition, the applicant should reflect the results of characterization appropriately in quality control as required by, for example, submitting partial change application.

- Information on the types and content (%) of the target cells
- Information on the types and contents (%) of the non-target cells
- Information on the types and contents of the cartilage matrix

The applicant submitted application for JACC before fully verifying the compliance of the raw materials used in the manufacture of JACC with the Standards for Biological Ingredients and without taking sufficient measures. In addition, although a change in raw materials requires prior assessment, no assessment of manufacturing conditions was performed for switching the raw material collagenase. The applicant's thoughts and responses with regard to the quality and safety assurance of the product are questionable. It is urgent to discuss appropriate conditions for the enzyme treatment.

2.B.(3) Responses to the findings pointed out by the committees of PAFSC at submission of confirmation application

When the confirmation application for JACC was reviewed, 64 findings requiring assessments and responses by the applicant (e.g., the conduct of studies) were pointed out by the Subcommittee on Cellular/Tissue-based Product, etc., the Committee on Pharmaceutical Affairs and Biotechnology, and

the Committee on Biotechnology of PAFSC. Of the findings, 5 needed to be clarified by the time of submission of marketing application. The applicant's responses are presented below.

Finding 1: The representative compressive elasticity modulus of the final product should be measured and presented later.

The applicant's response:
The amount of [redacted], a cartilage matrix, and the dynamic elasticity modulus were measured using [redacted] specimens created from rabbit cartilage tissue, which were similar to JACC, instead of JACC created from human cartilage tissue. Although a linear correlation was observed between these parameters at and after [redacted] weeks of incubation, the change in the dynamic elasticity modulus of JACC, which is derived from human cartilage tissue, cannot be measured because the content of [redacted] in the similar product derived from rabbit cartilage tissue at [redacted] weeks of incubation is at least approximately [redacted] fold that in JACC at the time of release for the clinical study. The applicant therefore determined that the compressive elasticity modulus is not appropriate for a release test. The use of the amount of [redacted] as a measure for the hardness of the cartilage can be justified based on the literature reporting that the amount of [redacted] correlates with Young's modulus or dynamic compression, etc. in cultured cartilage using hyaluronic acid or agarose.

PMDA's view:
The Committee on Pharmaceutical Affairs Biotechnology advised the applicant to understand the characteristics of JACC by measuring the compressive elasticity modulus of the human cartilage tissue-derived product but did not recommend inclusion of the compressive elasticity modulus in the release tests (specifications). The applicant measured the compressive elasticity modulus using a similar product derived from rabbit cartilage tissue and provided no specific information relevant to the issue pointed out by the Committee on Pharmaceutical Affairs Biotechnology. The applicant explained that JACC is a cellular/tissue-based product that is not intended to have a certain hardness at the time of release or immediately after transplantation but is expected to provide cartilage graft that becomes harder after transplantation. The applicant's explanation is understandable to some extent. The applicant's opinion was accepted because inclusion of the compressive elasticity modulus in the specification at product release is considered not essential. It is desirable to continuously collect information on this quality attribute.

Finding 2: Culture methods using serum-free and autologous serum media should be considered to switch from FBS to non-FBS media at the earliest possible time (results need to be organized by the time of submission of marketing application).

The applicant's response:
Culture methods were studied using human chondrocyte specimens cultured in serum-free media, FBS-added media, serum-free media with [redacted], [redacted], [redacted], or [redacted], and [redacted] media with [redacted], [redacted], [redacted], or [redacted]. No media that can constantly produce JACC has been identified to

date, other than FBS-added media. A washing step to reduce residual FBS has been added to the manufacturing process.

PMDA accepted the above response.

Finding 3: The acceptance criterion for the amount of residual bovine serum albumin in the final product should be included in the specification of the final product.

The applicant's response:
The acceptance criterion of ≤ [redacted] μg/cm^3 has been established for the amount of residual FBS based on the measurements of specimens obtained in the clinical study and the amount of residual FBS after the washing step.

PMDA accepted the above response. The justification for this acceptance criterion is presented in Section "2.B.(2).1) Justification for the proposed tests and acceptance criteria in the specification."

Finding 4: The specification should be established based on the concentration of glycosaminoglycan derived from aggrecan, which is the main proteoglycan in hyaline cartilage.

The applicant's response:
Since glycosaminoglycan is present universally in the cartilage, it is technically difficult to measure aggrecan-derived glycosaminoglycan alone. In addition, determination of glycosaminoglycan concentrations in a media is also difficult. The presence of atelocollagen and/or other matrices may prevent glycosaminoglycan from being released in media. The release tests include the measurement of the [redacted] concentration in a cell suspension prepared from cultured cartilage and [redacted] by immunostaining.

PMDA accepted the applicant's explanation to the effect that determination of the [redacted] concentration in media is difficult and therefore cell suspensions prepared from cultured cartilage is used. The justification for the acceptance criterion for the concentration is presented in Section "2.B.(2).1) Justification for the proposed tests and acceptance criteria in the specification."

Finding 5: Higher quality materials of animal origin should be used as far as possible.

The applicant's response:
Atelocollagen [redacted] was used. Highly antigenic telopeptide is removed from this [redacted]. In addition, an intradermal test is performed before use to reduce the risk for allergy. [redacted] contains swine trypsin and cow milk-derived lactose is used, but no replacement of higher quality is available.

PMDA accepted the above response.

3. Stability

3.A Summary of the submitted data

The following storage conditions for the clinical study of JACC were initially selected based on the results of tests using rabbit- and human-derived cultured cartilages: Storage at [redacted]°C to [redacted]°C in [redacted] for up to [redacted] hours (shelf life). Subsequently, stability studies were conducted to evaluate transport media for cultured cartilage. The stability of [redacted] and [redacted] added with [redacted] and [redacted] (hereinafter, "[redacted]") was compared after storage at [redacted]°C and [redacted]°C for [redacted], [redacted], [redacted], and [redacted] hours. Both media conformed to the acceptance criteria for all tests at all time points. The latter medium, however, produced less changes in the test items over time. On the basis of these results, [redacted] was selected as the transport medium for cultured cartilage and separately subjected to the following stability studies.

To verify the optimal storage conditions for JACC in the transport medium for cultured cartilage, [redacted] specimens each stored in [redacted] at [redacted]°C, [redacted]°C, or [redacted]°C, and [redacted]°C for [redacted], [redacted], or [redacted] hours were subjected to the in-process tests, release tests, and confirmation tests. While all specimens conformed to the acceptance criteria for all test items at [redacted]°C, [redacted]°C, and [redacted]°C for up to [redacted] hours of storage, [redacted] specimens failed to conform to the acceptance criterion for "[redacted] of chondrocytes" at [redacted]°C at [redacted] hours of storage.

On the basis of these results, the following storage conditions were selected: Storage at 8°C to 25°C in the transport medium for cultured cartilage. The shelf life of 80 hours was determined considering a safety margin.

3.B Outline of the review conducted by PMDA

PMDA asked the applicant to explain the reason for switching of the transport medium for cultured cartilage from [redacted], which was specified at the submission of confirmation application and in the clinical study, to [redacted] and justify this change.

The applicant's response:

The clinical study was conducted using the shelf life of [redacted] hours as determined based on the results of the stability studies for the submission of confirmation application. However, improvement of the storage conditions was attempted for the following reasons: (1) The shelf life of [redacted] hours is not long enough considering the possibility of situations such as shipping JACC to a wider area beyond the location of the study site, delay in shipping, a change in surgical schedule after the market launch is obtained; and (2) the stability studies conducted using the product manufactured using human cartilage tissue (derived from patients with gonarthrosis) for research after the clinical study revealed that some specimens failed to show the storage stability for [redacted] hours. As a result, the transport medium was switched to [redacted]. In addition, the shelf life was extended to 80 hours based on the results of the stability studies.

PMDA accepted the above response.

4. Electrical safety, biological safety, and other safety-related data

4.A Summary of the submitted data

4.A.(1) Biological safety

4.A.(1).1) Animal studies

4.A.(1).1).(i) Allogeneic cultured cartilage transplantation study in rabbits

A total of 60 Japanese White male rabbits (n = 20/group) aged 28 to 32 weeks were used to generate a rabbit model of full-thickness knee cartilage defect. Immediately after creation of a full-thickness cartilage defect of the knee (area [redacted] × [redacted] mm, depth [redacted] mm) in rabbits, each animal received treatment with allogeneic cultured cartilage over the defect, which was then covered with the periosteal patch (ACC-01 group). The study included the 2 control groups: animals with cartilage defect treated with collagen gel alone followed by coverage with the periosteal patch (collagen gel group); and animals with cartilage defect covered with the periosteal patch alone (defect group). All of the groups were monitored for clinical signs, food consumption, and body weight throughout the study, and subjected to hematology, clinical chemistry, organ weight, and histopathology at 28, 56, 84, 168, and 371 days post-treatment.

Four deaths occurred, which include 1 animal in the ACC-01 group (at 16 days post-treatment), 2 animals in the collagen gel group (at 13 days post-treatment for both), and 1 animal in the defect group (Day 38 post-treatment). The cause of death was related to surgical invasion for all animals but considered unrelated to the treatment with ACC-01.

The ACC-01 group showed no abnormal change in food consumption or body weight. At necropsy at 84 and 168 days post-treatment, the ACC-01 and collagen gel groups tended to have a delayed recovery of locomotor activity compared with the defect group. At necropsy at 84 days post-treatment, the ACC-01 group tended to have a delayed recovery of locomotor activity compared with the defect and collagen gel groups.

Hematology in the ACC-01 group at 28 days post-treatment showed increased platelet count and prolonged prothrombin time compared with the defect group, and prolonged prothrombin time compared with the collagen gel group. The ACC-01 group had decreased fibrinogen levels at 168 days post-treatment and increased monocyte percentages at 371 days post-treatment compared with the collagen gel group.

Clinical chemistry in the ACC-01 group revealed decreased alkali phosphatase levels at 84 days post-treatment compared with the collagen gel group and increased albumin levels at 371 days post-treatment compared with the defect group.

The tendency of the delayed recovery of locomotor activity, and the hematological and clinical chemistry findings mentioned above did not appear to be of particular significance.

Necropsy revealed the dilation of the cerebral ventricles in 1 animal in the ACC-01 group at 56 days post-treatment. This finding was not observed at and after 84 days post-treatment. The ACC-01 group had decreased brain weights at 168 days post-treatment compared with the collagen gel group. The brain weight did not differ among the 3 groups at 371 days post-treatment.

The above results indicate no systemic effects of allogeneic cultured cartilage ACC-01 in rabbits.

4.A.(1).1).(ii) Autologous cultured cartilage transplantation study in dogs

A total of 36 male beagle dogs (n = 12/group), aged 20 to 23 months, were used in the study. Cartilage tissue was collected from the knee of the left hindlimb of each animal and cultured. At 3 weeks after cartilage tissue collection, [redacted] of a full-thickness cartilage defect model (area, [redacted] × [redacted] mm; depth, [redacted] mm) was created in the right hindlimb knee. Immediately after creation of full-thickness cartilage defect in dogs, each animal received treatment with the autologous cultured cartilage over the defect, which was then covered with the periosteal patch harvested from the tibia of the right hindlimb (ACC-01 group). The study included the 2 control groups: animals with cartilage defect treated with collagen gel alone followed by coverage with the periosteal patch (collagen gel group); and animals with cartilage defect covered with the periosteal patch alone (defect group). All of the groups were monitored for clinical signs, food consumption, and body weight throughout the study, subjected to hematology and clinical chemistry at 13, 26, 39, and 53 weeks post-treatment, and necropsied at 26 and 53 weeks post-treatment.

Death occurred in 1 animal in the collagen gel group, but not in the ACC-01 and defect groups.

Systemic effects observed were assessed. Changes in body weight and food consumption did not substantially differ among the 3 groups up to 53 weeks post-treatment. At necropsy at 26 weeks post-treatment, the ACC-01 group tended to have a delay in the resolution of abnormal gait at Week 13 compared with the defect group. At necropsy at 53 weeks post-treatment, the ACC-01 group tended to have a delay in the resolution of abnormal gait compared with the defect and collagen gel groups.

Hematology and clinical chemistry in the ACC-01 group revealed prolonged activated thromboplastin time at 26 weeks post-treatment compared with the defect group and increases in white blood cell counts and neutrophil percentages at 53 weeks post-treatment compared with the collagen gel group.

Necropsy revealed the dilation of the cerebral ventricles in 1 animal and a small pancreas in another animal in the ACC-01 group at 53 weeks post-treatment. These changes are unlikely to be related to ACC-01.

The above results indicate no systemic effects of autologous cultured cartilage ACC-01 in dogs.

4.A.(1).2) Cytogenetic stability and tumorigenicity

4.A.(1).2).(i) Karyotype analysis

Chondrocytes were isolated from the knee cartilage of patients with gonarthrosis [redacted], seeded in a culture flask at the density of [redacted] cells/cm^2, and cultured in monolayer at [redacted]°C and [redacted]% [redacted] (pre-culture cells). Cultured cartilage derived from the above 3 patients was digested with a collagenase solution for test. The resultant cell suspension was seeded at the density of [redacted] to [redacted] cells/cm^2 and cultured in monolayer under the same condition. The obtained cultured cells were examined, in comparison with pre-culture cells, by Giemsa

staining and G band differential staining of 20 nuclear plates for the number of chromosomes, chromosomal structure, and chromosome aberrations.

Pre-culture cells from all patients had chromosome aberrations, and trisomy 7 was detected in 2 of 20 cells from each patient. In cultured cells from 1 patient, trisomy 7 was detected at the same frequency as pre-culture cells, showing no difference between before and after culture. The remaining 2 patients had the normal karyotype. The trisomy of pre-culture cells was considered attributable to the primary disease of the patients (Castellanos, et al. *Osteoarthritis Cartilage.* 2004;12:982-985). Karyotype analysis showed no increase in the frequency of trisomy from before to after culture.

Based on the above, the number of chromosomes, chromosomal structure, and the frequency of chromosome aberrations did not differ before and after culture, and the chromosome aberrations observed before culture was not detected after culture, indicating that culturing chondrocytes using the manufacturing process established for JACC does not affect the cytogenetic stability of chondrocytes.

4.A.(1).2).(ii) Soft agar colony formation test

To investigate the tumorigenicity of cells constituting cultured cartilage, a soft agar colony formation test was conducted. Chondrocytes isolated from the culture cartilage that were prepared using the test tissue subjected to the karyotype analysis and chondrocytes cultured (passages) for the transplantation study in nude mice were subcultured times to obtain cell suspensions from cells after and passages, respectively. Chondrocyte, MRC-5 cell, and HeLa cell suspensions were seeded on % soft agar media at the density of , , and cells/flask, respectively, and the media were incubated at °C and % for days. After incubation, the number of colonies were counted. The proliferative capacity of anchorage-dependent cells was assessed by culturing cells in liquid media at the density of and cells/flask for chondrocytes, and cells/flask for HeLa and MRC-5 cells.

The colony formation rates in the liquid media ranged from 108% to 110% for HeLa cells, from 2.4% to 5.6% for chondrocytes, and from 27.4% to 32.6% for MRC-5 cells. On the other hand, the colony formation rate in the soft agar medium ranged from 97.6% to 103% for HeLa cells, while no colony of chondrocytes and MRC-5 cells was detected.

The above results demonstrated that chondrocytes proliferated anchorage-dependently and remained non-transformed. Cells constituting the cultured cartilage were shown to have no tumorigenicity.

4.A.(1).2).(iii) Transplantation study in nude mice

This study was conducted to investigate the tumorigenicity of cells constituting cultured cartilage. Cultured cartilage prepared from chondrocytes of patients with gonarthrosis was subjected to collagenase treatment to disperse cells. The cells were cultured in monolayer at °C and %, and subcultured or times. The resulting cultured cells were subcutaneously implanted to nude mice (n = 10/group) at the density of 1×10^7 cells/animal. Animals were necropsied under diethyl ether anesthesia between 7 and 14 days post-transplantation and at 21 days post-transplantation for histopathology. Suspended HeLa cells were used as the positive control.

Nodes were observed in accordance with the WHO guidelines (WHO Expert Committee on Biological Standardization: forty-ninth report. [WHO technical report series; 897], 1998).

Necropsy on Day 21 post-transplantation revealed enlarging nodes in all animals in the HeLa cell transplantation group and gradual nodal shrinkage in all animals in the chondrocyte transplantation group. Histopathology of animals necropsied between Days 7 and 14 post-transplantation showed that retractile nodes in the chondrocyte transplantation group were nodes of chondrocytes. No metastasis to other organs was found. Based on the above, cells constituting the cultured cartilage have no tumorigenicity.

4.A.(1).3) Cells cultured beyond the pre-specified period

Chondrocytes from patients with gonarthrosis [redacted] were cultured for [redacted] and [redacted] days ([redacted] specimens each), beyond the usual duration of incubation ([redacted] days), in the manufacturing process. The cultured cartilage thus-obtained was subjected to the in-process tests, release tests, and confirmation tests to assess its conformity to the specification. All specimens cultured for [redacted] and [redacted] days conformed to the acceptance criteria for all tests. However, [redacted] of [redacted] specimens cultured for [redacted] days did not conform to the acceptance criteria for some tests; e.g., the presence of [redacted] cultured cartilage with [redacted] falling outside the acceptable range. On the basis of the above results, the maximum duration of incubation of cultured cartilage was defined as [redacted] days. All specimens cultured for [redacted] days conformed to the acceptance criteria for the in-process tests, release tests, and confirmation tests of the cultured cartilage other than [redacted].

4.A.(2) Safety of residual biological materials, antibiotics, etc. in the final product

The following excipients may potentially remain in the final product: FBS, and gentamicin sulfate and Amphotericin B which are added in the cartilage culture medium. The safety of these excipients has been evaluated.

4.A.(2).1) Safety with regard to the amount of residual BSA

Since all of the 32 specimens manufactured for the clinical study conformed to the provisional acceptance criterion for the amount of residual BSA selected for the clinical study (≤[redacted] μg/cm^3) and the observed values ranged from below the limit of detection ([redacted] μg/cm^3) to [redacted] μg/cm^3, the final acceptance criterion for the amount of residual BSA was determined to be ≤[redacted] μg/cm^3 [for the justification for this criterion, see Section "2.B.(2).1) Justification for the proposed tests and acceptance criteria in the specification"].

To discuss the safety with regard to the amount of residual BSA in JACC, the amount of BSA in Apligraf, an allogeneic cultured dermal substitute approved/marketed in the US, and its safety were compared with those of JACC. The amount of residual BSA per sheet of Apligraf (ϕ75 mm) is estimated to be approximately 2250 to 2700 μg (Center for Devices and Radiological Health, FDA, US. Apligraf [Graftskin], summary of safety and effectiveness data, 1998), which is equivalent to [redacted] to [redacted] times that contained in JACC to be used for the largest possible area of the recipient site (10 cm^2) ([redacted] μg according to the acceptance criterion for density of [redacted] μg/cm^3 in the shipping specification). Since

Apligraf-related allergic adverse event has been reported only in 1 patient to date, JACC is very unlikely to cause allergy-related safety problems.

The risk of allergy caused by bovine serum cannot be fully ruled out as long as FBS-added culture media are used in the manufacture of JACC. To advise precautions against the risk, the applicant specified the following measures in the Warning and Contraindications sections of the proposed package insert:

- Advise about the risk of allergic reaction due to the use of JACC.
- Question the patient about a history of beef allergy prior to transplantation.
- Perform a beef allergy test prior to transplantation. Do not transplant JACC to patients who tested positive.
- Obtain consent from the patient after providing the patient with written information and informing the patient that JACC cannot be transplanted to the patient if it does not conform to the acceptance criterion for the amount of residual BSA.

Specifically, the package insert describes the risk for anaphylaxis and allergy to cow or bovine-derived materials, and the allergy induction potential at the second or subsequent transplantation because of sensitization during the first transplantation in patients who require multiple transplantations.

4.A.(2).2) Measurement of the amount of residual antibiotics

Residual gentamicin sulfate and Amphotericin B in cultured cartilage manufactured from chondrocytes from patients with gonarthrosis [redacted] were assayed by liquid chromatography-tandem mass spectrometry. This cultured cartilage was manufactured through the manufacturing process proposed at submission of confirmation application, and therefore it was not washed with [redacted].

The amounts of residual antibiotics were below the lower limit of quantitation (gentamicin sulfate [redacted] mg/mL, Amphotericin B [redacted] μg/mL). The amounts of residual antibiotics per specimen ([redacted] μL) were <[redacted] mg for gentamicin sulfate and <[redacted] μg for Amphotericin B. When the area of the recipient site is 10 cm^2 (2.4 cm^3), the theoretical amounts of residual gentamicin sulfate and Amphotericin B are [redacted] mg and [redacted] μg, respectively, which are below the acceptable limits of these antibiotics ([redacted] mg for gentamicin sulfate and [redacted] mg for Amphotericin B, calculated from the minimum daily dose of intravenous infusion). The manufacturing process for the clinical study and that proposed in the marketing application include additional washing with [redacted], which is expected to reduce the amounts of these residual antibiotics. However, since the potential for gentamicin sulfate and Amphotericin B to induce allergic reaction cannot be fully ruled out, JACC is indicated only for patients who have no history of hypersensitivity to these antibiotics.

4.A.(2).3) Safety evaluation of other raw materials that may remain in the final product

[redacted] contained in the media used to manufacture JACC conforms to the Japanese Standards of Quasi-drug Ingredients. The acceptable limit of this material is 2 mg/kg (100 mg/50 kg), one hundredth of 200 mg/kg, as determined based on the literature reporting that a single intraperitoneal dose of 200 mg/kg of [redacted] caused no abnormality ([redacted]) and considering a safety margin. The medium for cartilage culture contains [redacted] μg/mL of [redacted]

[redacted]. Even when the medium occupies JACC to be used for the largest possible area of the recipient site of 10 cm^2 (2.4 cm^3), the content of [redacted] is as small as [redacted] μg, which is [redacted] of the acceptable limit (100 mg/50 kg). This substance is considered to raise no safety concerns.

[redacted] is used for disinfection of cartilage tissue, [redacted] solution and collagenase solution at a concentration of [redacted] units/mL are used to isolate chondrocytes from cartilage tissue in the early steps of the manufacturing process of JACC. These substances are unlikely to remain in the final product for the following reasons: (i) The medium is subsequently changed every [redacted] to [redacted] days; (ii) the culture continues for ≥[redacted] weeks; and (iii) the product is washed in the final process. In addition, the tumorigenicity studies of JACC manufactured using these substances (the soft agar colony formation study and the transplantation study in nude mice) revealed no transformation. These substances have a high safety profile.

4.B Outline of the review conducted by PMDA

4.B.(1) Tendency toward a delay in the recovery of locomotor activity or the resolution of abnormal gait in the allogeneic cultured cartilage transplantation study in rabbits and the autologous cultured cartilage transplantation study in dogs

There was a tendency toward a delay in the recovery of locomotor activity or the resolution of abnormal gait in the ACC-01 and collagen gel groups compared with the defect groups in the studies in rabbits and dogs. PMDA therefore asked the applicant to discuss a causal relationship of this tendency with JACC.

The applicant's response:

A substance having a certain volume was implanted into the recipient site of rabbits and dogs in the ACC-01 and collagen gel groups, while no such substance was implanted in animals in the defect groups. In the transplantation groups (ACC-01 and collagen gel groups), the weight, during postoperative joint movement, is more easily transmitted to the inside of the defect via the joint surface than the non-transplantation group (defect group). This probably caused symptoms for a certain period of time, which might have interrupted locomotor activity or delayed the resolution of abnormal gait (Tables 7 and 8). In clinical practice, patients are instructed to avoid putting the weight on the knee for approximately 1 month so that no symptoms corresponding to these findings occur. Even if these conditions occur in patients, they are transient postoperative symptoms and do not deny the safety of JACC in terms of treatment outcome and long-term prognosis, or compromise its therapeutic benefits.

Table 7. Time to recovery of locomotor activity in the rabbit transplantation study

	Defect	Collagen gel	ACC-01
Time to recovery of locomotor activity (mean ± standard deviation [SD])	6.3 ± 2.1 (n = 19)	7.5 ± 2.6 (n = 19)	7.7 ± 1.8 (n = 19)

(Data from animals necropsied on Days 28, 56, 84, 168, and 371 combined; dead animals [unscheduled] excluded from the calculations)

Table 8. Time to resolution of abnormal gait in the dog transplantation study

	Defect	Collagen gel	ACC-01
Time to resolution of abnormal gait (mean ± SD)	14.8 ± 1.8 (n = 12)	19.1 ± 3.7 (n = 11)	17.3 ± 4.5 (n = 11)

(Data from animals necropsied on Weeks 26 and 53 combined)

PMDA's view:

The applicant's interpretation about the tendency toward a delay in the recovery of locomotor activity and the resolution of abnormal gait in JACC-treated animals is poorly justified and is merely speculation. Nevertheless, these events can be managed provided that patients avoid applying the weight on the affected area for 1 month postoperative and that JACC is used at medical institutions that can provide appropriate weight-bearing management, such as during rehabilitation, because the delayed recovery of locomotor activity and the resolution of abnormal gait within 1 month postoperative was observed only in the animal studies but not in the clinical study (described later in Section "6.B.(6) Safety evaluation").

4.B.(2) Effects on safety by the change in cultured cartilage transport medium

As aforementioned under Section "3.A. Summary of the submitted data," the cultured cartilage transport medium was changed in the course of the development of JACC. Since the nonclinical safety studies were conducted using the product shipped in the pre-change media, PMDA asked the applicant to explain the safety of the new transport medium for cultured cartilage.

The applicant's response:

The new transport medium for cultured cartilage is [redacted]. Most of the constituents of this medium are used as pharmaceutical compounds. During transplantation, a maximum of [redacted] mL of the transport medium for cultured cartilage is incorporated into the joint. The amounts of the pharmaceutical constituents contained at the above amount of the medium are below their respective daily doses, except for [redacted]. The amounts of non-pharmaceutical constituents ([redacted], [redacted], and [redacted]), and [redacted] (approximately [redacted] times the daily dose of [redacted] contained in pharmaceutical drugs) are sufficiently lower than their respective LD_{50}. Therefore, the safety of the new transport medium for cultured cartilage can be assured without conducting a new safety study.

PMDA accepted the above applicant's explanation.

5. Performance

5.A Summary of the submitted data

Primary pharmacodynamics

5.A.(1) Efficacy studies in animals

5.A.(1).1) Allogeneic cultured cartilage transplantation study in rabbits

As described in Section "4.A.(1).1).(i) Allogeneic cultured cartilage transplantation study in rabbits," a biological safety study was conducted in a rabbit model of full-thickness knee cartilage defect using allogeneic cultured cartilage ACC-01 and collagen gel. In this study, the efficacy of JACC was also evaluated. Animals in the ACC-01, collagen gel, and defect groups (n = 20/group) were necropsied under anesthesia at 28, 56, 84, 168, and 371 days post-treatment (4 animals each), and cartilage defects

at each recipient site were assessed according to the cartilage defect scoring system by Wakitani et al. (Wakitani score; Wakitani S, et al. *J. Bone Joint Surg. Am.* 1994;76:579-592) based on histopathological findings and the production of cartilage matrix by [redacted] staining, and histopathological assessment. In the histopathological assessment, the lesions were rated over time using a 5-point scale ("None," "Very slight," "Mild," "Moderate," and "Marked") for the following parameters: cartilage formation, bone formation, fibrosis, granulation, foreign-body giant cells, bleeding, infiltration of inflammatory cells/mononuclear cells, and lipomatous metaplasia.

The Wakitani score-based assessment revealed decreased scores in the ACC-01 group compared with the defect group at 28 and 371 days post-treatment ($P < 0.05$, Steel-Dwass test) and with the collagen gel group at 28 days post-treatment ($P < 0.01$), showing significant differences. Animals with transplanted cultured cartilage showed improvement in the defect compared with the control groups. At 56, 84, and 168 days post-treatment, the ACC-01 group tended to have lower scores than other 2 groups, without a significant difference.

Assessment by [redacted] staining showed [redacted] staining-positive areas at all postoperative time points in the ACC-01 group, which were not found in the collagen gel and defect groups.

The major histopathological findings observed in the study are described below.

The ACC-01 group showed a sign of cartilage formation at 28 days post-treatment and moderate to marked cartilage formation at 84 days post-treatment. At 168 days post-treatment, the ACC-01 group had moderate cartilage formation at 5 of 8 sites, while cartilage formation was not observed in the defect group and only 1 of 6 sites in the collagen gel group. At 371 days post-treatment, the ACC-01 group had moderate cartilage formation at 2 of 8 sites, while no cartilage formation was observed in either defect or collagen gel group. On the other hand, bone formation at the recipient site did not differ among the 3 groups at 28 and 56 days post-treatment. At 84 days post-treatment, the defect and collagen gel groups had moderate bone formation at 3 of 8 and 3 of 6 sites, respectively, while the ACC-01 group had no bone formation. At 168 days post-treatment, the ACC-01 group had moderate bone formation at 6 of 8 sites, and the collagen gel group at 2 of 6 sites. At 371 days post-treatment, moderate bone formation was observed at 5 of 8 sites in the ACC-01 group, 2 of 8 sites in the defect group, and 7 of 8 sites in the collagen gel group. Moderate fibrosis at the recipient site was observed in the defect and collagen gel groups at 28, 84, and 168 days post-treatment, and in the defect group at 56 and 371 days post-treatment, while the ACC-01 group had no fibrosis at any time point. The ACC-01 group more frequently had moderate infiltration of inflammatory cells/mononuclear cells than the defect and collagen gel groups after 56 days post-treatment. This finding was not observed at 371 days post-treatment.[5]

The above results indicate that allogeneic cultured cartilage ACC-01 repairs full-thickness knee cartilage defect in this rabbit model of full-thickness knee cartilage defect.

[5] Amended after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics (before amendment of the "site" in this paragraph, "animal")

5.A.(1).2) Autologous cultured cartilage transplantation study in dogs

As described in Section "4.A.(1).1).(ii) Autologous cultured cartilage transplantation study in dogs," a biological safety study was conducted in a dog model of full-thickness knee cartilage defect using autologous cultured cartilage ACC-01 and collagen. In this study, the efficacy of JACC was also evaluated. The ACC-01, collagen gel, and defect groups (n = 12/group) were necropsied under anesthesia at 26 and 53 weeks post-treatment (6 animals each), and cartilage defects at each recipient site were assessed by Wakitani's scoring system and the production of cartilage matrix by [redacted] staining, and histopathology. In the histopathological assessment, the lesions were rated over time using a 5-point scale ("None," "Very slight," "Mild," "Moderate," and "Marked") for the following parameters: cartilage formation, bone formation, fibrosis, granulation, bleeding, cell infiltration, and lipomatous metaplasia.

The Wakitani score revealed lower scores in the ACC-01 group than the collagen gel group at 26 weeks post-treatment, showing significantly better repair of the defect ($P < 0.05$, Steel-Dwass test). At 53 weeks post-treatment, the ACC-01 group tended to have lower scores than the defect and collagen groups, without a significant difference.

Assessment by [redacted] staining showed [redacted]-positive areas at 26 and 53 weeks post-treatment in the ACC-01 group. In the defect group, [redacted]-positive areas were observed at 53 weeks post-treatment. These areas were poorly stained with an unclear boarder between the bottom of the defect and the graft bed. In the collagen gel group, [redacted]-positive areas were observed at the bottom of the defect at 53 weeks post-treatment. These areas were poorly stained.

The major histopathological findings observed in the study are described below.

All of the groups had mild cartilage formation at 26 and 53 weeks post-treatment. The defect and collagen gel groups had a lower frequency of cartilage formation than the ACC-01 group. Bone formation at the recipient site did not differ among the 3 groups at 28 weeks post-treatment. At 53 weeks post-treatment, marked bone formation was observed at 7 of 12 sites in the ACC-01 group, 3 of 10 sites in the defect group, and 1 of 12 sites in the collagen gel group. The defect and collagen gel groups had a lower frequency of bone formation than the ACC-01 group. Mild fibrosis was observed at 2 of 10 sites in the ACC-01 group at 26 weeks post-treatment, while marked fibrosis was observed at 4 of 12 sites in the defect group and 3 of 8 sites in the collagen gel group. At 53 weeks post-treatment, moderate to marked fibrosis at the recipient site was observed at 2 of 12 sites in the ACC-01 group, 4 of 10 sites in the defect group, and 4 of 12 sites in the collagen gel group.[6]

The above results indicate autologous cultured cartilage ACC-01 repairs full-thickness knee cartilage defect in this dog model of full-thickness knee cartilage defect.

[6] Amended after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics (before amendment of the "site" in this paragraph, "animal")

5.A.(2) Histology using specimens from the clinical study

To verify the production of cartilage-specific matrix in cultured cartilage, which characterizes JACC, tissue sections were prepared using 32 specimens from the clinical study and examined for the production of [redacted] by immunostaining and of [redacted] by [redacted] staining.

The production of the cartilage matrix was assessed by [redacted] immunostaining and [redacted] staining of 32 specimens transplanted in the clinical study. [redacted] was produced in [redacted] specimens. All of the 32 specimens were tested positive for [redacted] staining, indicating the production of [redacted].

5.A.(3) Overall evaluation of efficacy

On the basis of the results in 5.A.(1) and 5.A.(2), the applicant considers that JACC has the capability of producing the cartilage matrix and repairs the articular cartilage with hyaline cartilage formation.

5.B Outline of the review conducted by PMDA

5.B.(1) Efficacy studies in animals

On the basis of the results of the ACC-01 transplantation studies in rabbit and dog models of full-thickness knee cartilage defect, the applicant concluded that the transplantation of JACC repairs the full-thickness knee cartilage defect with hyaline cartilage formation. PMDA asked the applicant to explain the justification of the conclusion.

The applicant's response:

The efficacy and safety of JACC in humans can be sufficiently predicated based on the results of the studies using cultured cartilage derived from rabbits and dogs. However, the animal studies have the following limitations due to the differences between animals and humans:

- The results merely suggest one aspect of a wide spectrum of pathological conditions in humans.
- Postoperative management (non-weight bearing and rehabilitation) similar to those in humans cannot be given to animals.
- There are anatomical differences in the structure of the knee and weight-bearing situation.
- Age-matched assessment is impossible because of the difference in life expectancy.

Taking account of the above points, there are limitations in extrapolating the results from the animal studies directly to humans. However, the efficacy of JACC in humans can be predicted by monitoring the postoperative clinical course. The transplantation studies in rabbits and dogs showed the common trend in which the significant difference was observed shortly after transplantation in the assessment by Wakitani score but not in the medium- and long-term. As a defect the depth of [redacted] mm reaching the subchondral bone was created in all animals, the defect group also had a transient cartilage formation, possibly resulting in the unclear difference between the defect and ACC-01 groups. Although the rabbit transplantation study demonstrated no significant difference between the ACC-01 group and the 2 control groups on Days 56, 84, and 168, the ACC-01 group had lower scores for 2 (cell morphology and stainability of matrix) of the parameters of Wakitani score at all time points. These results show the occurrence of cartilage formation at the recipient site to repair the cartilage defect.

PMDA's view:

It is well known that there are limitations in extrapolating data from animal studies directly to humans. Nevertheless, the findings from the animal studies can be important information in evaluating the efficacy and safety of JACC in humans. Despite the applicant's claim that JACC repaired the full-thickness cartilage defect with hyaline cartilage formation, however, it is difficult to draw the conclusion from the animal study data submitted, because of the problems shown below.

- The following information is not available regarding the cartilage-like tissue formed after transplantation of ACC-01:
  - Type (e.g., [redacted], [redacted], and [redacted]), content, presence in tissue, and distribution of the cartilage matrix produced
  - Type (e.g., [redacted] and [redacted] double-positive cells, [redacted]/[redacted]-positive cells, and [redacted] staining-positive cells), composition ratio, and change in the number of cells in the cartilage tissue
  - Kinetic properties, including hardness
- The method employed in the animal studies has significant difficulties in proving that the cartilage-like tissue formed after transplantation of ACC-01 is hyaline cartilage.
- [redacted] staining showed substantial variation in the stainability of the cartilage matrix in the ACC-01 group depending on the observation time point. The results were not consistent.

The above conclusion of PMDA was supported by the expert advisors at the Expert Discussion. PMDA has concluded that the submitted primary pharmacodynamics studies failed to confirm the treatment concept of JACC ("Chondrocytes contained in JACC and cartilage matrix produced by chondrocytes repair cartilage defect"). The applicant should provide the relevant information and precautionary advice in the package insert of JACC and other information materials. The applicant should also ensure that patients are provided with written information and fully informed of the treatment with JACC to give informed consent prior to the use of JACC.

6. Clinical data

6.A Summary of the submitted data

The applicant submitted pivotal efficacy and safety evaluation data, in the form of the results from 1 Japanese clinical study (Study J-TEC002). In addition, the applicant submitted reference data including the results of research on the long-term safety of JACC in patients enrolled in the clinical study and 1 Japanese clinical research [for the reference data, see Section "IV. Results of Compliance Assessment Concerning the New Medical Device Application Data"].

Japanese clinical study, J-TEC002 (Attached document 6-1; study period, August 2004 to September 2006)

A multicenter, open-label, uncontrolled study was conducted at 5 study sites in Japan to evaluate the efficacy, safety, and usefulness of JACC in patients with cartilage defect aged ≥20 years[7] (target sample size, ≥30 patients).

Of 33 patients enrolled in the study, 32 patients who received JACC (20 patients with traumatic cartilage defect, 6 patients with osteochondritis dissecans [4 for the knee and 2 for the elbow], and 6 patients with gonarthrosis) were included in safety evaluation. The remaining 1 patient who was found to have skin infection (erysipelas) prior to transplantation was excluded from analysis. Table 9 presents the baseline characteristics of the 32 patients treated with JACC. A total of 30 patients were evaluated for efficacy and usefulness and the remaining 2 patients were excluded from analysis (1 patient who underwent reoperation at Month 3 post-transplantation because of the detachment of JACC and another 1 patient who completed the study but had no arthroscopic data at Month 12 post-transplantation).

Table 9. Baseline characteristics of patients treated with JACC

Baseline characteristic	Category	Number of patients (%)
Diagnosis	Traumatic cartilage defect	20 (62.5)
	Osteochondritis dissecans	6 (18.8)
	Gonarthrosis	6 (18.8)
Ligament injury in patients with traumatic cartilage defect	No	14 (70.0)
	Yes	6 (30.0)
Time to ligament reconstruction in patients with ligament injury	<3 years	3 (50.0)
	≥3 years	2 (33.3)
	Unknown	1 (16.7)
Time from ligament reconstruction to transplantation of JACC in patients with ligament injury	<1 year	4 (66.7)
	≥1 year	2 (33.3)
Prior treatment of meniscus[8]	No	23 (76.7)
	Resected	6 (20.0)
	Sutured	1 (3.3)
Duration of illness	<1 year	18 (56.3)
	≥1 year	13 (40.6)
	Unknown	1 (3.1)
Site (details, largest defect)	Medial condyle of femur	18 (56.3)
	Lateral condyle of femur	5 (15.6)
	Femoral trochlea	1 (3.1)
	Patella	6 (18.7)
	Humeral trochlea or capitellum	2 (6.3)
Number of defects	1	29 (90.6)
	2	2 (6.3)
	3	1 (3.1)
Area of recipient site (at surgery)	≥1 and <2 cm^2	6 (18.8)
	≥2 cm^2	26 (81.3)

The procedures for JACC transplantation are shown below.

JACC was manufactured with 0.1 to 0.3 g of normal cartilage tissue collected by arthroscopy from the patient's knee according to the area of the recipient site. To transplant JACC, the defect site was opened to remove the degenerative cartilage around the cartilage defect and expose the subchondral bone.

[7] The study population was patients with traumatic cartilage defect, osteochondritis dissecans, or gonarthrosis (the area of the recipient site, 1-10 cm^2) who had a knee function score of ≤74 points as determined according to the knee (elbow) function scoring scale (Table 10) and who did not or were unlikely to respond to conventional treatment.

[8] The baseline characteristics of 30 patients, excluding 2 patients who received JACC at the elbow joint, are shown.

Subsequently, JACC that was previously trimmed to fit the shape of the recipient site was filled in the defect site, which was then covered with the periosteal patch collected from the tibia, etc. and sutured. The patient's knee was checked for detachment of the sutured periosteal patch and leakage of JACC while flexing and extending the knee. Then the wound was closed to complete the procedure.

The primary efficacy endpoint was improvement from baseline to end of follow-up (Month 12 postoperative) according to the efficacy evaluation criteria (Table 14) using a 4-point scale ("Very effective," "Effective," "Neither effective nor ineffective," and "Ineffective") which is a matrix index of the grading criteria for knee (elbow) function improvement (Table 11) using a 4-point scale ("Markedly improved," "Improved," "Unchanged," and "Aggravated") based on the total points of the knee (elbow) function scoring scale (Table 10) consisting of pain during flexion/extension, pain at rest, and knee (elbow) flexion/extension, and the arthroscopic assessment system (Table 12) using a 4-point scale (Grades I to IV) according to the International Cartilage Repair Society (ICRS) cartilage repair assessment system (Table 13).

Table 10. Knee (elbow) function scoring scale

Knee function		Elbow function	
Function	Point score	Function	Point score
Pain during knee flexion/extension:		Pain during elbow flexion/extension:	
None	50	None	50
Mild	35	Mild	35
Moderate	20	Moderate	20
Severe	0	Severe	0
Pain at rest:		Pain at rest:	
None	25	None	25
Mild	15	Mild	15
Moderate	0	Moderate	0
Knee range of motion:		Elbow range of motion:	
No problems	25	≥120°	25
Slightly impaired	16	90°-120°	15
<90°	8	<90°	0
Impossible	0		

(Maximum total score, 100 points)

Table 11. Grading criteria for knee (elbow) function improvement

		End of follow-up			
		100-90	89-75	74-60	<60
Baseline	74-60	Markedly improved	Improved	Unchanged	Aggravated
	<60	Markedly improved	Markedly improved	Improved	Unchanged

(100 to 90 points, very good; 89 to 75, good; 74 to 60 points, neither good nor poor; <60, poor)

Table 12. Arthroscopic assessment system

Grade I (Normal)	Grade II (Nearly normal)	Grade III (Abnormal)	Grade IV (Severely abnormal)
12	11-8	7-4	3-0

(total points)

Table 13. ICRS cartilage repair assessment

Observation and criteria	Point score
Degree of defect repair:	
In level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
0% repair of defect depth	0
Integration to border zone:	
Complete integration with surrounding cartilage	4
Demarcating border <1 mm	3
3/4 of graft integrated with surrounding cartilage, 1/4 with a notable border ≥1 mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border ≥1 mm	1
From no contact to 1/4 of graft integrated with surrounding cartilage	0
Macroscopic appearance:	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracks	2
Several, small or few but large fissures	1
Total degeneration of grafted area	0

(Maximum total score, 12 points)

Table 14. Efficacy evaluation criteria (primary endpoint)

		Knee (elbow) function			
		Markedly improved	Improved	Unchanged	Aggravated
Arthroscopic assessment	Grade I	Very effective	Effective	Neither effective nor ineffective	Ineffective
	Grade II	Very effective	Effective	Neither effective nor ineffective	Ineffective
	Grade III	Effective	Neither effective nor ineffective	Ineffective	Ineffective
	Grade IV	Neither effective nor ineffective	Ineffective	Ineffective	Ineffective

The results of the primary efficacy endpoint determined according to the efficacy evaluation criteria are presented below (Table 15).

Table 15. Efficacy results (patients evaluable for efficacy)

N	Very effective		Effective		Neither effective nor ineffective		Ineffective	
	n	%	n	%	n	%	n	%
30	25	83.3	3	10.0	2	6.7	0	0.0

The major secondary efficacy endpoints were a change from baseline in knee or elbow function assessment at the end of follow-up (Month 12 postoperative) according to the Lysholm Knee Score (Table 16) or Mayo Clinic Performance Index (Table 17), as well as arthroscopic assessment and imaging examination by MRI.

Table 16. Lysholm Knee Score

• Limp (maximum score, 5 points)	
None	5
Slight or periodical	3
Severe and constant	0
• Support (maximum score, 5 points)	
None	5
Stick or crutch	2
Weight-bearing impossible	0
• Locking (maximum score, 15 points)	
No locking and no catching sensations	15
Catching sensation but no locking	10
Locking occasionally	6
Locking frequently	2
Locked joint	0
• Swelling (maximum score, 10 points)	
None	10
On severe exertion	6
On ordinary exertion	2
Constant	0
• Instability (maximum score, 25 points)	
Never giving way	25
Rarely during athletics or other severe exertion	20
Frequently during athletics or other severe exertion	15
Occasionally in daily activities	10
Often in daily activities	5
Every step	0
• Pain (maximum score, 25 points)	
None	25
Inconstant and slight during severe exertion	20
Marked during severe exertion	15
Marked on or after walking ≥2 km	10
Marked on or after walking <2 km	5
Constant	0
• Stair-climbing (maximum score, 10 points)	
No problems	10
Slightly impaired	6
One step at a time	2
Impossible	0
• Squatting (maximum score, 5 points)	
No problems	5
Slightly impaired	4
≤90°	2
Impossible	0

(Maximum total score, 100 points)

Table 17. Mayo Clinic Performance Index

Observation and criteria	Point score	Observation and criteria	Point score
Pain:	(maximum score, 45 points)	Stability:	(maximum score, 10 points)
None	45	Normal (stable)	10
Mild	30	Moderate instability <10°	5
Moderate	15	Gross instability ≥10°	0
Severe	0		
Elbow motion:	(maximum score, 20 points)	Daily function:	(maximum score, 25 points)
Arc ≥100°	20	Combing hair	5
Arc 50°-100°	15	Feeding oneself	5
Arc <50°	5	Hygiene	5
Immobility	0	Putting on shirt	5
		Putting on shoes	5

(Maximum total score, 100 points)

The Lysholm Knee Score was determined in 29 patients treated with JACC in the knee at baseline and Months 3, 6, and 12 postoperative. The scores for 7 of the 8 items (those other than “Support”) of the Lysholm Knee Score were higher at Month 12 than baseline.

The Mayo Clinic Performance Index was determined in 2 patients treated with JACC in the elbow at baseline and Months 3, 6, and 12. The scores were higher at all postoperative time points than baseline.

Table 18 shows the grade of cartilage repair of the knee or elbow as assessed by arthroscopy at Month 12 postoperative. Of 30 patients, 7 patients were classified as Grade I (Normal) and 21 patients Grade II (Nearly normal). Patients with Grade I and Grade II accounted for 93.3% of the study population (Table 18).

Table 18. Arthroscopic assessment (knee/elbow) (patients evaluable fore efficacy)

N	Grade I		Grade II		Grade III		Grade IV	
	n	%	n	%	n	%	n	%
30	7	23.3	21	70.0	1	3.3	1	3.3

MRI confirmed cartilage repair at the recipient site in 14 of 30 patients based on signal intensity.

The safety of JACC was rated using a 4-point scale ("Very safe," "Safe," "Possibly unsafe," and "Unsafe")[9] based on JACC-related adverse events. In addition, overall usefulness was rated using a 4-point scale of "Very useful," "Useful," "Neither useful nor unfavorable," and "Unfavorable" based on the efficacy and safety ratings (Table 19).

Table 19. Evaluation criteria for usefulness

		Efficacy			
		Very effective	Effective	Neither effective nor ineffective	Ineffective
Safety	Very safe	Very useful	Useful	Neither useful nor unfavorable	Unfavorable
	Safe	Useful	Useful	Neither useful nor unfavorable	Unfavorable
	Possibly unsafe	Neither useful nor unfavorable	Neither useful nor unfavorable	Unfavorable	Unfavorable
	Unsafe	Unfavorable	Unfavorable	Unfavorable	Unfavorable

The safety of JACC was rated in 32 patients. The ratings were "Very safe" for 29 patients (90.6%), "Safe" for 2 patients (6.3%), "Possibly unsafe" for 1 patient (3.1%), and "Unsafe" for 0 patients.

The results of usefulness evaluation of JACC are presented below (Table 20).

Table 20. Usefulness assessment (patients evaluable for usefulness)

N	Very useful		Useful		Neither useful nor unfavorable		Unfavorable	
	n	%	n	%	n	%	n	%
30	23	76.7	5	16.7	1	3.3	1	3.3

A total of 29 adverse events (defined as any unfavorable symptoms associated with tissue collection and/or transplantation surgery) (16 of 32 patients [50%]) occurred (Table 21). No deaths were reported. A total of 7 serious adverse events (5 of 32 patients [15.6%]; 1 event of graft delamination, 2 events of decreased range of motion, 1 event of arthralgia, 1 event of joint swelling, 1 event of suicide attempt, and 1 event of depression) occurred. Of these, 4 events (2 of 32 patients [6.3%]; 2 events of decreased range of motion, 1 event of arthralgia, and 1 event of joint swelling) were considered related to JACC. Adverse event for which a causal relationship to cartilage tissue collection cannot be ruled out was 1 event of erysipelas.

[9] Very safe: No study device-related adverse event occurred.
Safe: A mild study device-related adverse event(s) requiring no intervention occurred.
Possibly unsafe: A study device-related adverse event(s) requiring intervention but no long-term treatment.
Unsafe: A study device-related adverse event(s) requiring intervention and long-term treatment.

Table 21. Adverse events and malfunctions (patients evaluable for safety)

Event term	Adverse event (N = 32)	Treatment-related (N = 32)
Graft delamination	1 patient (1 event)	0 patients
Decreased range of motion (serious)	2 patients (2 events)	2 patients (2 events)
Arthralgia (serious)	1 patient (1 event)	1 patient (1 event)
Joint swelling (serious)	1 patient (1 event)	1 patient (1 event)
Suicide attempt (serious)	1 patient (1 event)	0 patients
Depression (serious)	1 patient (1 event)	0 patients
Oedema peripheral (non-serious)	2 patients (2 events)	0 patients
Feeling hot (non-serious)	1 patient (1 event)	0 patients
Nasopharyngitis (non-serious)	4 patients (4 events)	0 patients
Nuclear magnetic resonance imaging abnormal (non-serious)	1 patient (1 event)	0 patients
Pityriasis rosea (non-serious)	1 patient (1 event)	0 patients
Headache (non-serious)	2 patients (3 events)	0 patients
Abdominal pain upper (non-serious)	1 patient (1 event)	0 patients
Insomnia (non-serious)	1 patient (1 event)	0 patients
Hypertrophic scar (non-serious)	1 patient (1 event)	0 patients
Dermatitis contact (non-serious)	1 patient (1 event)	0 patients
Post lumbar puncture syndrome (non-serious)	1 patient (1 event)	0 patients
Influenza (non-serious)	1 patient (1 event)	0 patients
Back pain (non-serious)	2 patients (2 events)	0 patients
Eczema (non-serious)	1 patient (1 event)	0 patients
Thermal burn (non-serious)	1 patient (1 event)	0 patients

6.B Outline of the review conducted by PMDA

Since the proposed intended use and indications of JACC do not contain basic information necessary to review the efficacy and safety of JACC, PMDA asked the applicant to specifically explain the expected target disease of JACC, in relation to the conventional standard treatments in Japan.

The applicant's response:

"Traumatic cartilage defects, osteochondritis dissecans, and gonarthrosis are all characterized by cartilage defect as a pathological condition, though they have different etiologies. For this reason, it is reasonable to select them as the target diseases of JACC. The proposed indication of JACC based on the results of its clinical study is patients with full-thickness cartilage defect in the knee. The clinical study has demonstrated the usefulness of JACC in the treatment of cartilage defects of 1 to 10 cm^2. For cartilage defects <1 cm^2, microfracture is the first-line therapy. When long-term prognosis is taken into consideration, microfracture should not be used for the treatment of cartilage defect >2 cm^2. Autologous osteochondral mosaicplasty is indicated for cartilage defects approximately 2 cm^2. Some literature reports that autologous osteochondral mosaicplasty can be used for the treatment of cartilage defects up to 4 cm^2. Surgeons need to choose an appropriate technique for the treatment of cartilage defects (i.e., JACC or conventional techniques), according to the size of each defect. However, given that (1) some research has suggested that the use of microfracture for the treatment of cartilage defects approximately 1 to 2 cm^2 is associated with a poor prognosis; (2) the limited availability of donor sites for tissue collection for autologous osteochondral mosaicplasty may cause wound healing problems at the donor sites; and (3) cartilage defects have various characteristics, the proposed area of defect was selected to

include defect sizes that are treatable by conventional techniques so that surgeons can choose an appropriate treatment technique.

On the basis of the above, the following indications are proposed for JACC:

Primary disease:	Traumatic cartilage defect, osteochondritis dissecans, and early stage gonarthrosis
Defect area:	1 to 10 cm^2
Defect site:	Weight-bearing femoral condyle and femoropatellar joint
Complications:	Patients with any complication, including fracture, ligament injury, and meniscus injury, must be treated for the complication before transplantation of JACC.
Conventional treatments:	Patients who have not received any conventional treatment for cartilage defect and patients with poor response to conventional treatment (conservative or surgical therapy)

To evaluate the efficacy and safety of JACC in the treatment of the proposed target diseases, PMDA considers that the submitted clinical study data have issues to be addressed. The issues are discussed in sections below.

6.B.(1) Target diseases

PMDA asked the applicant to justify the design of the clinical study because the efficacy and safety of JACC had been evaluated in 1 clinical study including patients with traumatic cartilage defect (20 patients; knee for all patients), those with osteochondritis dissecans (6 patients; 4 for the knee, 2 for the elbow), and those with gonarthrosis (6 for the knee), although these diseases have different pathological conditions and conventional therapies differ depending on the disease.

The applicant's response:

The intended use and indications of JACC are to fill a cartilage defect and repair the defect with cartilage matrix produced by chondrocytes contained in JACC. To achieve the purpose of cartilage defect repair, it is not necessary to consider various patient and baseline disease characteristics, such as the primary disease of patients and its severity. Although various factors can result in cartilage defect, the common goal of treating these diseases is to fill and repair the cartilage defect. The plausible cause of osteochondritis dissecans is shear force that is repeatedly applied onto the joint during sports and other activities. It is reasonable to evaluate the efficacy of JACC in the treatment of osteochondritis dissecans together with traumatic cartilage defect because the two diseases are similar in terms of cartilage defect caused by external force. As with the case with traumatic cartilage defect and osteochondritis dissecan, the efficacy of JACC in filling and repairing an articular cartilage defect is evaluated in patients with gonarthrosis. It is therefore appropriate to evaluate the efficacy of JACC in the treatment of the three diseases.

PMDA's view:

Traumatic cartilage defect and osteochondritis dissecans are defined as a localized cartilage defect caused by external force and are commonly treated by bone marrow stimulation techniques (including

microfracture) and autologous osteochondral mosaicplasty to fill the cartilage defect. On the other hand, gonarthrosis is a progressive disease characterized by knee joint deformity and inflammation accompanied by the degeneration of cartilage tissue, resulting from the abrasion of the cartilage caused by continued weight bearing, etc. Conventional therapies include high tibial osteotomy and joint prosthesis replacement, which are intended to correct leg alignment. An appropriate technique is decided according to the age, alignment change, the severity of cartilage degeneration, etc.

As explained by the applicant, traumatic cartilage defect and osteochondritis dissecans have much in common, including their etiology and pathological conditions and the purpose and types of therapies. In addition, as discussed in the Expert Discussion, there is no difference in the expected outcome of treatment with the conventional therapies and JACC between the 2 diseases. There appears to be no significant problem in evaluating the efficacy of JACC in the treatment of traumatic cartilage defect and osteochondritis dissecans. On the other hand, gonarthrosis differs from traumatic cartilage defect and osteochondritis dissecans in terms of etiology, pathological condition, types of conventional therapies, and the purpose of treatment, as well as safety risk. The safety risk is associated with several factors such as pathological conditions, the weight applied on JACC because of knee joint deformity, and the concern that it is difficult to reconstruct and maintain cartilage-like tissue at a defect site with inflammation or deformity. For these reasons, the efficacy and safety of JACC in filling and repairing an articular cartilage defect should not be evaluated in patients with gonarthrosis together with those with traumatic cartilage defect and those with osteochondritis dissecans. The efficacy and safety of JACC should be evaluated according to the diseases characteristics and treatment goal.

The elbow and knee should also be assessed separately for cartilage defect repair because these joints substantially differ in terms of weight loads on the joint and effects of symptoms and diseases on the patient's quality of life (QOL).

6.B.(2) Comparison with treatment outcome with conventional therapies

To evaluate the efficacy of JACC and verify its clinical positioning, the applicant should analyze data from patients with the proposed target diseases treated with JACC versus conventional therapies that are standard in Japan. However, the applicant did not do so. PMDA asked the applicant to justify their determination that the efficacy of JACC can be appropriately evaluated in a study with no control group.

The applicant's response:

Cartilage defects to be treated in Study J-TEC002 are unlikely to heal spontaneously or with conservative therapy. On the other hand, microfracture is not intended to repair cartilage defects. Autologous osteochondral mosaicplasty is not considered to be a common therapy for cartilage defect, because autologous osteochondral mosaicplasty is inevitably associated with the amount of autologous tissue to be harvested at the donor site and measures to minimize the risk of tissue collection. It is known that general orthopedic surgeons are reluctant to perform this technique because of limitations to the amount of tissue to be harvested. In terms of the efficacy after transplantation, autologous osteochondral mosaicplasty can be superior to JACC because it is autologous tissue transplantation. However, JACC is favorable in terms of the amount of tissue to be harvested (i.e., the smaller amount of tissue with JACC). As JACC is a novel product, few orthopedic surgeons have detailed knowledge of JACC.

Autologous osteochondral mosaicplasty is also an uncommon therapy and is indicated only for selected patients. Considering the learning curve of surgeons training in surgical procedures, it is infeasible to conduct a clinical study that requires surgeons thoroughly familiar with both procedures. The clinical study of ChondroCelect (TiGenix), a similar product to JACC, included a control group of patients treated with microfracture and 118 patients were recruited over 4 years at 13 study sites in 4 countries (Saris DBF et al. *Am. J. Sports Med.* 2008;36:235-246). If a clinical study using autologous osteochondral mosaicplasty as control is conducted in Japan, it will take ≥10 years as a study period. Because neither similar medical device nor guideline for clinical evaluation of treatment of joint defects is available, it is unlikely to obtain an objective outcome even if a controlled clinical study is conducted. The results of Study J-TEC002 are almost consistent with those of clinical research by Ochi et al.[10] (Ochi M et al. *J. Bone Joint Surg. Br.* 2002:84:571-578). The reproducibility of the efficacy results have been assured. In addition, because of the characteristics of surgical procedure and differences in surgical schedule, it is difficult and non-logical to conduct a controlled clinical study in a blind manner.

PMDA's view:
To introduce JACC to Japan, in principle, the applicant should appropriately evaluate the efficacy of JACC in a clinical study(ies) and clarify the clinical positioning of JACC. Therefore, if an established conventional treatment option is available in Japan, a clinical study should be designed taking the conventional treatment into consideration.

Although no treatment guideline for traumatic cartilage defect or osteochondritis dissecans clearly specifies the maximum defect area that can be treated by conventional therapies, such as microfracture and autologous osteochondral mosaicplasty, these are considered as standard treatments for cartilage defects <4 cm^2 based on the information reported in literature on these diseases in Japan and overseas (Matsusue Y. *6. Treatment of Joint cartilage injury. Orthopedics Knack & Pitfalls. Knack & Pitfalls of knee joint surgery [Kurosaka M. ed.]*. Bunkodo; 2005:188-192) and the discussion in the Expert Discussion. Therefore, to evaluate the clinical positioning of JACC in the treatment of traumatic cartilage defect and osteochondritis dissecans with a defect area of <4 cm^2, the clinical study should have been designed so that which treatment option, JACC or conventional therapy, should be selected can be explained; e.g., a clinical study that compares the efficacy of JACC, microfracture, and autologous osteochondral mosaicplasty or a clinical study having an appropriate efficacy target. On the other hand, cartilage defects ≥4 cm^2 are managed by conservative therapy or subjected to osteotomy to modify the weight-bearing condition on the joint. No treatment to fill cartilage defects is established. Therefore, the clinical study should have been designed so that the efficacy results of JACC that justify its introduction to clinical settings can be evaluated; e.g., a clinical study using appropriate conservative therapy as a control or a clinical study having an efficacy target beyond that of conservative therapy. The evaluation method for autologous cultured cartilage products issued by the European Medicines Agency (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/05/WC500090887.pdf) also proposes different study designs for cartilage defects <4 cm^2 and those ≥4 cm^2.

[10] The results of the clinical research by Ochi et al. were submitted as reference data. Of 25 patients treated with cartilage cultured using atelocollagen, similar to JACC, at the medial and lateral femoral condyles, 22 had a Lysholm Knee Score of ≥90 points.

PMDA considers that a clinical study in patients with gonarthrosis should be designed so that the efficacy of JACC can be compared with conventional therapy based on appropriate endpoints because radical therapy, such as joint replacement, is often indicated for this disease.

The applicant explained that (1) it is infeasible to conduct a clinical study that requires surgeons thoroughly familiar with novel JACC as well as autologous osteochondral mosaicplasty; (2) the duration of a controlled study is expected to be long based on the overseas clinical study of a similar product; and (3) it is difficult to conduct a controlled clinical study in a blind manner. However, even if a blind study is infeasible, efficacy evaluation in a controlled study of JACC versus conventional therapy as a concurrent control has certain significance. In addition, even if the conduct of a controlled clinical study itself is difficult, there is a room for discussion on the study design; e.g., the efficacy of JACC can be evaluated by specifying an efficacy target based on the outcome of conventional therapies. There is no reason that justifies the applicant's determination that the design of Study J-TEC002 is appropriate.

6.B.(3) Knee (elbow) function scoring scale

PMDA asked the applicant to justify the use of the knee (elbow) function scoring scale (Table 10) in the efficacy evaluation of JACC.

The applicant's response:

The Lysholm Knee Score (Table 16) is a clinical assessment scale, consisting of 8 items (limp, support, locking, pain, swelling, instability, stair-climbing, and squatting [maximum total score, 100 points]). This scale was used by Ochi et al. for assessment of cartilage defects (Ochi M et al. *J. Bone Joint Surg. Br.* 2002;84:571-578). A recent study also reported that this scale can be used for assessment of cartilage defects (Kocher MS et al. *J. Bone Joint Surg. Am.* 2004;86:1139-1145). Patients with a cartilage defect, however, generally show neither instability nor locking but only mild swelling, and only few patients limp and require a walking stick. An assessment scale that considers all of these conditions may decrease the sensitivity of treatment response assessment. To solve this problem, the knee (elbow) function scoring scale was created by excluding these items from Lysholm Knee Score so that the joint function can be assessed focusing on pain to accurately evaluate the response to JACC (maximum total score, 100 points).

PMDA's view:

The applicant has explained that the knee (elbow) function scoring scale used by the applicant is based on Lysholm Knee Score but focuses more on pain and joint movement. The items of this scoring scale and the scoring scale are substantially simplified from the Lysholm Knee Score. The scientific validity of this simplified scoring system and the relationship between the score of each item and its clinical significance have not been discussed based on sufficient clinical data. The knee (elbow) function scoring scale has not been validated. The results of assessment based on this scoring scale should not be used in the efficacy evaluation of JACC. The Lysholm Knee Score used as a secondary endpoint in Study J-TEC002 can assess a change in score over time. However, this scale does not necessarily reflect clinical symptoms in young individuals, who are highly active, and is developed to assess clinical symptoms of the anterior cruciate ligament. For these reasons, this scale is not necessarily appropriate for clinical evaluation of cartilage injury (Japanese Orthopaedic Association's Clinical Practice Guideline). This

scale is, however, used in clinical practice for assessment of clinical symptoms of traumatic cartilage defect and osteochondritis dissecans. As explained by the applicant, there is a publication reporting the use of this scale to assess clinical symptoms of cartilage defects. For patients in a chronic stage with fixed symptoms, improvement of 1 level in each item is known to be a clinically significant change. In addition, each score change can be interpreted to have clinical significance. For these reasons, the scale can be used for the efficacy evaluation of JACC.

6.B.(4) Efficacy evaluation method in Study J-TEC002

PMDA's view on the efficacy evaluation of JACC:

As described in Sections 6.B.(1) to 6.B.(3), it is difficult to evaluate the efficacy of JACC in the proposed indications (i.e., traumatic cartilage defect, osteochondritis dissecans, and gonarthrosis) on the basis of the submitted clinical study results because Study J-TEC002 included different patient populations having different diagnoses and defect sites that should be separately evaluated for efficacy and did not appropriately evaluate the efficacy according to the difference in the characteristics of the target diseases.

The interpretation of the results of Study J-TEC002 was discussed at the Expert Discussion. The expert advisors supported the PMDA's conclusion that the efficacy and clinical positioning of JACC cannot be explained by the submitted study results.

The expert advisors also raised the following comments: In rare cases of the proposed target diseases of JACC for which no treatment is available, data from each patient between before and after transplantation can be analyzed to assess the effects of JACC on patients, thereby explaining the clinical positioning of JACC. Consequently, PMDA discussed this issue as shown below.

PMDA concluded that it is worth discussing the potential of JACC as a new treatment option for patients with a rare disease who have inadequate response to conventional therapy by making best use of the results from 33 patients in Study J-TEC002 because the proposed target diseases of JACC include diseases that are not adequately responsive to conventional therapies and diseases that cannot be studied in a clinical study because of a limited number of patients. PMDA decided to evaluate the efficacy of JACC in patients with traumatic cartilage defect and osteochondritis dissecans, who are unlikely to be adequately responsive to conventional therapies and who have a relatively large cartilage defect area. The details of the evaluation are presented in Section 6.B.(5).

6.B.(5) Efficacy evaluation by PMDA

On the basis of the discussion in Section 6.B.(4), patients with a cartilage defect of the knee due to traumatic cartilage defect or osteochondritis dissecans enrolled in Study J-TEC002 were included in the efficacy evaluation of JACC. The effects of the transplanted JACC on clinical symptoms and morphologic improvement were investigated in the individual patients for the issues described below.

6.B.(5).1) Study population

Of 38 patients included in the registry for the study, 5 were excluded from the study because of screening failures. A total of 33 patients from whom the cartilage was collected for preparation of JACC were included in safety evaluation. Of them, 32 patients were included in efficacy evaluation, and 1 patient

who was not treated with JACC because of an adverse event (erysipelas) was excluded from analysis. In addition, based on the discussion in Sections 6.B.(1) and 6.B.(2), 8 patients (6 patients with gonarthrosis and 2 patients treated with JACC at the elbow) were excluded from analysis, and 24 patients were included in efficacy evaluation for individual patients. The characteristics of 24 patients are summarized below (Table 22).

Table 22. Patient's baseline characteristics

Patient ID	Age	Sex	Duration of illness[11]	Site of cartilage defect	Area of cartilage defect (area of recipient site) (cm^2)[12]	Degeneration of subchondral bone	Prior treatment of cartilage defect	Timing of prior treatment of cartilage defect[13]	Concurrent injury and medical history	Surgery for concurrent injury and medical history	Timing of surgery for concurrent injury and medical history[14]
Osteochondritis dissecans											
1	30	Male	192 months	Medial condyle of left femur	4.7 (6.9)	Yes	Osteochondrosynthesis	16 years ago	None	Osteosynthesis	16 years ago
2	25	Female	2 months	Lateral condyle of left femur	1.8 (2.0)	No	None	-	Discoid meniscus of left knee	Meniscectomy, removal of loose body	10 years ago
3	34	Female	3 months	Medial condyle of right femur	3.1 (2.6)	Yes	None	-	Right meniscus injury	Meniscorrhaphy	7 years ago
4	29	Male	24 months	Medial condyle of left femur	2.4 (2.7)	Yes	None	-	Left osteochondritis dissecans	Removal of loose body	9 months ago
Traumatic cartilage defect											
5	42	Female	Unknown	Medial condyle of left femur	3.1 (3.5)	No	None	-	Bilateral anterior cruciate ligament damage, left meniscus injury	Anterior cruciate ligament reconstruction, meniscectomy	20 years ago, 3 months ago
6	30	Male	3 months	Lateral condyle of left femur	1.5 (1.5)	Yes	None	-	Fracture of lateral condyle of left femur	Osteosynthesis	3 months ago
7	49	Female	60 months	Right patella	1.8, 1.8 (1.1, 1.2)	No	Intra-articular injection	Unknown	Internal derangement of right knee	(arthroscopy)	3 years ago
8	33	Female	13 months	Left patella	2.5 (2.4)	No	Intra-articular injection	Unknown	Internal derangement of left knee	(arthroscopy)	8 months ago
9	26	Male	36 months	Medial condyle of right femur	4.9 (3.9)	Yes	None	-	None	None	-
10	36	Female	11 months	Medial condyle of left femur	1.8 (1.3)	No	Intra-articular injection	Unknown	Left medial meniscus injury	None	-
11	26	Male	24 months	Lateral condyle of left femur	3.1 (2.4)	No	None	-	Left anterior cruciate ligament damage	Anterior cruciate ligament reconstruction	21 months ago
12	21	Male	7 months	Medial condyle of right femur	4.0 (4.0)	No	None	-	Right anterior cruciate ligament damage	Anterior cruciate ligament reconstruction	At the time of tissue collection
13	21	Female	60 months	Left patella	1.8 (2.0)	Yes	None	-	Left patella dislocation	Medial patellofemoral ligament reconstruction	At the time of transplantation
14	45	Male	12 months	Medial condyle of left femur	2.9 (2.9)	No	Intra-articular injection	Unknown	Left medial meniscus injury	Partial meniscectomy	1 year ago
15	30	Male	3 months	Medial condyle of left femur	1.6 (2.0)	Yes	None	-	None	None	-
16	23	Male	3 months	Medial condyle of right femur	1.8 (1.6)	No	None	-	Right anterior cruciate ligament damage Right meniscus injury	Anterior cruciate ligament reconstruction, Partial meniscectomy	3 months ago
17	24	Female	24 months	Medial condyle of right femur	1.8 (1.3)	No	Intra-articular injection	Unknown	Right medial meniscus injury Cartilage injury of medial condyle of right femur	Partial meniscectomy Fixation of cartilage graft	33 months ago
18	41	Male	10 months	Medial condyle of left femur	3.9 (4.2)	Yes	Intra-articular injection	Unknown	Left anterior cruciate ligament damage	Anterior cruciate ligament reconstruction	3 months ago
19	22	Female	3 months	Medial condyle of right femur	4.5 (3.8)	No	None	-	None	None	-
20	21	Female	3 months	Lateral condyle of left femur	1.2 (2.0)	Yes	None	-	Left patella dislocation	Medial patellofemoral ligament reconstruction	At the time of transplantation

21	55	Female	14 months	Left patella	4.9 (4.2)	Yes	Intra-articular injection	Unknown	Left patella fracture	Osteosynthesis, medial and lateral partial meniscectomy	14 months ago, at the time of tissue collection
22	40	Male	36 months	Lateral condyle of right femur	4.7 (4.9)	No	None	-	Right medial and lateral meniscus injury	Partial meniscectomy	3 years ago
23	37	Female	3 months	Left patella, femoral trochlea	3.1, 1.5 (4.1, 2.9)	No	None	-	Right anterior cruciate ligament damage (contralateral)	Anterior cruciate ligament reconstruction (contralateral)	30 months ago
24	22	Female	60 months	Left patella	4.9 (4.2)	Yes	Osteochondrosynthesis		Left patella dislocation/fracture	Invasive osteosynthesis, arthrorisis of patella	5 years ago

[11] Time from diagnosis of defect to transplantation of JACC

[12] In patients with more than one cartilage defect, the area of each defect is presented.

[13] The timing of prior treatment of cartilage defect relative to the day of transplantation of JACC. All patients received intra-articular injection of hyaluronic acid. The last day of injection was "Unknown." Since the protocol of Study J-TEC002 included the exclusion criterion "Patients who received intra-articular injection of hyaluronic acid after 1 week before baseline knee (elbow) function evaluation (9 weeks before scheduled transplantation)", the patient appeared to have received the drug >9 weeks before transplantation of the product.

[14] The timing of surgery for concurrent injury and medical history relative to the day of transplantation of JACC. When ≥2 surgeries were performed on concurrent injury, they are described in the table in the order of the name of surgery described in the previous cell.

6.B.(5).2) Evaluation methods

Clinical symptoms (Lysholm Knee Score) and morphological findings (MRI images and arthroscopy [arthroscopic assessment system]) in patients included in individual efficacy evaluation were examined at baseline and Month 12 postoperative. In addition, PMDA selected and investigated the effects of factors that might affect the assessment of clinical symptoms (painkiller use, cartilage defect area, and timing of surgery for cartilage defect and complications) and factors that might affect the evaluation of morphological findings based on imaging data (cartilage defect area and concurrent degeneration of subchondral bone).

Endpoints

(i) Lysholm Knee Score

Changes in the Lysholm Knee Score were analyzed as shown below.

(a) Total points of Lysholm Knee Score

The change from baseline in the total points of Lysholm Knee Score at Month 12 was assessed.

(b) Patients with normalization of Lysholm Knee Score items

The percentage of patients who had a level of each Lysholm Knee Score item lower than the normal function level (maximum score of each item) at baseline and who achieved the normal level at Month 12 was determined.

(c) Patients with marked improvement in Lysholm Knee Score items

The percentage of patients who had a level of each Lysholm Knee Score item by ≥2 levels lower than the normal function level (maximum score of each item) at baseline and who had a marked improvement in the item at Month 12 was determined. Marked improvement was defined as a ≥2 level improvement leading to the normal function in ≥2 items. Baseline disease characteristics were investigated in patients with marked improvement.

(d) Patients with worsening of Lysholm Knee Score items

The percentage of patients with a decrease in the level of each Lysholm Knee Score item at Month 12 from baseline was determined. Worsening was defined as a decrease in the level of each item at Month 12 from baseline. Patients with a decrease in the level of any item were defined as patients with worsened symptoms. Their baseline disease characteristics were investigated.

(e) Factors that is likely to affect Lysholm Knee Score

The effects of painkiller use, cartilage defect area, and surgery for concurrent injury on the total points of Lysholm Knee Score were investigated. Patients whose last use of painkillers was not confirmed at Month 12 were classified as patients possibly under influence of painkillers. Patients were classified into 2 subgroups based on whether their defect size exceeded 4.0 cm^2 (the maximum area of a defect for which autologous osteochondral mosaicplasty as a conventional therapy can be indicated): those with a cartilage defect area <4.0 cm^2 and those with a cartilage defect area ≥4.0 cm^2. The expert adviser commented that surgical treatment is likely to strongly affect knee function within 3 months postoperative. On the basis of the expert adviser's comment,

patients who were treated with JACC within 3 months after surgical treatment of concurrent injury and the other patients were separately assessed.

(ii) Image review

Images were reviewed as shown below. Among MRI images submitted by the applicant, those taken with techniques suitable for assessment of the cartilage, such as T2-weighted and proton density-weighted sequences, were selected for review.

(a) MRI-based assessment of cartilage repair tissue at cartilage defect site (recipient site for JACC)
The cartilage defect site (recipient site for JACC) was examined to detect normal cartilage tissue at Month 12.

(b) MRI-based assessment of height of cartilage defect site (recipient site for JACC)
The percentage of patients who had the cartilage defect site (recipient site for JACC) integrated with the surrounding cartilage and that of patients who did not were determined at Month 12. Baseline disease characteristics were investigated in patients with thickening of the transplanted cartilage. Similar information was collected by arthroscopy, which directly observed the periosteal patch to assess the height of the recipient site. Since the condition of the periosteum at Year 1 is unknown, it is difficult to distinguish the transplanted cartilage from the periosteum based on the arthroscopic findings. For this reason, MRI findings were used to assess the recovery of the height of the recipient site for JACC.

(c) Inflammatory findings based on MRI images
The percentage of patients who had MRI evidence of inflammation of the tissue surrounding the cartilage defect at baseline and did not have such evidence at Month 12 was determined. In patients who appeared to have new inflammatory findings after the transplantation of JACC, baseline disease characteristics were investigated. Inflammatory findings include the retention of joint fluid.

(d) Arthroscopic findings
Arthroscopic findings were graded at Month 12 according to the arthroscopic assessment system based on the ICRS's cartilage repair assessment. The frequency of each grade was determined. Baseline disease characteristics were also investigated in patients with no improvement to Grade III or IV.

6.B.(5).3) Results

(i) Lysholm Knee Score

The results of assessment of clinical symptoms using the Lysholm Knee Score are as shown below (Table 23).

(a) Total points of Lysholm Knee Score
The total points of the Lysholm Knee Score increased from baseline at Month 12 in 23 of 24 patients (the remaining 1 patient having no score data because of re-transplantation).

(b) Patients with normalization of Lysholm Knee Score items

The percentage of patients with a low score for each Lysholm Knee Score item at baseline was as follows: 100% (24 of 24 patients) for pain, 83.3% (20 of 24 patients) for stair-climbing, 70.8% (17 of 24 patients) for locking, 66.7% (16 of 24 patients) for squatting, 62.5% (15 of 24 patients) for limp, 58.3% (14 of 24 patients) for instability, 41.7% (10 of 24 patients) for swelling, and 12.5% (3 of 24 patients) for support.[15] Further, the percentage of patients with a low score at baseline who had improvement to the normal function level for these items at Month 12 was as follows: 86.7% (13 of 15 patients) for limp, 35.3% (6 of 17 patients) for locking, 71.4% (10 of 14 patients) for instability, 40.0% (8 of 20 patients) for stair-climbing, 66.7% (2 of 3 patients) for support, 70.0% (7 of 10 patients) for swelling, 41.7% (10 of 24 patients) for pain, and 75.0% (12 of 16 patients) for squatting.

(c) Patients with marked improvement in Lysholm Knee Score items

The percentage of patients who had a decreased score for each Lysholm Knee Score item by ≥2 levels at baseline was as follows: 87.5% (21 of 24 patients) for pain, 45.8% (11 of 24 patients) for instability, 41.7% (10 of 24 patients) for stair-climbing, 20.8% (5 of 24 patients) locking, and 16.7% (4 of 24 patients) each for limp and swelling. No patient had decreased scores for squatting and support. The percentage of patients who had marked improvement in these items at Month 12 was as follows: 33.3% (7 of 21 patients) for pain, 63.6% (7 of 11 patients) for instability, 75.0% (3 of 4 patients) for limp, 60.0% (3 of 5 patients) for locking, 20.0% (2 of 10 patients) for stair-climbing, and 50.0% (2 of 4 patients) for swelling.

Five patients (1 patient with osteochondritis dissecans, 4 patients with traumatic cartilage defect) had marked improvement. The 1 patient with osteochondritis dissecans had a cartilage defect area of 1.8 cm^2 and had 4-level improvement in pain and instability. Two patients with traumatic cartilage defect had a cartilage defect area of ≥4.0 cm^2; 1 patient with multiple defects having ≥2-level improvement in locking and instability and 1 patient having ≥2-level improvement in instability and pain. The other 2 patients with traumatic cartilage defect had a cartilage defect area of <4.0 cm^2; 1 patient having ≥2-level improvement in limp, instability, stair-climbing, swelling, and pain (subsequently experiencing suicide attempt) and 1 patient having ≥2-level improvement in limp, locking, swelling, and pain. The former patient received the transplantation of JACC within 3 months after osteosynthesis, while the latter patient was possibly under the influence of painkillers.

(d) Patients with worsening of Lysholm Knee Score items

The percentage of patients with a decrease in the level of each Lysholm Knee Score item from baseline at Month 12[16] was as follows: 16.7% (4 of 24 patients) for stair-climbing; 12.5% (3 of 24 patients) for squatting; 8.3% (2 of 24 patients) each for limp, locking, instability, support, and swelling; and 4.2% (1 of 24 patient) for pain. Symptoms worsened in 9 patients with traumatic cartilage defect, including 1 patient who had undergone re-transplantation because of graft

[15] The patient who had been re-transplanted because of graft delamination was handled as a case of unconfirmed improvement because of a missing score at Month 12.

[16] The patient who had been re-transplanted because of graft delamination had a missing score at Month 12. This patient was included in the analysis as a patient with decreases in the score levels for items because the lack of efficacy may have resulted in graft delamination.

delamination. Of them, 2 patients had decreases in the score levels for more than one items; 1 patient with graft delamination and 1 patient who underwent transplantation at multiple sites and had decreases in the score levels for limb, stair-climbing, and support. Two patients had a decrease in the score level only for stair-climbing. Both patients had a cartilage defect area of <4.0 cm^2. One of them was treated with transplantation of JACC within 3 months after surgical treatment. Two patients had a decrease in the score level only for squatting. The sizes of their cartilage defects were 3.9 and 4.7 cm^2. One patient each had a decrease in the score level for instability, locking, or swelling. All of these patients had a cartilage defect size of <4.0 cm^2. Patients treated with transplantation of JACC within 3 months after surgical treatment all had decreases in the score levels for locking and swelling.

(e) Factors that is likely to affect Lysholm Knee Score

Of the 24 patients included in individual efficacy evaluation, 2 patients with traumatic cartilage defect may have been under the influence of painkillers, with 16- and 42-point increases in the total points of the Lysholm Knee Score. Other patients also had increases in the total points of the Lysholm Knee Score.

The cartilage defect size was <4.0 cm^2 in 16 patients and ≥4.0 cm^2 in 8 patients, including 1 patient with missing data who had undergone re-transplantation because of graft delamination. All of the patients had increases in the total points of the Lysholm Knee Score regardless of the cartilage defect size.

All of the 8 patients who were treated with transplantation of JACC within 3 months after surgical treatment were patients with traumatic cartilage defect, including 1 patient with missing data due to re-transplantation because of graft delamination. Patients treated with transplantation of JACC within 3 months after surgical treatment as well as other patients had increases in the total points of the Lysholm Knee Score.

None of the other factors particularly affected the Lysholm Knee Score.

(f) Others

The following factors may have affected assessment of clinical symptoms based on the Lysholm Knee Score: Injury site (femoral medial condyle in 13 patients, femoral lateral condyle in 5 patients, patella in 6 patients, including 1 patient with multiple injuries in the patella and femoral trochlea), multiple injuries (2 patients), and degeneration of subchondral bone (11 patients). None of these patient's baseline characteristics particularly affected the Lysholm Knee Score.

(ii) Image review

PMDA's review of submitted image findings is summarized below (Table 24).

(a) MRI-based assessment of cartilage repair tissue at cartilage defect site (recipient site of JACC)

Of the 24 patients included in individual efficacy evaluation, 22 patients had evaluable MRI images at Month 12. Of them, only 1 patient (4.2%) with traumatic cartilage defect had a tissue newly covering the cartilage defect with a signal intensity almost comparable to that of the normal

cartilage tissue. This patient had a 2.9-cm^2 cartilage defect in the medial condyle of the left femur, with an increase in the total points of the Lysholm Knee Score from 73 points at baseline to 91 points at Month 12, as well as improvement in arthroscopic findings.

(b) MRI-based assessment of height of cartilage defect site (recipient site of JACC)

Of the 22 patients with evaluable MRI images at Month 12, eight patients (36.4%) showed integration of repair tissue with the surrounding tissue of the cartilage defect site at Month 12 although whether the formed tissue was normal cartilage remained unclear, and all of them were patients with traumatic cartilage defect. Seven patients (31.8%) had no integration of repair tissue with the surrounding tissue of the cartilage defect site at Month 12.

The recipient site of JACC was higher than the original cartilage in 7 of 22 patients (31.8%). Of them, 2 patients with traumatic cartilage defect were diagnosed with thickening or swelling; 1 patient who was treated with transplantation of JACC for a 4.5-cm^2 cartilage defect in the femoral medial condyle and 1 patient who was treated with transplantation of JACC for a 4.9-cm^2 cartilage defect in the left patella accompanied by degeneration of subchondral bone.

(c) Inflammatory findings based on MRI images

Inflammation around the cartilage defect at baseline was observed in 13 patients. Of them, 5 patients (38.5%) had a clear improvement based on MRI images at Month 12, while 8 patients (61.5%) persistently had inflammation. After transplantation of JACC, inflammation was newly observed in 2 patients with traumatic cartilage defect who had retention of joint fluid. These 2 patients had the cartilage defect sizes of 3.1 cm^2, and 3.1 and 1.5 cm^2 (multiple defects). None of them had degeneration of subchondral bone. One patient with osteochondritis dissecans had severe bone marrow oedema. The cause and pathological condition of this event were unknown.

(d) Changes in arthroscopic findings

Patients' arthroscopic findings at Month 12 were graded according to the arthroscopic assessment system based on the ICRS's cartilage repair assessment. A total of 22 patients had evaluable arthroscopic findings, which were rated as follows: Grade I (12 points) in 7 patients (31.8%),[17] Grade II (11-8 points) in 14 patients (63.6%),[18] and Grade IV (3-0 points) in 1 patient (4.5%). The patient with a Grade IV change based on arthroscopic assessment was the patient with osteochondritis dissecans who was treated with transplantation of JACC for a 2.4-cm^2 cartilage defect accompanied by degeneration of subchondral bone. This patient had a severe depression of the subchondral bone and graft delamination.

[17] Amended after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics (before amendment, "Grade I [12 points] in 6 patients [27.3%]")

[18] Amended after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics (before amendment, "Grade II [11-8 points] in 15 patients [68.2%]")

Table 23. Lysholm Knee Score

Patient	Total		Limp		Locking		Instability		Stair-climbing		Support		Swelling		Pain		Knee motion		Reference information			
	Maximum score, 100 points		Maximum score, 5 points		Maximum score, 15 points		Maximum score, 25 points		Maximum score, 10 points		Maximum score, 5 points		Maximum score, 10 points		Maximum score, 25 points		Maximum score, 5 points					
	Base-line	Month 12	Base-line	Month 12	Base-line	Month 12	Base-line	Month 12	Base-line	Month 12	Base-line	Month 12	Base-line	Month 12	Base-line	Month 12	Base-line	Month 12	Cartilage defect size (cm^2)[19]	Painkiller use at time of evaluation[20]	Surgical treatment within 3 months postoperative[21]	Others
Osteochondritis dissecans																						
1	74	95	3	5	15	15	25	25	6	10	5	5	10	10	5	20	5	5	4.7	-	No	
2	44	91	3	5	6	10	5	25	6	6	5	5	10	10	5	25	4	5	1.8	-	No	
3	54	95	3	5	15	15	10	25	6	10	5	5	6	10	5	20	4	5	3.1	-	No	
4	76	100	5	5	10	15	25	25	6	10	5	5	6	10	15	25	4	5	2.4	-	No	Graft hypertrophy, delamination
Traumatic cartilage defect																						
5	76	81	5	5	10	10	25	25	6	10	5	5	6	2	15	20	4	4	3.1	-	Yes	
6	31	99	0	5	10	15	5	25	0	10	2	5	0	10	10	25	4	4	1.5	-	Yes	Suicide attempt
7	53	55	5	3	10	10	25	25	2	0	5	2	2	6	0	5	4	4	1.8, 1.8	-	No	Panic disorder, Multiple transplantations
8	67	81	5	5	10	10	25	25	2	6	5	5	6	10	10	15	4	5	2.5	-	No	
9	86	100	5	5	15	15	20	25	6	10	5	5	10	10	20	25	5	5	4.9	-	No	
10	54	96	0	5	6	15	20	25	2	6	5	5	2	10	15	25	4	5	1.8	Unknown	No	
11	79	96	5	5	15	15	25	25	10	6	5	5	10	10	5	25	4	5	3.1	-	No	
12	83	100	3	5	15	15	15	25	10	10	5	5	10	10	20	25	5	5	4.0	-	Yes (at the time of tissue collection)	
13	81	95	5	5	15	10	25	25	6	10	5	5	6	10	15	25	4	5	1.8	-	Yes (at the time of transplantation)	
14	73	91	3	5	15	15	25	25	6	6	5	5	10	10	5	20	4	5	2.9	-	No	
15	65	95	3	5	10	15	25	25	2	10	5	5	6	10	10	20	4	5	1.6	-	No	
16	58	81	3	5	6	10	10	20	10	6	5	5	10	10	10	20	4	5	1.8	-	Yes	
17	66	82	3	5	6	15	25	20	2	2	5	5	10	10	10	20	5	5	1.8	Unknown	No	
18	40	80	0	5	10	10	5	20	0	6	5	5	10	10	5	20	5	4	3.9	-	Yes	
19	64	74	3	3	10	10	15	20	6	6	5	5	10	10	10	15	5	5	4.5	-	No	
20	61	91	3	5	10	10	10	25	2	6	2	5	10	10	20	25	4	5	1.2	-	Yes (at the time of transplantation)	
21	33	-	0	-	10	-	15	-	0	-	2	-	2	-	0	-	4	-	4.9	-	Yes (at the time of tissue collection)	Re-transplantation after graft, delamination
22	65	85	3	5	10	10	20	25	2	6	5	5	10	10	10	20	5	4	4.7	-	No	Partial periosteal elevation
23	52	91	5	5	6	15	10	25	6	6	5	5	10	10	5	20	5	5	3.1,1.5	-	No	Multiple transplantations
24	61	100	5	5	10	15	15	25	10	10	5	5	6	10	5	25	5	5	4.9	-	No	

[19] In patients with more than one cartilage defect, the size of each defect is presented.

[20] Although the applicant has explained that no patient used a painkiller on the day of assessment of the Lysholm Knee Score, the effects of painkillers on the score are unknown because the timing of the last painkiller use is unknown.

[21] "Yes" for patients who received surgical treatment within 3 months before the transplantation of JACC and "No" for patients who did not.

Table 24. Image finding

Patient	MRI							Arthroscopy	Reference information	
	Baseline			Month 12				Month 12	Cartilage defect size (cm^2)[22]	Degeneration of subchondral bone
	Site of cartilage defect	Inflammatory finding	Others	Cartilage regeneration	Height of transplantation site	Inflammatory finding	Others	ICRS's cartilage repair assessment[23]		
Osteochondritis dissecans										
1	Medial condyle of left femur	-	Not performed (metal)	No	-	-	Not performed (metal)	10 (4.4.2)	4.7	Yes
2	Lateral condyle of left femur	Yes	Contusion of subchondral bone, retention of joint fluid	No	Low	No	Bone marrow oedema, high signal on subchondral bone	10 (4.3.3)	1.8	No
3	Medial condyle of right femur	Yes	Retention of joint fluid, irregular signal intensity of cartilage	No	Low	Yes	Retention of joint fluid, high signal on surface	9 (3.3.3)	3.1	Yes
4	Medial condyle of left femur	Yes	Depression of subchondral bone, retention of joint fluid	No	Low	Yes	Depression at the center of cartilage surface (thickening at the recipient site followed by detachment) Regeneration of subchondral bone, retention of joint fluid	2 (2.0.0)	2.4	Yes
Traumatic cartilage defect										
5	Medial condyle of left femur	Yes	Cartilage thinning	No	Normal	Yes	Cartilage-like tissue (high signal), retention of joint fluid	10 (4.2.4)	3.1	No
6	Lateral condyle of left femur	No	Cartilage defect at a fractured site	No	High	No	Mild hyperplasia at transplantation site, intraosseous cartilage (fracture site)	11 (4.4.3)	1.5	Yes
7	Right patella	Yes	Severe inflammation, retention of joint fluid	No	High	Yes	Cyst under bone cortex, retention of joint fluid, cartilage-like tissue (low signal)	Not performed	1.8, 1.8	No
8	Left patella	Yes	Retention of joint fluid	No	Low	No	Engraftment (low signal), decreased blood flow in subchondral bone (same to low signal)	10 (3.3.4)	2.5	No
9	Medial condyle of right femur	No	Contusion of subchondral bone, irregular surface	No	Normal	No	Cartilage-like tissue (same to low signal), smooth surface	12 (4.4.4)	4.9	Yes
10	Medial condyle of left femur	Yes	Irregular surface, low signal on subchondral bone, retention of joint fluid	No	High	No	Slight thickening at recipient site	10 (4.3.3)	1.8	No
11	Lateral condyle of left femur	No	Unclear subchondral bone area, cartilage thinning	No	Low	Yes	Cartilage-like tissue (low signal), depression of the surface of the transplanted cartilage, retention of joint fluid	12 (4.4.4)	3.1	No
12	Medial condyle of left femur	No	Irregular subchondral bone, high signal on cartilage	No	High	No	Thickening of cartilage-like tissue (same to low signal), depression of subchondral bone	12 (4.4.4)	4.0	No
13	Left patella	No	Irregular subchondral bone, non-weight-bearing part	No	Normal	No	Cartilage-like tissue integrated with the surrounding cartilage	10 (4.4.2)	1.8	Yes
14	Medial condyle of left femur	Yes	Partial cartilage defect (only high signal)	Yes	Normal	Yes	Possibly normal cartilage (same to low signal), retention of joint fluid	9 (4.4.1)	2.9	No
15	Medial condyle of left femur	No	Depression of subchondral bone, with an unclear border	No	Normal	No	Cartilage-like tissue (low signal), high signal at the border	12 (4.4.4)	1.6	Yes
16	Medial condyle of right femur	Yes	Low signal on bone marrow, cartilage thinning	No	Normal	Yes	Retention of joint fluid, cartilage-like tissue (same signal),	12 (4.4.4)	1.8	No
17	Medial condyle of right femur	Yes	Unclear bone marrow - subchondral bone border, cartilage thinning, retention of joint fluid	No	High	Yes	Cartilage-like tissue (same to low signal), low signal on adjacent bone marrow	9 (4.2.3)	1.8	No
18	Medial condyle of left femur	Yes	Low signal on bone marrow, cartilage thinning	No	Normal	Yes	Cartilage-like tissue (same signal), low signal on subchondral bone	12 (4.4.4)	3.9	Yes
19	Medial condyle of left femur	Yes	Low signal on bone marrow, irregular subchondral bone	No	High	No	Swelling, thickening, and irregular surface of recipient site (same to low signal)	12 (4.4.4)	4.5	No
20	Lateral condyle of left femur	No	High signal on cartilage surface	No	Normal	No	Reactive remodeling of subchondral bone, cartilage-like tissue (same signal)	11 (4.4.3)	1.2	Yes
21	Left patella[9]	No	Cartilage defect	No	-	-	Not performed	Not performed	4.9	Yes

22	Lateral condyle of right femur	No	Low signal on bone marrow, cartilage thinning	No	Low		Partially irregular surface, graft missing	9 (2.4.3)	4.7	No
23	Left patella Femoral trochlea	No	Low signal on bone marrow, irregular signal intensity on patella	No	Low	Yes	Possibly cartilage-like tissue on patella, unclear image of trochlea, retention of joint fluid	11 (4.4.3)	3.1, 1.5	No
24	Left patella	Yes	Inflammation of subchondral bone, retention of joint fluid	No	High	No	Thickening of recipient site (high signal on cartilage-like tissue)	11 (4.4.3)	4.9	Yes

[22] In patients with more than one cartilage defect, the area of each defect is presented.
[23] The figures in parenthesis represent the scores (points) of the ICRS's cartilage repair assessment items (degree of defect repair, border zone, and surface) (see Table 13).

6.B.(5).4) Summary of Efficacy

All of the 23 patients with evaluable clinical symptoms at Month 12 had increases in the total points of Lysholm Knee Score. Many patients had a recovery to normal function or ≥2-level improvement of for each item. In particular, patients often had improvement in the score levels for pain, limp, and instability, and the items of their related clinical symptoms, suggesting a possible contribution of JACC to alleviation of symptoms, especially pain. Although there are limitations in identifying factors that may affect the assessment of these clinical symptoms because of the limited number of patients, the total points of the Lysholm Knee Score increased from baseline after transplantation of JACC regardless of patient's baseline characteristics. The results showed that the patient's baseline characteristics did not substantially influence improvement in clinical symptoms after transplantation of JACC in patients included in the evaluation by PMDA. The maximum cartilage defect size in the patients included in individual efficacy evaluation was 4.9 cm^2. The study provided promising results showing that JACC tends to improve clinical symptoms even in patients with a large cartilage defect.

There are factors that affect improvement of clinical symptoms other than the patient's baseline characteristics investigated in the PMDA's individual efficacy evaluation. In particular, the beneficial effects of strengthening the muscle and supporting tissue through rehabilitation given as a standard medical procedure on motor function are well known by healthcare professionals. Concomitant rehabilitation may have contributed to the increased scores for clinical symptoms after transplantation of JACC.

On the other hand, the assessment of MRI findings from Study J-TEC002 showed tendencies toward the integration of defect repair tissue to the surrounding cartilage and improvement in inflammation after transplantation compared with baseline. The arthroscopic assessment also demonstrated that the cartilage defect tended to be repaired, although the cartilage defect covered with a periosteal patch was only observed by arthroscopy. As a result of review by PMDA and the Expert Discussion, however, it was concluded that neither images showing JACC changing into the normal cartilage-like tissue nor findings indicating the characteristics of the hyaline cartilage were obtained at Month 12 post-transplantation, although there are limitations to reviewing MRI data. MRI images from the study varied among patients because the imaging conditions were not adequately discussed in advance at the planning stage of the clinical study. Such images are not necessarily suitable for detection of the cartilage defect. PMDA has concluded that Study J-TEC002 failed to provide MRI data supporting the cartilage reconstruction by JACC, although MRI images supporting cartilage repair might have been obtained under appropriate imaging conditions.

Carticel® approved in 1997 in the US and ChondroCelect™ approved in 2009 in Europe are both indicated for "repair of cartilage defect." In their clinical studies, morphological assessment using tissue sections and biopsies has demonstrated formation of hyaline cartilage-like tissue, unlike the results of Study J-TEC002.

Study J-TEC002 provided no data supporting cartilage regeneration by JACC and the applicant did not submit data on the effects of JACC on the clinical course of patients after Month 12. These issues remain

a challenge. However, the clinical symptom scores tended to show a clinically significant improvement at Month 12 at least in the patients included in individual efficacy evaluation.

6.B.(6) Safety evaluation

6.B.(6).1) Safety information

PMDA asked the applicant to explain the safety analysis of Study J-TEC002 by clarifying the patient population, the definition of adverse events, and determination of a causal relationship of each adverse event with JACC.

The applicant's explanation:
Adverse events related to JACC were defined as "any unfavorable symptom associated with tissue collection or transplantation surgery" and did not include events that might have occurred without transplantation of JACC. Patients who provided tissue specimens but were not treated with transplantation of JACC were excluded from the safety analysis.

PMDA asked the applicant to organize safety information as shown below because adverse events should be "any unfavorable event occurring during the study period" regardless of its causality with JACC and all relevant information should be collected accordingly.

(a) Add 1 patient who provided a tissue specimen but was not treated with transplantation of JACC to the safety analysis population (33 patients in total).
(b) Re-organize information regarding all unfavorable events occurring during the study period that were reported in the case report form but not classified as adverse events by the investigator.
(c) Fully discuss adverse events caused by JACC itself, as well as those due to any transplantation-related procedure and those for which a causal relationship with JACC cannot be ruled out.

The applicant performed safety analysis accordingly and provided the following safety information.

A total of 33 patients (32 treated with transplantation of JACC and 1 patient who provided cartilage tissue but was not treated with transplantation of JACC) were included in the safety analysis. A total of 64 adverse events occurred in 22 of 33 patients (66.7%), while 13 malfunctions occurred in 5 of 33 patients (15.2%). Serious adverse events occurred in 5 of 33 patients (15.2%) (12 events; 3 events of application site pain, 2 events of graft delamination, 2 events of joint range of motion decreased, 2 events of post procedural swelling, 1 event of graft hypertrophy, 1 event of suicide attempt, and 1 event of depressive symptom). Malfunctions occurred in 3 of 33 patients (9.1%) (10 malfunctions; 3 events of application site pain, 2 events of graft delamination, 2 events of joint range of motion decreased, 2 events of post procedural swelling, and 1 events of graft hypertrophy). Table 25 presents malfunctions reported in Study J-TEC002.

Table 25. Malfunctions reported in Study J-TEC002

Event term (seriousness)	Malfunction
Application site pain (serious)	3 events in 3 patients
Graft delamination (serious)	2 events in 2 patients
Joint range of motion decreased (serious)	2 events in 2 patients
Post procedural swelling (serious)	2 events in 2 patients
Red swelling of arm (non-serious)	2 events in 2 patients
Graft hypertrophy (serious)	1 event in 1 patient
Application site warmth (non-serious)	1 event in 1 patient

6.B.(6).2) Safety evaluation

On the basis of the applicant's response, PMDA reviewed information on malfunctions possibly related to JACC.

(a) Graft hypertrophy

In Study J-TEC002, graft hypertrophy was reported in 1 patient as a serious adverse event, which was followed by graft delamination accompanied by pain, swelling, and decreased joint range of motion. This patient experienced haemorrhage from the degenerative subchondral bone during transplantation and had incomplete suturing of the periosteal patch to the intercondylar area. As the above adverse events occurred at Month 8 postoperative, the patient underwent removal of loose body. Histology revealed formation of fibrocartilage on the detached surface of the loose graft. The applicant has explained the possibility of thickening of the periosteal patch used to cover the graft and suggested that bone marrow cell-derived mesenchymal stem cells from subchondral bone haemorrhage may have proliferated or differentiated into fibrocartilage. The applicant also explained the necessity of providing appropriate advice because the differentiation of JACC may have resulted in the formation of fibrocartilage seen in the loose graft.

PMDA's view:

Information on graft hypertrophy should be provided to healthcare professionals because similar events were reported in similar clinical research (clinical research of autologous cultured chondrocyte Carticel [Zaslav K et al. *Am. J Sports Med.* 2009;37:42-55], clinical research by Ochi et al.) although it is difficult to discuss the pathology and etiology of this hypertrophy based on the data from the 1 patient in Study J-TEC002. To prevent the risk of thickening of the periosteal patch, precautions during transplantation, including adjustment thickness of the periosteal patch, are necessary. Special precautions should be taken for transplantation in patients with degenerative subchondral bone or haemorrhage, and those at risk for haemorrhage if the possibility of tissue proliferation due to subchondral haemorrhage cannot be ruled out. As explained by the applicant, the possible differentiation of JACC into fibrocartilage in the loose graft should be communicated to healthcare professionals appropriately. In addition, since MRI images obtained at Month 12 showed graft hypertrophy and swelling (1 patient each), and findings of graft elevation that was not clear thickening but exceeded the surrounding cartilage (5 patients) although these were not reported as adverse events, The applicant should collect further information regarding hypertrophy at the transplantation site.

(b) Graft delamination

Graft delamination was reported as a serious adverse event in 2 patients. The event in 1 patient was reported with graft hypertrophy. The event occurred in the other patient when the patient bent

the knee at Month 3. Delamination of JACC associated with movements occurred only in 1 patient and the cause of this event remains unknown because no histological examination was performed. However, PMDA has concluded that precautions about weight-bearing movements after surgery are necessary if graft delamination can potentially occur even after Month 3 because of weight bearing. This event should also be included in the package insert etc. and further information should be collected.

(c) Decreased joint range of motion

Joint range of motion decreased was reported as a serious adverse event in 2 patients. The event in 1 patient was reported with graft hypertrophy and delamination. The other patient with gonarthrosis had the limited joint range of motion prior to transplantation and the symptom aggravated after transplantation of JACC. Aggravated symptoms after transplantation of JACC were rarely reported, and this event was transient and improved in 2 months postoperative although gonarthrosis takes a different clinical course from traumatic cartilage defect and osteochondritis dissecans. PMDA considers that information regarding this event should be provided and further information should be collected.

(d) Others

PMDA has defined "graft ossification" and "post-transplantation retention of joint fluid" as significant malfunctions, although they were not reported as adverse events in Study J-TEC002. The applicant explained that graft ossification occurred in 1 patient in the clinical research by Ochi et al. (Ochi M, et al. *J. Bone Joint Surg. Br.* 2002;84:571-578) and that it is reasonable to think that this ossification was caused by the infiltration of cells having osteogenic potential from the subchondral bone and bone marrow around the recipient site. The applicant also explained that even if the ossification of the product progresses, it will not be a significant problem.

PMDA's view:

Graft ossification is a clinically significant event because it may lead to new symptoms, such as limited joint function. No histological examination was performed in the patient who experienced this event in the clinical research by Ochi et al. and the mechanism of graft ossification and other relevant information are unknown. Differentiation of JACC itself into osteocytes and the effects of the periosteal patch used for transplantation might also have been involved in the graft ossification. Precautions about this event should be provided and further information should be collected.

In addition, assessment of MRI images in the individual efficacy evaluation by PMDA revealed retention of joint fluid that persisted up to Month 12, with no outcome data available. This event was probably associated with inflammation. Taking the above finding into account, relevant information regarding this event should be communicated to healthcare professionals appropriately and further information should be collected.

6.B.(6).3) Long-term safety

PMDA asked the applicant to explain the long-term safety of JACC.

The applicant conducted extended follow-up of patients treated with JACC after the end of the 12-month follow-up period to evaluate the long-term safety of JACC. Safety information collected from 26 of 32 patients after 23 months of follow-up and 19 of 32 patients after ≥35 months of follow-up. Safety information were also collected from 7 of 16 patients who had ≥48 months of follow-up at the time of the follow-up survey (up to 51 months of follow-up). The safety information collected from these patients was submitted as reference data. On the basis of this information, the applicant explained that no serious adverse event occurred in these patients who were followed up for long periods of time.

PMDA's view:
Since MRI images of some patients at Month 12 in Study J-TEC002 showed the cartilage whose height exceeded the normal cartilage and retention of joint fluid probably because of inflammation, these events may lead to adverse events, including graft hypertrophy/delamination and persistent inflammatory reaction, during the long-term clinical course. Because the mechanism of efficacy of JACC remains unexplained, adverse events that are not predictable from the safety information from Study J-TEC002 may occur and it is also difficult to discuss measures for reducing the risk of such adverse events.

The submitted reference data show that no serious adverse event occurred in patients who were followed up for extended periods of time, including the patient followed for up to 51 months, which is important information. However, the long-term safety of JACC should be continuously evaluated considering the limited number of patients.

6.B.(6).4) Summary of safety

Based on the above results, none of the adverse events reported in patients in Study J-TEC002 was associated with a significant malfunction that is considered strongly related to JACC and the events were resolving or resolved spontaneously or after some treatment. PMDA concluded that the safety profile of JACC is tolerated up to Month 12 post-transplantation. Considering the limited number of patients evaluated and the limited safety data available, however, further information should be collected. In particular, graft hypertrophy and delamination, decreased joint range of motion, graft ossification, and retention of joint fluid should be assessed as events of special interest.

6.B.(7) Clinical positioning of JACC

The data submitted have not clearly demonstrated the regeneration of the cartilage by JACC. The clinical symptom score tended to improve in patients included in the evaluation by PMDA (cartilage defect size: ≥1.2 cm^2 and ≤4.9 cm^2) although the extent of the contribution of JACC to the improvement in score remains unclear. The safety information up to Month 51, although it is reference data, indicates no significant concern about the use of JACC.

It is difficult to evaluate the efficacy of JACC on the basis of the submitted clinical study results because Study J-TEC002 has problems in the design including selection of the target diseases and evaluation method of efficacy. In addition, the efficacy and safety of JACC could not be compared with those of the conventional treatment because the protocol defined no efficacy target to verify the efficacy of JACC or hypothesis to be tested. The data submitted do no support the clinical positioning of JACC, including

the patient population for whom the conventional treatment is indicated, proposed by the applicant. At present, there is no evidence that supports the use of JACC in patients who are eligible for standard surgical therapies. JACC should not be indicated for patients for whom other treatment options are available.

In Japan, the current standard surgical options for the treatment of traumatic cartilage defect and osteochondritis dissecans, which are the target diseases of JACC, are microfracture and autologous osteochondral mosaicplasty. It is, however, known that these techniques are not always available to patients with a relatively large cartilage defect. Patients with a large cartilage defect are more likely to be young people or adults with a high level of physical activity who are diagnosed with a sport traumatic injury. Such patients are not only restricted from daily activities and sports because of clinical symptoms, such as pain and limited joint range of motion, but also may experience the relatively early progression to gonarthrosis.

Based on the discussion, etc. described in Section "6.B.(4) Efficacy evaluation method in Study J-TEC002," the individual efficacy evaluation conducted by PMDA for the data obtained before and after transplantation of JACC is retrospective and has limitations. Nevertheless, this evaluation by PMDA indicates that JACC can only be a surgical treatment option for patients with a large cartilage defect, specifically a defect ≥4 cm^2; autologous osteochondral mosaicplasty cannot be recommended for the treatment the defect size ≥4 cm^2. JACC has clinical significance in terms of providing a new treatment opportunity to improve clinical symptoms in such patients.

IV. Results of Compliance Assessment Concerning the New Medical Device Application Data

The new medical device application data were subjected to a document-based compliance inspection and a data integrity assessment in accordance with the provisions of the Pharmaceutical Affairs Act.

The clinical study report (Attached document 6-1) and the addendum to the clinical study report (Attached document 6-2) were initially submitted as evaluation data in support of the application. The inspection revealed that although data through Month 24 were submitted, the data from Month >12 through Month 24 included in Attached document 6-2 were not GCP-compliant because the sponsor (applicant) had submitted the notification of study completion to PMDA after the end of the follow-up period at Month 12 postoperative. Consequently, the data from Month >12 through Month 24 were eliminated from the evaluation data and handled as reference data.

In addition, the applicant explained that the submitted data include the results of tests, etc. not specified in the protocol. These results were also eliminated from the evaluation data and handled as reference data.

The inspection and assessment revealed no particular problems in other data. PMDA thus concluded that there were no obstacles to conducting its review based on the application documents submitted, provided that the above data are eliminated from the evaluation data.

V. Others

The documents submitted for the application of JACC lack information/explanation about the following key issues in regulatory review:

- Appropriateness of the manufacturing process, quality characterization, and quality control,
- Appropriateness of the discussion on the results of the primary pharmacodynamics study,
- Concept of the target patient population and its rationale,
- Conventional treatment options for the target patient populations, and concept of the clinical positioning of JACC and its rationale,
- Justification for choice of the study population,
- Efficacy evaluation in the clinical study, and
- Safety evaluation in the clinical study.

PMDA asked the applicant to explain each of these issues for regulatory review, but the applicant submitted no satisfactory answer. It took time for PMDA to organize the details of the application and key issues to be discussed. The proposed objective of development of JACC was "cartilage tissue repair." However, the applicant did not perform characterization, etc. of cultured chondrocytes in the quality study and detailed histology, etc. to discuss the efficacy of JACC in the nonclinical studies. Since data on the properties and function involved in cartilage tissue repair were not submitted, it took time for PMDA to verify the applicant's justifications for the proposed indication. The application was submitted before the applicant fully verified the conformance of the raw materials used in the manufacture of JACC to the Standards for Biological Ingredients and took no appropriate measure necessary for switching the raw material after the regulatory submission. These also appear to have delayed the review. As described in Section "6.B.(4) Efficacy evaluation method in Study J-TEC002," PMDA concluded that it is difficult to evaluate the efficacy of JACC on the basis of the submitted clinical study results because Study J-TEC002 has problems in the design. This conclusion of PMDA was supported by the expert advisors at the Expert Discussion, PMDA further requested the applicant to discuss the efficacy, safety, and clinical positioning of JACC, but no appropriate measure to address the problems was taken by the applicant. As a result, PMDA's reviewers had to conduct the individual efficacy evaluation in order to investigate the potential of identifying the clinical positioning of JACC after the problems were pointed out at the Expert Discussion. This also delayed the PMDA's review.

PMDA considers that it is difficult to efficiently proceed with the review and draw a conclusion without the applicant's logical explanations about key issues for regulatory review of a proposed product. The applicant is expected to recognize the incompleteness of their responses and file an application after establishing a complete system in the future.

In addition, as described in Section "IV. Results of Compliance Assessment Concerning the New Medical Device Application Data," the clinical study results include some data not collected/prepared in compliance with the GCP. In order to ensure that failures in administrative procedures and management of clinical studies do not recur, the applicant should make every effort to comply with the GCP.

VI. Overall evaluation

PMDA has concluded that the nonclinical and clinical data submitted do not show evidence that JACC filled the defective area of cartilage and repaired the cartilage tissue by produced cartilage matrices, which is the proposed development objective of JACC.

PMDA also has concluded that it is difficult to evaluate the efficacy of JACC on the basis of the submitted clinical study results alone because the clinical study included different patient populations having different diagnoses and defect sites that should be separately evaluated for efficacy and did not appropriately evaluate the efficacy according to the difference in the characteristics of the target diseases.

However, when the efficacy of JACC was individually evaluated in each patient from the clinical point of view, the clinical symptoms of traumatic cartilage defect or osteochondritis dissecans tended to improve at Month 12 postoperative, although the extent of the contribution of JACC remains unclear.

The safety of JACC can be assured up to Month 12 postoperative although it has risks for infection, etc. because of its nature as a biological product.

As described above, JACC tended to improve the clinical symptoms of traumatic cartilage defect or osteochondritis dissecans. No standard surgical treatment is currently available for patients with traumatic cartilage defect or osteochondritis dissecans who have a cartilage defect of ≥ 4 cm^2. JACC has clinical significance in the treatment of only these diseases because it can offer a new treatment option for the patient populations.

To market JACC, the applicant is required to take the necessary measures shown below for the following reasons: (1) This is the first cellular/tissue-based product in the orthopedics field in Japan; (2) currently, only limited efficacy data are available; and (3) collecting efficacy and safety findings of JACC is not only helpful in ensuring the proper use of JACC but also valuable and useful for the future development of products in the same field, etc.

- Surgeons and medical institutions
  JACC should be used by surgeons with full knowledge/experience in treating traumatic cartilage defect and osteochondritis dissecans of the knee at medical institutions with facilities that enable such surgeons to perform relevant procedures after they become fully familiarized with handling of JACC through training, etc. provided by the marketing authorization holder. Patients eligible for treatment with JACC should be selected after full assessment of the patient's pathological condition and the availability of other treatment options.

- Information to be provided to patients and/or families
  Patients and/or their families should be thoroughly informed of accurate information regarding JACC, including the following: Only limited information is available regarding the characteristics and clinical study results of JACC; and no data showing the formation of the cartilage tissue after

transplantation of JACC are available. Prior to the use of JACC, written informed consent should be obtained after an appropriate written explanation is given.

- Post-marketing use-results surveys
  Since the number of patients included in the clinical study was limited, a use-results survey involving all patients treated with JACC should be conducted for a certain period in the post-market stage. Information on the safety and/or proper use of JACC should be provided to healthcare professionals, academic societies, etc. in a timely manner, as necessary.

- Quality improvement
  Taking into account that the relationship between the quality attributes of JACC and its safety or efficacy remains unclear, detailed data and scientific findings/information regarding the quality attributes of JACC should be continuously collected to review the quality control procedures, including the specifications of JACC, as necessary.

As a result of the above review, PMDA has concluded that JACC may be approved after modifying the intended use and indication as shown below, with the following conditions of approval.

Intended Use or Indication
Alleviation of clinical symptoms of traumatic cartilage defect or osteochondritis dissecans (excluding gonarthrosis) of the knee only when no other treatment options are available[24], and it is used at a cartilage defect with a defect size of ≥4 cm^2

Conditions of Approval
1. The applicant is required to take appropriate measures to ensure that the product will be used in eligible patients by surgeons with a full understanding of its efficacy and safety and sufficient knowledge and experience in the treatment of traumatic cartilage defect and osteochondritis dissecans of the knee at medical institutions with facilities that enable such surgeons to perform relevant procedures.
2. The applicant is required to conduct use-results surveys involving all patients treated with the product for a certain period in the post-market stage to collect data on the efficacy and safety of the product, and take appropriate measures as necessary.

The product is classified as a medical device with a new structure. The re-examination period should be 7 years considering the characteristics of the product and the number of eligible patients. The product is an autologous cellular product. It is classified as a biological product based on the raw materials, etc. used in the manufacturing process.

PMDA has concluded that the present application should be deliberated at the Committee on Medical Devices and *In vitro* Diagnostics.

[24] Amended after the end of the meeting of the Committee on Medical Devices and *In-vitro* Diagnostics (before amendment, “no other treatment options are available”)