Review Report

August 6, 2007
Pharmaceuticals and Medical Devices Agency

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

[Category]	Medical products 4 Orthopedic products
[Generic name]	Other surgical/orthopedic materials (autologous cultured epidermis)
[Brand name]	JACE
[Applicant]	Japan Tissue Engineering Co., Ltd. (J-TEC)
[Date of application]	October 6, 2004
[Reviewing office]	Office of Biologics

Review Results

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Results of review

JACE consists of autologous cultured keratinocytes, ranging from [redacted] to [redacted] cell layers thick, produced using Green's technique, in which keratinocytes isolated from the patient's own skin tissue are co-cultured with irradiated 3T3-J2 cells derived from mouse embryo as a feeder layer. The product is indicated for use in patients with serious, extensive burns who do not have sufficient donor skin available for autografting and is applied to the wound surface of deep dermal (deep second-degree) or full-thickness (third-degree) burns after dermis has been reconstructed with allografts etc. in order to close the wounds via engraftment/epithelialization.

A multi-center, open-label, uncontrolled clinical study was conducted in 2 patients to confirm the efficacy and safety of the product in the treatment of severe burns. As a result, epithelialization of the wounds treated with JACE was observed and there were no particular safety problems. However, due to the very limited data obtained from the clinical study, it is considered necessary to impose the following conditions for approval: the conduct of a post-marketing clinical study and use-results surveys in all patients treated with JACE.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the following intended use and that it is appropriate for the application to be deliberated at the Committee on Medical Devices/In vitro Diagnostics.

Intended use

JACE is indicated for use in patients with serious, extensive burns when sufficient donor sites for autologous skin grafts are not available and the total area of deep dermal and full-thickness burns is 30% or more of the total body surface area. JACE is applied onto the reconstructed dermis in a full-thickness burn wound to facilitate the closure of the wound. Dermis should be reconstructed with allografts, as a general rule. JACE should be used for deep dermal burn wounds only when full-thickness and deep dermal burns coexist and it is difficult to treat them separately.

Conditions for approval

1. Appropriate measures should be taken to ensure that the product will be used, with an understanding of its efficacy and safety, by physicians with adequate knowledge/experience for treating severe burns, at medical institutions capable of treating the intended population appropriately.

2. Due to the very limited number of patients treated in the clinical study, a post-marketing clinical study should be conducted to confirm the efficacy and safety of the product and the results should be reported promptly.

3. Due to the very limited number of patients treated in the clinical study, use-results surveys in all patients treated with JACE should be conducted until the end of the re-examination period as a rule, efficacy and safety information on the product should be collected early after market launch, and the results should be reported periodically.

4. The results etc. of the post-marketing clinical study and use-results surveys should be disclosed promptly. Also, the information should be provided to relevant physicians and medical institutions adequately and incorporated into a patient information leaflet appropriately.

Review Report (1)

June 21, 2007

I. Product for Review

[Category] Medical products 4 Orthopedic products
[Generic name] Other surgical/orthopedic materials (autologous cultured epidermis)
[Brand name] JACE
[Applicant] Japan Tissue Engineering Co., Ltd. (J-TEC)
[Date of application] October 6, 2004
[Proposed intended use] Wound closure of severe burns (e.g. extensive burns)
[Items warranting special mention] Priority Review
[Reviewing office] Office of Biologics

II. Product Overview

JACE consists of autologous cultured keratinocytes, ranging from [redacted] to [redacted] cell layers thick, produced using Green's technique, in which keratinocytes isolated from the patient's own skin tissue are co-cultured with irradiated 3T3-J2 cells derived from mouse embryo as a feeder layer. After deep dermal or full-thickness burn wounds in severely burned patients are pre-treated with allografts etc., JACE is applied onto the wound surface where a dermal layer is present in order to close the wounds via engraftment/epithelialization.

Figure 1
Autologous cultured epidermis suspended by a carrier

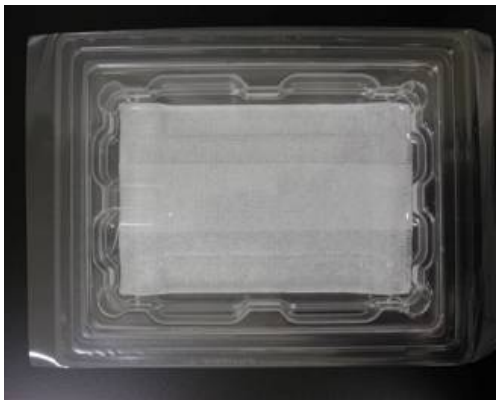

Figure 2 Appearance

III. Summary of the Submitted Data and Outline of the Review by the Pharmaceuticals and Medical Devices Agency

With regard to this application, the data submitted by the applicant and the applicant's responses to the questions from the Pharmaceuticals and Medical Devices Agency (hereinafter referred to as PMDA) are outlined below.

As described later [see *Outline of the review by PMDA, the applicant's response, and the quality of the data and responses submitted*], there were a lot of deficiencies in the data submitted at the time of filing an application for approval of the medical device and each of the *Summary of the submitted data* sections in the Review Report (1) is described based on a completely revised version of the data for regulatory approval.

1. Origin or history of development and usage conditions in foreign countries

1.(1) Product overview

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

Burns are traumatic skin damage caused by various factors and the mortality increases according to the size of the burn, and it has been reported that the mortality among patients with a Burn Index (hereinafter referred to as "BI." BI = full-thickness burn area [%] + 1/2 × dermal burn area [%]) of ≥50 and <60 exceeds 65% and the overall mortality among patients with a BI ≥50 reaches 86% (Japanese Journal of Acute Medicine. 2003;27:3-6). Burns are classified by the depth of the injury into epidermal (first-degree), superficial dermal (superficial second-degree), deep dermal (deep second-degree), and full-thickness (third-degree) burns. Deep dermal burns involve damage to the deeper portions of the dermis and take a long period of time (3-4 weeks) to epithelialize. In the case of full-thickness burns, almost all layers of the dermis are lost and epithelialization does not occur. If there are wounds that are not closed by epithelialization, the general condition may be aggravated due to bacterial infections etc. and such wound infections may cause a deep dermal burn to develop into a full-thickness burn. Therefore, early wound closure is important.

Deep dermal and full-thickness burns are treated with skin autografts such as patch grafts and mesh grafts for wound closure. Since patients with extensive burns do not have enough donor skin available to cover the wounds, repeated autografting is necessary. Until donor skin sites can be reharvested, the wound is temporarily covered with artificial skin, allogeneic cryopreserved cadaver skin obtained from a skin bank (hereinafter referred to as "cadaver skin") or fresh allogeneic skin from close relatives, to protect the wound, reconstruct dermis, and prevent infections. As a cadaver skin graft or fresh skin allograft sloughs off within 2-3 weeks after grafting due to immune rejection, the epidermal layer is removed at one to several weeks after grafting and replaced with an autograft. However, there are problems: As donor skin sites for autografting create new wounds, the risk of infections etc. arise; and repeated autografting is highly invasive.

With respect to keratinocyte cultivation, Green et al. of Harvard Medical School, the US, reported a method of co-cultivation with mouse embryo-derived cells (3T3 cells) as a feeder layer in 1975 and since then, it has been called "cultured epidermal autografts generated using Green's technique." In Japan, clinical application of cultured epidermal autografts generated

using Green's technique to extensive burns was first reported in 1985. There is a report that cultured epidermal autografts generated using Green's technique have clinically been applied to more than 80 patients with skin conditions including scars, nevi, burns, tattoo, and ulcers at the Department of Oral Surgery, Nagoya University School of Medicine (Mater Sci Eng. 1998;C6: 211-219). JACE is a cultured epidermal autograft generated using Green's technique developed by technology transfer from the Department of Oral Surgery, Nagoya University School of Medicine. The manufacturing method of JACE and that adopted by the Department of Oral Surgery, Nagoya University School of Medicine are not identical, but similar (*Note by PMDA:* Published articles on cultured epidermal autografts generated using Green's technique whose manufacturing method is considered similar to that of JACE, as well as the above reports, were referred to for a regulatory review of the application for JACE, as needed [see 6.B *Outline of the review by PMDA*]).

An application for approval of JACE has been filed based on the data from a clinical study in patients with severe burns, which was conducted in Japan in 2004.

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

JACE has not been marketed in any country as of June 2007 and no cultured epidermal autograft generated using Green's technique has been commercialized in Japan to date. Outside Japan, BioSurface's "Epicel®" (current Genzyme Biosurgery, US) was introduced into the market in the US in 1987 and 552 patients were treated with Epicel® between 1989 and 1996. While Epicel® has been designated as a Humanitarian Use Device (HUD),[1)] Premarket Approval Application (PMA),[2)] Humanitarian Device Exemption (HDE),[3)] or Biologics License Application (BLA)[4)] has not been approved so far. In South Korea, Tego Science's "Holoderm®" was approved in 2002.

Outline of the review by PMDA, the applicant's response, and the quality of the data and responses submitted

The information and data presented in the data package submitted at the time of filing an application for regulatory approval were very limited and the summary technical documentation consisted of 72 pages. PMDA asked questions mainly about the following items in the course of a regulatory review and a revised version of the summary technical documentation consisting of more than 470 pages, incorporating the responses to these questions, was submitted as of March

1) Humanitarian Use Device: A medical device that is intended to benefit patients by treating or diagnosing a disease or condition that affects or is manifested in fewer than 4,000 individuals in the United States per year.
2) Premarket Approval Application: The effectiveness and safety of a class III medical device according to the US Classification of Medical Devices are reviewed and the product can be marketed if the premarket approval application is approved.
3) Humanitarian Device Exemption Application: It is exempt from the effectiveness requirements of a PMA. An HUD can be marketed after approval of HDE.
4) Biologics License Application: The efficacy and safety of biological product or biologics are reviewed and the product can be marketed if the application is approved.

16, 2007.

- Intended population
- Existing therapies for the target conditions and the clinical positioning of JACE
- Usage method
- As to allogeneic skin grafts to be used for pre-treatment, the status of use of a skin bank and allogeneic skin grafts in Japan
- Response to the instructions from the Committee on Pharmaceutical Affairs Biotechnology, and its subordinate body, the Subcommittee on Cell/Tissue-based Pharmaceuticals etc., of the Pharmaceutical Affairs and Food Sanitation Council during the deliberation of an application for confirmation of the safety and quality of the investigational device (*kakunin-shinsei*) filed with the Minister of Health and Welfare before the submission of a clinical trial plan notification (hereinafter referred to as "confirmation application") based on "Quality and Safety Assurance of Cell/Tissue-derived Medical Devices and Cell/Tissue Pharmaceuticals" (MHW/PMSB [*Iyaku*] Notification No. 906 dated July 30, 1999)
- Response to the Guideline for epithelial tissue engineering using 3T3J2 cell line and 3T3NIH cell line as feeder cells (MHLW/HPB/RDD [*Iseiken*] Notification No. 0702001 dated July 2, 2004) (hereinafter referred to as "Guideline for Xenotransplantation") based on "Guideline on Infectious Disease Issues in Xenotransplantation for Public Health Service*"
  * MHLW/HPB/RDD *(Iseiken)* Notification No.0709001 dated July 9, 2002
- Specific manufacturing method
- The details of quality control methods and their justification
- Differences in the manufacturing method and quality control between the investigational device and the device to be marketed after approval (hereinafter referred to as "the to-be-marketed device")
- The impact of manufacturing method changes on the efficacy and safety of the device
- The manufacturing method of the device used for the validation of production processes, characterization, quality control, and safety studies
- Justification for setting stability attributes
- Specific information on the clinical study subjects, e.g. pre-treatment, concomitant therapy, the patient's symptoms, the wound condition, and the JACE-grafted sites.
- Efficacy/safety assessment criteria for the clinical study
- Adverse events observed in the clinical study (only one event was documented)
- Similar products in Japan and foreign countries and differences between them and JACE
- Explanation of the risks/benefits of JACE

Not only the data for regulatory approval, but also the applicant's responses to the questions from PMDA had problems: Objective assessments based on the data were not clearly distinguished from inferences/expectations; the text was difficult to understand grammatically; and inconsistencies or discrepancies in the written information were found sporadically, etc. Consequently, it took considerable amount of time to understand/grasp the content. PMDA considers that these many, wide-ranging problems were not just a matter of the data format, but were attributable to the applicant's very poor understanding of the key issues for assuring the safety and efficacy of the product.

In addition, as JACE falls under the category of cell/tissue-based medical devices, a confirmation application was filed as of December 18, 2000 and as a result of a deliberation at a meeting of the Committee on Pharmaceutical Affairs Biotechnology of the Pharmaceutical Affairs and Food Sanitation Council on March 5, 2002, its quality and safety for starting clinical trials were confirmed (http://www.mhlw.go.jp/shingi/2002/03/txt/s0305-1.txt). However, a large number of instructions issued by the Committee [see 2.B.(1) Response to the instructions from the Committee regarding the confirmation application] were not mentioned in the data for regulatory approval and an application for approval of the medical device was filed without dealing with those instructions, which included not only the issues of which the applicant insisted they were unaware, but also the issues to which the applicant had responded that they would examine and handle the instructions appropriately by the time of regulatory submission. Such a situation is extremely unusual. Those instructions were issued again during the regulatory review and were handled by the applicant later and as a result, the information necessary for evaluation was not presented until then, which was one of the main causes of a prolonged review time.

2. Physicochemical properties and specifications

2.A *Summary of the submitted data*

The information on materials management, specific manufacturing method, and process controls was not presented at the time of regulatory submission and the following statements are based on a revised version of the summary technical documentation as of March 16, 2007 incorporating the data and information additionally submitted in the course of the regulatory review.

2.A.(1) Raw materials

2.A.(1).1) Skin tissue

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

As a source material, skin tissue is taken from the patient's normal skin other than the burned areas at the medical institution. Although the anatomic site from which the skin tissue is taken is not specified, a piece of full-thickness skin should be excised in order to ensure that highly proliferative, basal layer cells in the lower epidermis and cells around hair follicles are obtained.

The area of skin required to produce 10 sheets of JACE is 3 cm^2 when grafting is performed within 20 days and 1 cm^2 when grafting is performed from 21 days onwards. The minimum area of skin taken should be 1 cm^2 and if a series of skin grafting operations are performed, skin required for all the grafting operations should be taken at a time. A piece of skin tissue taken is placed into a tissue transport tube and soaked in tissue transport medium ([redacted] containing antibiotics and [redacted]% fetal bovine serum [FBS]). The tube is capped, packaged in a dedicated heat-insulated transport container, and sealed, and then transported to the applicant's manufacturing site.

Before receiving the skin tissue at the manufacturing site, it should be checked that the heat-insulated transport container is kept sealed and that the container is returned to the manufacturing site within 62 hours after sending out the heat-insulated transport container to the medical institution. Subsequently, it should be checked that the tissue transport medium is free of turbidity (accompanied by yellowing) associated with bacterial contaminants. Although the accepted skin tissue may be kept at [redacted]°C-[redacted]°C until the start of manufacture, the manufacture should be started within [redacted] hours after the skin tissue was taken. A portion of the skin tissue (≥1 mm^3) should be put into a cryopreservation tube and stored at below −80°C for the expiration period of the product plus 10 years so as to investigate a cause in the event of infections in the grafted patient.

Since skin tissue is derived from the patient's own skin, donor screening is not performed.

2.A.(1).2) Feeder cells

2.A.(1).2).i) Preparation of cell banks

Feeder cells to be used in the manufacture of JACE are 3T3-J2 cells cloned from Swiss 3T3 cell line established from 17- to 19-day-old fetuses of Swiss mouse in 1963 and the original frozen cell stock was prepared in [redacted] 19[redacted]. The applicant was directly given 3T3-J2 cells obtained after [redacted]-[redacted] subcultures of the original frozen cell stock, by Green, the originator of the cell line in [redacted] 20[redacted], and prepared a master cell bank (MCB), a master working cell bank (MWCB) and a working cell bank (WCB) from these cells.

2.A.(1).2).ii) Characterization and control of cell banks

For the MCB, isoenzyme analysis, karyological analysis, soft agar colony forming assay, sterility test, test for the presence of mycoplasma, and virus testing (extended S^+L^- focus assay; extended XC plaque assay; electron microscopy; reverse transcriptase assay; *in vitro* test using Vero cells etc. [cytopathic effects, hemadsorption and hemagglutination]; *in vivo* test using guinea pigs, embryonated eggs, etc.; mouse antibody production test; and assay for the presence of bovine viruses using bovine nasal turbinate cells etc.) were performed. For the MWCB, sterility test and test for the presence of mycoplasma were performed. For the WCB, sterility test, test for the presence of mycoplasma, and isoenzyme analysis were performed. For cells that

underwent [redacted] passages from the thawing/seeding of the MCB beyond the limit of *in vitro* cell age for production use (CAL) ([redacted] passages), karyological analysis, soft agar colony forming assay, sterility test, test for the presence of mycoplasma, and virus testing (extended S^+L^- focus assay, extended XC plaque assay, electron microscopy, reverse transcriptase assay, *in vitro* tests and *in vivo* tests as performed for the MCB) were performed. As a result, these were confirmed to be of mouse origin and have no tumorigenic potential and no evidence of contamination with bacteria, fungi, mycoplasma, and endogenous and adventitious viruses was observed.

In addition, in accordance with "Guideline for Xenotransplantation," tests for the presence of retroviruses (extended S^+L^- focus assay, extended XC plaque assay, electron microscopy, reverse transcriptase activity assay) were performed on X-ray irradiated feeder cells, and the test results were negative for retrovirus. It has also been confirmed that the cells differentiate into giant cells and are shed in cell culture over time and there are no viable cells remaining on Day [redacted] of culture. The above-mentioned guideline requires an investigation of the chromosome number and tumorigenicity of feeder cells remaining in the culture vessel after cultured tissue is removed. However, in the manufacture of JACE, most of the feeder cells are shed as keratinocytes grow and become confluent. Also, the feeder cells remain on the top of a cultured epidermal sheet, not on the side of the basal layer (on the side of the flask) and do not remain in the culture flask. Thus, this investigation has not been conducted.

The cell banks are stored at below −[redacted]°C. An additional MCB will not be prepared. MWCB and WCB will be prepared in the same manner as before if there are only a few containers left or the quality of the cell bank is deteriorated due to troubles etc. of the cell bank facility. If a new MWCB is prepared, sterility test, test for the presence of mycoplasma, proliferative capacity assay (population doubling time) and morphology observation should be performed and if a new WCB is prepared, isoenzyme analysis, *in vitro* tests and *in vivo* tests, in addition to the above tests, should be performed to confirm conformance to the acceptance criteria. Furthermore, sterility test, test for the presence of mycoplasma, proliferative capacity assay (population doubling time) and morphology observation should be performed every 10 years for the MCB, every 5 years for the MWCB, and once a year for the WCB to confirm conformance to the acceptance criteria.

2.A.(1).2).iii) The number of passages of 3T3-J2 cells

Although Green advised not to use 3T3-J2 cells beyond [redacted] passages, since the passage number as the starting point was unknown, it has been stipulated that cells beyond [redacted] passages from the thawing/seeding of the MCB should not be used. The *in vitro* cell age at the time of thawing the MCB is [redacted] passages from the cells provided by Green, but it has been confirmed that cell morphology and linear logarithmic growth are maintained for CAL ([redacted] passages from the thawing of the MCB). When the device was manufactured using 3T3-J2 feeder cells at [redacted] passage or [redacted] passage from the thawing of the MCB [the manufacturing method proposed in the

confirmation application; see 2.B.(2) Manufacturing method changes], it was confirmed that there were no differences in "morphological observation of the cells in the culture flask," "appearance inspection of cultured epidermal sheet," "cell viability of cultured epidermal sheet" or "colony forming assay of cultured epidermal sheet."

2.A.(1).3) Biological raw materials other than skin tissue and feeder cells

Fetal bovine serum (to supplement a culture medium for the growth of keratinocytes, a medium for the preparation of feeder cells, tissue transport media, and cryopreservation medium for keratinocytes), and calf serum or bovine serum (to supplement a culture medium for the growth of 3T3-J2 cells) should be sourced from Australia or New Zealand and virus testing compliant with FDA 9CFR113.53 or EMEA/CPMP/BWP/1793/02 should be conducted. Also, γ-ray irradiation of the raw materials should be performed before use. Trypsin should be derived from porcine pancreas and virus testing compliant with FDA 9CFR113.53 should be conducted and γ-ray irradiation should be performed before use. Human epidermal growth factor (genetical recombination), cholera toxin, human insulin (genetical recombination), dispase, and antibiotics are derived from microorganisms. Both trypsin and dispase contain lactose derived from the US-sourced and New Zealand-sourced cow's milk.

2.A.(2) Manufacture and release of JACE

2.A.(2).1) Manufacturing method

2.A.(2).1).i) Preparation of feeder cells

The WCB of 3T3-J2 cells is thawed and suspended in a culture medium for the growth of 3T3-J2 cells (containing antibiotics and % calf serum or bovine serum), seeded at a viable cell density of - cells/cm^2, and incubated at °C in % . Within days of incubation and , the cells are detached using % trypsin-EDTA solution and recovered by centrifugation, and seeded in a new flask for subculturing. For the preparation of feeder cells, the cells are recovered in the same manner and suspended in a medium for the preparation of feeder cells (containing % FBS) to make mL/ mL tube, and the entire surface of tube is irradiated with a dose of Gy using an X-ray irradiator. These cells are seeded at a viable cell density of - cells/cm^2 and incubated for - hours to prepare feeder cells. Only the cells up to passages from the MCB should be used and the cells beyond passages should be discarded.

2.A.(2).1).ii) Isolation/cultivation of keratinocytes

After excess fat tissue and are removed from the accepted skin tissue, the tissue is immersed in diluted -fold with buffer solution ( ) for minutes and immersed in a disinfectant solution for tissue ( ) for - minutes repeatedly times, and then rinsed with times. After the skin tissue is cut into about , these are agitated in trypsin % solution at °C for minutes,

the supernatant is [redacted] with [redacted], keratinocytes are isolated from the skin tissue ([redacted]) and recovered by centrifugation, and suspended in a culture medium for the growth of keratinocytes ([redacted] mixture medium [redacted] containing [redacted], human insulin [genetical recombination], and [redacted]%FBS). This isolation/recovery of keratinocytes by trypsinization are repeated [redacted] times and all of the cell suspensions are centrifuged at a time, [redacted] by [redacted], and then keratinocytes are resuspended in a culture medium for the growth of keratinocytes, seeded in the feeder cell flask at a viable cell density of [redacted] cells/cm^2, and incubated at [redacted]°C in [redacted].

After colony formation (between [redacted] and [redacted]) is confirmed, subculturing is performed depending on the growth state of keratinocytes and the release date. The primary culture is generally subcultured on Day [redacted] of incubation and subsequent subcultures are passaged on Day [redacted] of incubation. For subculturing, keratinocytes are detached using trypsin [redacted]% solution and the cells recovered by centrifugation are suspended in a culture medium for the growth of keratinocytes, seeded at a viable cell density of [redacted] cells/cm^2 and incubated. As an in-process control, the proliferative capacity is assessed at passage by checking that the viable cell density is ≥ [redacted] of the seeding density.

If the timing of release of the product needs to be adjusted or a sufficient number of keratinocytes for the manufacture of the total number of sheets to be released are obtained at passage, keratinocytes should be suspended in cryopreservation medium for keratinocytes ([redacted] containing [redacted]%FBS and [redacted]%[redacted]) at a viable cell concentration of [redacted] cells/mL, and aliquots of [redacted] mL should be stored at –[redacted] ± [redacted]°C. The cells are thawed in time for the date of release and it should be checked that the cell viability is ≥[redacted]%, and then the cells are used for the production of cultured epidermal sheets. If JACE grafts are applied in a series of operations at different times, the process step of thawing the cryopreserved keratinocytes and producing cultured epidermal sheets should be repeated.

2.A.(2).1).iii) Production of cultured epidermal sheets

[redacted] days prior to release, keratinocytes are suspended in a culture medium for the growth of keratinocytes, seeded in a [redacted] cm^2 feeder cell flask at a viable cell density of [redacted] cells/cm^2, and incubated at [redacted]°C in [redacted]%[redacted]. Keratinocytes to be seeded at this point should be [redacted] passages from the primary culture.

2.A.(2).2) Primary packaging, labeling, and release

2.A.(2).2).i) Sheet detachment and rinsing

The cultured epidermal sheet flask is rinsed with [redacted] times, added with [redacted]% [redacted] solution, and treated at [redacted] for [redacted] minutes. Then, a cell sheet detachment solution diluted [redacted]-fold

with [redacted] ([redacted]) is added to the culture flask and treated at [redacted]°C in [redacted] [redacted]% [redacted] for [redacted]–[redacted] minutes. [redacted] is confirmed and is rinsed with cultured epidermis storage medium ([redacted] containing [redacted]) [redacted] times. Then a carrier (Material: [redacted], Size: 8 × 10 cm) is placed on the cultured epidermal sheet, the edges of the cultured epidermal sheet are folded back, and the sheet is detached together with the carrier from the culture flask. The cultured epidermal sheet attached to the carrier is rinsed by being dipped into [redacted] [redacted] containing [redacted]. As an in-process inspection, it should be checked that the sheet shape is maintained and that there are no damages, perforations or defects, etc.

2.A.(2).2).ii) Packaging and release

A cultured epidermal sheet attached to the carrier facing up is placed onto a cover sheet (Material: [redacted]), and the cultured epidermal sheet with its edges folded back onto the carrier is wrapped in the cover sheet. It is transferred to a cultured epidermis container and the container is filled with cultured epidermis storage medium. The container is capped and heat-sealed with a top film, and a label containing necessary information is attached to it. Aside from the sheets to be released, [redacted] sheets of cultured epidermis should be stored at below –[redacted]°C for its expiration period plus 10 years.

The container is packaged in a dedicated heat-insulated transport container and the heat-insulated transport container is kept at [redacted] ± [redacted]°C until the results of release testing become available. A heat-insulated transport container that has been confirmed to be capable of maintaining the storage temperature at 10°C-25°C for at least [redacted] hours even under stressed conditions (ambient temperature [redacted]°C or –[redacted]°C) should be used.

Before receiving the product at the medical institution, the tissue code indicated on the primary package should be checked. Also, it should be checked that the heat-insulated transport container is kept sealed and visual inspection should be performed to confirm that there is no damage to or leakage from the package. Then the product is kept at 10°C-25°C until ready for use.

2.A.(2).3) Measures to prevent mix-ups during the manufacturing process

In order to prevent mix-ups involving patients' skin tissues and cells, identification management of [redacted] etc. by [redacted] is employed, simultaneous manipulations of the cells sourced from two or more different patients in the same cell culture room is prohibited, identification management in [redacted] by [redacted] and [redacted] is employed, a follow-up is done based on the production record, the use of the manufacturing facility and equipment is controlled, and education and training are provided to the production personnel.

2.A.(3) Physicochemical properties

At the time of regulatory submission, the following biological safety studies had been performed using the product produced by the manufacturing method proposed in the confirmation application [see 2.B.(2) Manufacturing method changes]: colony forming assay; [redacted]; [redacted] confirmation test; assay for residual mitomycin C; *in vitro* mitomycin C [redacted] and the feeder cell residual rate; determinations of the residual amounts of fetal bovine serum, antibiotics, and mitomycin C; the proliferation inhibition of X-ray irradiated feeder cells; morphology observation and cellular proliferation of human epidermal cells manufactured using X-ray irradiated feeder cells; and the appearance, physical property, cell viability etc. and the feeder cell residual rate of cultured epidermal sheets. However, an investigation of the percentages of component cells and physiologically active substances such as cytokines, which was instructed at the Committee on Pharmaceutical Affairs Biotechnology's meeting on the confirmation application, was not conducted until after regulatory submission. For example, the data on the percentages of component cells were added in a revised version of the summary technical documentation submitted as of May 10, 2006.

The results of studies on the properties including those performed after regulatory submission are as follows.

2.A.(3).1) Percentages of component cells

For [redacted] samples each of the primary cultured cells, subcultured cells, and cultured epidermal sheets, [redacted] of keratinocytes ([redacted]), Langerhans cells ([redacted]), Merkel cells ([redacted]), and fibroblasts ([redacted]) were performed. Also, [redacted] and [redacted] were performed for melanocytes and fat cells, respectively, and the residual feeder cells were measured as cells that [redacted] and [redacted] by [redacted]. Keratinocytes accounted for [redacted]% at the primary culture, fibroblasts tended to increase slightly at the [redacted] passage and the [redacted] passage, but decreased thereafter, melanocytes and Langerhans cells both accounted for about [redacted]% at the primary culture, but decreased with passage. Merkel cells or fat cells did not exist from the primary culture. In the final product, keratinocytes accounted for [redacted]% on average, fibroblasts and feeder cells were both [redacted]% on average, and melanocytes, Langerhans cells, Merkel cells, and fat cells were confirmed not to exist.

2.A.(3).2) Physiologically active substances such as cytokines and their quantities

Cytokines in culture supernatant (over 24 hours after the medium was changed) at the time of release per cultured epidermal sheet were measured by ELISA and the mean values of [redacted] samples were [redacted] ng/day for interleukin 1α (IL-1α), [redacted] ng/day for interleukin 1β (IL-1β), [redacted] ng/day for platelet-derived growth factor AA (PDGF-AA), [redacted] ng/day for transforming growth factor α (TGF-α), [redacted] ng/day for transforming growth factor β1 (TGF-β1), [redacted] ng/day for vascular endothelial growth factor (VEGF), and below the detection limits for keratinocyte

growth factor (KGF), basic fibroblast growth factor (b-FGF), and interleukin 6 (IL-6). There was no clear correlation between the production of the detected cytokines and the epidermal cell growth rate.

2.A.(3).3) Colony forming assay and [redacted] test

As an indicator of cellular proliferative capacity, the colony formation rate (the percentage of viable cells that form a colony) was evaluated at different manufacturing process steps of cultured epidermal sheets for 2 clinical study subjects: the primary culture, subculture, and cultured epidermal sheet. Their colony formation rates were [redacted]% and [redacted]% at the primary culture, but both were increased to about [redacted]% at passage. It has been discussed that this resulted from selection and growth of proliferative cells in the primary culture at passage. Abnormal colonies, which are smaller than normal colonies and not in round shape, are formed by more differentiated cells. Colony forming assay showed that abnormal colonies scarcely existed at the primary culture and tended to increase with passage. In the cultured epidermal sheet, the colony formation rates were decreased to [redacted]% and [redacted]%, respectively and there was a trend towards an increase in the proportion of abnormal colonies. This fact has been discussed as follows: It is inferred that epidermal cells are stratified in cultured epidermal sheets and that proliferative cells are localized to the side of the basal layer. Thus, the proportion of proliferative cells among the total cells was decreased. For other [redacted] samples of cultured epidermal sheets produced after [redacted] passages, the colony formation rate was [redacted]%.

On the other hand, colony forming assays may underestimate the cellular proliferative capacity because the number of viable cells or differences in the size of colony depending on the status of differentiation are not reflected and cells are seeded at a low density. Therefore, the applicant has devised and conducted [redacted] test on [redacted] samples of cultured epidermal sheets produced after [redacted] passages. Namely, cultured epidermal sheets were [redacted], [redacted], [redacted] cultured, and [redacted] was determined. As a result, [redacted]% for all samples.

2.A.(3).4) Cell stability beyond the standard manufacturing period ([redacted] passages) (karyological analysis)

Karyological analysis of 3 samples of cultured epidermal sheets produced from keratinocytes at the initial stage of cultivation (1st passage) and 3 samples of cultured epidermal sheets produced from keratinocytes at a later stage of cultivation (5th passages) was performed. As a result, no chromosomal abnormalities were detected.

2.A.(3).5) Tumorigenesis

Since a translocation was observed in one case by karyological analysis performed for the

confirmation application, soft agar colony forming assay and nude mice inoculation test in addition to karyotyping were performed on 3 samples of cultured epidermal sheets produced after 5 passages including the cryopreservation step. As a result, no tumorigenicity was observed.

2.A.(3).6) The amounts of substances remaining in cultured epidermal sheets

The residual amounts of cholera toxin ([redacted] samples) and fetal bovine serum (bovine serum albumin in [redacted] samples were measured) to supplement culture media for the growth of keratinocytes were measured by [redacted] and were below the detection limit ([redacted] ng/sheet) and [redacted] μg/sheet, respectively. When measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS), benzylpenicillin was below the detection limit ([redacted] units/sheet), kanamycin sulfate was [redacted] ± [redacted] μg/sheet, streptomycin sulfate was [redacted] μg/sheet, and amphotericin B was below the detection limit ([redacted] μg/sheet).

See 4.A. *Summary of the submitted data* for discussions on the safety in 4)-6) above.

2.A.(4) Quality control including specifications

Receiving inspection/in-process tests/release testing/confirmation tests in the manufacturing process are as follows. Tests denoted with an asterisk (*) were not specified at the time of regulatory submission but added in the course of regulatory review. In the following tables, when only the culture flasks that fail to meet the acceptance criteria are required to be disposed of, “partial disposal” is indicated, and when all flasks are required to be disposed of in the event that any flask fails to meet the acceptance criteria, “total disposal” is indicated.

Table. Receiving inspection for skin tissue

	Sample	Test procedure etc.
Check the state of tissue transport*	Heat-insulated transport container containing a skin tissue transport tube	(a) The heat-insulated container is kept sealed, and (b) The container is returned to the manufacturing site within 62 hours after sending out the heat-insulated transport container to the medical institution. [Discard the skin tissue in the event of failure to meet (a) or (b).]
Check the appearance of skin tissue	Tissue transport tube containing skin tissue	(a) There is no damage to/leakage from the tissue transport tube, (b) Skin tissue remains immersed in tissue transport medium, and (c) Tissue transport medium is free of turbidity (accompanied by yellowing) associated with bacterial contaminants. [In the event of failure to meet (a) or (b), contact/consult with the medical institution. If (c) is not met, discard the skin tissue.]

Table. In-process tests

	Sample	Test procedure etc.
Morphological observation of the cells in the 3T3-J2 cell culture flask*	All of the 3T3-J2 cell culture flasks [redacted] and [redacted]	1) Visual observation (a) Color of the medium, (b) Turbidity of the medium, (c) Mold, (d) Foreign body contamination, (e) Leakage of the medium due to flask breakage, (f) Damage to/loss of the label [(a)-(f), Partial disposal] 2) [redacted] (≥[redacted] fold) (a) [redacted], (b) [redacted], (c) [redacted], (d) [redacted] [(a), Partial disposal; (b)-(d), Total disposal]
Morphological observation of the cells in the feeder cell flask*	All of the feeder cell flasks [redacted]	1) Visual observation (a) Color of the medium, (b) Turbidity of the medium, (c) Mold, (d) Foreign body contamination, (e) Leakage of the medium due to flask breakage, (f) Damage to/loss of the label [(a)-(f), Partial disposal] 2) [redacted] (≥[redacted] fold) (a) [redacted], (b) [redacted]. [(a), Partial disposal; (b), Total disposal]
Morphological observation of the cells in the culture flask*	All of the culture flasks at [redacted]	1) Visual observation (a) Color of the medium, (b) Turbidity of the medium, (c) Mold, (d) Foreign body contamination, (e) Damage to/loss of the label, (f) Leakage of the medium due to flask breakage, [(a)-(e), Partial disposal; (f), Partial disposal for [redacted], and [redacted] for other process steps] 2) [redacted] (≥[redacted] fold) (a) [redacted], (b) [redacted], (c) [redacted], (d) [redacted], (e) [redacted], (f) [redacted] [(a), Partial disposal; (b)-(f), Total disposal] 3) [redacted] (a) [redacted], (b) [redacted] [(a) (b), Total disposal]
Physical property test at the time of sheet detachment/rinsing	All of the cultured epidermal sheets at the time of sheet detachment/rinsing	(a) Detachment operation, (b) The shape is maintained, (c) Damage, (d) Durability [(a)-(d), Partial disposal]

Table. In-process tests (Continued)

	Sample	Test procedure etc.
Check the proliferative capacity of keratinocytes*	All of the culture flasks at passage	≥[redacted]-fold the seeding density of keratinocytes at the previous passage (Total disposal)
Check the cell viability at the time of thawing/seeding of keratinocytes*	Cell cryopreservation tubes thawed at the time of thawing/seeding of keratinocytes	The cell viability at the time of thawing/seeding of keratinocytes is ≥[redacted]% (Partial disposal)

Table. Specifications (Release testing)

	Sample	Test procedure etc.
Test for total viable count	A mixture of media after cultivation at [redacted]	JP Microbial Limit Test, Test for total viable count (Membrane Filtration Method) (Total disposal)
Test for the presence of mycoplasma	A mixture of media after cultivation at [redacted]	Detection by PCR (Total disposal)
Appearance inspection of cultured epidermal sheets	All culture flasks for cultured epidermal sheets [redacted]	(a) [redacted], (b) [redacted], (c) [redacted], (d) [redacted] [(a), (c), (d), Total disposal; (b), Partial disposal]
Bacterial endotoxins test	A mixture of culture supernatants in the culture flasks for cultured epidermal sheets [redacted]	JP General Tests, Processes and Apparatus, Bacterial endotoxins test, gel-clot technique (Total disposal)
[redacted] tests	All of [redacted]	(a) [redacted], (b) [redacted], (c) [redacted], (d) [redacted], (e) [redacted] [(a)-(e), Partial disposal]

Tests on cultured epidermal sheet	[redacted] final product	Viable cell density determination, cell viability determination*, assay for residual bovine serum albumin*, determination of the feeder cell residual rate* and determination of keratinocyte content*, physical property test [(a) [redacted], (b) [redacted], (c) [redacted]] (Total disposal)

Table. Confirmation tests

	Sample	Test procedure etc.
Test for the presence of mycoplasma	A mixture of all culture medium supernatants [redacted]	DNA staining test (using indicator cells)
Sterility test	[redacted]	JP General Tests, Processes and Apparatus, Sterility test, Membrane Filtration

2.A.(5) Justification for manufacturing method changes

Although the investigational device was produced by the manufacturing method described in the confirmation application, the manufacturing method is changed for the to-be-marketed device, for which the application for regulatory approval has been filed, as shown in the table below.

Table. Changes in the manufacturing method

Change	Confirmation application, Investigational device	Manufacturing approval application	Purpose
1) Antibiotics to supplement the media*	Benzylpenicillin potassium Kanamycin sulfate Amphotericin B	Streptomycin sulfate is added.	To prevent microbial contamination due to bacterial contaminants from the skin tissue harvested
2) Treatment method for the skin tissue harvested	[redacted] method	[redacted] method	To reduce the treatment time
3) Procedure for feeder preparation	Mitomycin C treatment	X-ray irradiation	To increase the safety
4) Package	Contained in flasks (Cultured and shipped in flasks)	Packaged product ([redacted])	To improve convenience in medical practice
5) Reduction of bovine-derived materials	Use of bovine serum albumin as [redacted] of [redacted] in [redacted]	Switched to fetal bovine serum from the same lot as that to supplement the media	To reduce TSE risk

6) [redacted] Solution	Dulbecco's phosphate buffered saline	Physiological saline	To change from a reagent to a JP-listed agent

* [redacted] medium, [redacted] medium, [redacted] medium

Regarding the above changes, the following investigations had been conducted at the time of regulatory submission [see 2.B.(2) Manufacturing method changes, for the investigations conducted after regulatory submission].

2.A.(5).1) Additional antibiotic to supplement the medium

It has been shown that the growth inhibition of [redacted] and other bacteria which could not be reduced by [redacted] antibiotics and the proliferative capacity of keratinocytes at passage and cultured epidermal sheets ([redacted] and colony forming assay) are comparable before and after the addition of the antibiotic.

2.A.(5).2) Change in the treatment method for skin tissue harvested

It has been shown that a sufficient amount of keratinocytes for seeding in primary culture can be obtained by [redacted] method.

2.A.(5).3) Change in the procedure for feeder preparation

It has been shown that the proliferative capacity of keratinocytes at passage and cultured epidermal sheets ([redacted] and colony forming assay), the appearance, physical property, and cell viability of cultured epidermal sheets, [redacted] of feeder cells, and the residual rate of feeder cells in cultured epidermal sheets are comparable between the use of mitomycin C-treated feeder cells and X-ray irradiated feeder cells.

2.A.(5).4) Change in the packaging

The stability of the packaged product was studied [see 3.A *Summary of the submitted data*].

2.B *Outline of the review by PMDA*

2.B.(1) Response to the instructions from the Committee regarding the confirmation application

Concerning the confirmation application for the product, the Committee on Pharmaceutical Affairs Biotechnology and the Subcommittee on Cell/Tissue-based Pharmaceuticals etc. instructed the applicant to investigate the following five points (http://www.mhlw.go.jp/shingi/2002/03/txt/s0305-1.txt).

Instruction 1. The necessity of setting a specification for the purity of the cells in the product (types of cells, relative abundances)

Taking account of the deliberations at the meetings of the Committee on Pharmaceutical Affairs

Biotechnology and the Subcommittee on Cell/Tissue-based Pharmaceuticals etc., the applicant had responded that the content of melanocytes which had been reported to remain in cultured epidermis would be measured and the necessity of setting a specification would be reviewed (http://www.mhlw.go.jp/shingi/2002/03/txt/s0305-1.txt). However, only the results of microscopy were included in the data for approval application, and except for feeder cells, the percentages of different types of cells that are present in the product and the impact of their presence were not described.

Instruction 2. Genetic stability of keratinocytes in the cryopreservation/cell culture process

With respect to the potential for the occurrence of chromosomal abnormalities associated with the manufacturing method, since the results of karyological analysis of cultured epidermis, which were submitted at the time of filing the confirmation application, revealed a translocation in one of [redacted] samples [Note by PMDA: While the data for approval application reads 3 samples, the data for confirmation application describe about a total of [redacted] samples including [redacted] samples after [redacted] passages and [redacted] samples after <[redacted] passages], the applicant was instructed to further perform karyological analysis. Then, the results of karyological analysis of the investigational devices for 2 clinical study subjects and the results of soft agar colony forming assay for the one case with a translocation detected by karyological analysis as documented in the data for confirmation application, were submitted in the data for approval application, in which no detailed explanations were given.

Instruction 3. The necessity of an investigation of the time course of JACE engraftment, e.g. histological changes in the process of engraftment

This issue was not mentioned in the data for approval application.

Instruction 4. An investigation of an allergy test against mice since murine cells are used as feeder cells

This issue was not mentioned in the data for approval application.

Instruction 5. The necessity of setting a specification for the device functions, e.g. the cell viability, the proliferative capacity, and the ability to produce physiologically active substances such as cytokines, of the final product

This issue was not mentioned in the data for approval application.

PMDA asked the applicant to explain what kind of investigations had been conducted in response to each instruction by the time of regulatory submission and how their results were incorporated into safety assurance/quality control, etc.

Instruction 1

The applicant explained about types of cells that may be present in the product based on the

results of microscopy as follows:
Melanocytes have been confirmed to be present in JACE by an in-process test (microscopy), but its content has not been measured. JACE is indicated for the treatment of severe burns and is intended to cover the wounds by the proliferation of grafted keratinocytes. As melanocytes do not contribute to the wound closure compared to keratinocytes, its content does not matter. Also, since melanocytes migrate from the surrounding normal skin, there has been no report that a grafted area of skin becomes white after cultured epidermis is applied, regardless of melanocyte content in the product. It has been reported that Langerhans cells or Merkel cells do not exist in cultured epidermal autografts generated using Green's technique and microscopy of JACE also revealed that [redacted] cells were all [redacted] melanocytes. Thus, it is considered that these types of cells do not exist. However, a study for the presence of these types of cells in JACE by electron microscopy has never been performed and their presence can not be ruled out. Even if Langerhans cells that are [redacted] exist, there should be little impact, as JACE is autologous and shows no immune rejection. Merkel cells involved in [redacted] of skin do not work alone and have no direct impact on graft performance. As to fibroblasts, [redacted] cells have never been observed in the cell culture process for JACE to date. Even in the case of the introduction from skin tissue, if fibroblast feeder cells are [redacted], it is inferred that the proliferation of fibroblasts derived from human skin tissue is inhibited and that very few fibroblasts remain in the final product. Also, even though very few fibroblasts derived from skin tissue are present, they do not affect engraftment as they are autologous. There has been no report that fat cells are introduced into cultured epidermal autografts generated using Green's technique and fat cells [redacted] have never been observed in the cell culture process for JACE or in the final product. Since tissue is processed after the fat layer of skin tissue as a source material is [redacted] and fat cells [redacted] removed due to [redacted], the possibility of the introduction of fat cells is very small. The residual rate of feeder cells in the final product was not high compared to reports on other cultured epidermal autografts generated using Green's technique. Because feeder cells have lost their proliferative capacity by X-ray irradiation and [redacted], they should slough off together with the stratum corneum (horny layer) of epidermis after grafting, [redacted]. Based on the above, there is no need to set a specification for the percentages of these types of cells in the final product. Moreover, as an in-process test, cellular morphology is monitored continuously, and cells are generally regarded as not being present if cells are not observed by microscopy. Thus, there is also no need to set an in-process test for the percentages of these types of cells.

PMDA asked the applicant to provide a justification for the test method, e.g. the detection sensitivity for each type of cell, if the applicant insists that morphological observation of cells by microscopy is adequate for the detection of cells other than keratinocytes.

The applicant responded as follows:

Since the detection sensitivity of microscopy is inadequate for cells other than keratinocytes and quantitative analysis is difficult, the percentages of different types of cells that may be present will be determined by such a technique as [redacted] for the primary cultured cells, subcultured cells, and cultured epidermal sheets. The results of this study were added in a revised version of the summary technical documentation submitted as of May 10, 2006 [see 2.A.(3).1) Percentages of component cells].

In addition, the applicant explained as follows:
The release specifications for JACE include keratinocyte content (≥[redacted]%) and the determination of the residual rate of feeder cells (≤[redacted]%), which ensures that the percentage of cells other than keratinocytes is less than [redacted]%. Thus, there is no need to set a specification for the percentages of other types of cells.

Instruction 2
The applicant explained the genetic stability of keratinocytes in the cryopreservation/cell culture process as follows and the information on a study with cultured epidermal sheets produced by the manufacturing method proposed in the approval application was added in a revised version of the summary technical documentation submitted as of May 10, 2006.

In one case with a translocation (derived from a patient with giant pigmented nevus), a marker chromosome originating from a translocation possibly involving the short arm of chromosome 8 was identified by the banding patterns, and the translocation occurred at a low frequency, i.e., in 2 out of 10 metaphase plates. In the patient with giant pigmented nevus, karyological analysis of the primary-cultured cells derived from the skin revealed chromosome rearrangements such as translocation, dicentric chromosome, ring chromosome, and a marker chromosome while karyological analysis of peripheral blood leukocytes of the same patient showed no abnormalities and there was a report that the patient was a genetic mosaic. The observed translocation was not “breakage type,” but “stable type” which allows the cells to divide normally, and the observed translocated chromosome was common to the relevant two metaphase plates. Thus, it was inferred that it might be genetic mosaicism in the collected tissue cells. Based on these findings, we have concluded that the chromosomal aberration observed in one case should be derived from the tissue provided. Also in soft agar colony forming assay, the relevant cells with a translocation were anchorage dependent for growth, which confirmed that the cells have not undergone transformation and have no tumorigenic potential after transplantation. Furthermore, as to the investigational devices for 2 patients in the clinical study and 3 samples produced after 5 passages by the manufacturing method proposed in the approval application, karyological analysis of the primary cultured cells and cultured epidermal sheets was performed for comparison. As a result, the modal chromosome number was 46, which is the same as normal human cells, and the Q-banding patterns of the chromosomes in all metaphase plates were also consistent with those of the normal human chromosomes and there were no

chromosomal abnormalities. These results have confirmed that the cytogenetic properties of human keratinocytes do not change in the cell culture process for JACE. In addition, using 3 samples of cultured epidermal sheets produced after 5 passages by the manufacturing method proposed in the approval application, soft agar colony forming assay and nude mice inoculation test were performed, which confirmed the absence of tumorigenicity [see 4.A *Summary of the submitted data*].

Instruction 3

Regarding an investigation of histological changes etc. after JACE is grafted, the applicant responded that they had not investigated it because they were unaware of this instruction from the Subcommittee and the Committee. PMDA asked the applicant to conduct this investigation and the results were added in a revised version of the summary technical documentation submitted as of May 10, 2006 [see 5.B.(1) Studies to support indications].

Instruction 4

Regarding an investigation of allergy test against mice, the applicant responded that they were unaware of this instruction from the Subcommittee and the Committee, but explained as follows:

Severely burned patients develop immune tolerance resulting from immune deficiency due to aggravated general condition and even if allergy test is performed after the burn injury, the response may be different from that in normal state. Similarly, as to the acquisition of allergy following JACE grafting, due to the breakdown of immune response resulting from infections etc. in severely burned patients, even if serologic test is performed after grafting, it will be difficult to detect IgE and IgG antibodies. Furthermore, since there are no commercial reagents to detect mouse-specific IgG antibody and commercial reagents for mouse-specific IgE are intended to detect antibodies to epithelium (epidermis, hair), urine proteins, and serum proteins, which are possible major allergens, it is unknown whether allergy associated with feeder cells can be determined. The two patients in the clinical study had no allergy-like symptoms and the results of a literature review on 748 cases of clinical use of cultured epidermal autografts generated using Green's technique, which is a similar product to JACE, in patients with severe burns, also showed that there were no reports of allergic adverse events associated with grafting. Based on the above, this matter will be addressed on the assumption that mouse allergy can be induced by JACE grafting in all patients, and the risk of the development of anaphylaxis or allergy to mouse or mouse-derived cells will be described in the instructions for use. In addition, in order to alert the physicians, it will clearly be stated in the instructions for use that when JACE is applied in a series of operations, allergy may occur in subsequent operations due to sensitization to an initial graft.

Instruction 5

In order to assess the cell viability and proliferative capacity of the final product, colony

forming assay as an indicator of the cell viability and proliferative capacity had been performed with the investigational device, but explanation about the setting of a specification was not provided in the data for approval application and was added in a revised version of the summary technical documentation submitted as of July 15, 2005. The ability to produce cytokines etc. was investigated according to PMDA's instruction after regulatory submission and the results were added in a revised version of the summary technical documentation submitted as of May 10, 2006 [see 2.B.(3) Setting of specifications (release testing) and "2.B.(4) In-process tests].

PMDA accepted the above responses to the instructions 1-5 from the Committee on Pharmaceutical Affairs Biotechnology and the Subcommittee on Cell/Tissue-based Pharmaceuticals etc.

2.B.(2) Manufacturing method changes

PMDA asked the applicant to explain whether the final products before and after the manufacturing process changes are comparable in terms of the quality. The applicant explained that the pre-change and post-change final products were confirmed to conform to the specifications [*Note by PMDA:* At the time of conducting this investigation, the residual amount of bovine serum albumin, the percentage of feeder cells, and keratinocyte content were not included in the specifications and thus were not checked]. PMDA asked the applicant to check other attributes, e.g. the percentages of component cells and secreted material such as physiological active substances and their quantities as well. These data were added in a revised version of the data for approval application submitted as of January 12, 2007 and were explained as follows:

Using skin tissues from [redacted] persons as source material, cultured epidermal sheets were produced by both the manufacturing methods proposed in the confirmation application and in the approval application. In addition to release testing and confirmation tests, tests were performed for evaluating the percentages of component cells (keratinocytes, melanocytes, Langerhans cells, Merkel cells, fibroblasts, fat cells, and feeder cells), physiologically active substances such as cytokines (b-FGF, IL-1α, IL-1β, IL-6, PDGF-AA, TGF-α, TGF-β1, VEGF, and KGF), cellular proliferative capacity ([redacted], [redacted], colony forming ability), residual substances in the final product (cholera toxin, antibiotics, feeder cells, bovine serum albumin), cellular tumorigenesis (soft agar colony forming assay, nude mice inoculation test), and cellular stability beyond the standard manufacturing period (karyological analysis of keratinocytes after [redacted] passages, and the conformance to the acceptance criteria for in-process tests, release testing, and confirmation tests), and it was confirmed that there are no differences that would affect the safety or performance between the pre-change and post-change products.

PMDA considers as follows:

In the post-change product (produced by the manufacturing method proposed in the approval application), [redacted] and [redacted] production increased about 2-fold compared to the pre-change

product (produced by the manufacturing method described in the confirmation application). However, because these are cytokines that are secreted in wound-healing process, the secretion of other cytokines is comparable, and the test results on the percentages of component cells, residual impurities in the final product, colony forming ability, etc. are comparable, the manufacturing method changes are unlikely to have a significant impact on the efficacy and safety. Even so, in the case of cell/tissue-based medical devices, efficacy and safety evaluation based on quality data alone has limitations. It is necessary to evaluate clinical data using the to-be-marketed device as soon as possible [see 6.B.(6) Post-marketing investigations]. This issue will be judged taking account of comments from the expert advisors of the Expert Discussion.

2.B.(3) Setting of specifications (release testing)

2.B.(3).1) Process-related impurities

Since the test for the residual amounts of substances that may cause allergy, such as feeder cells and culture media supplements, was not included in the specifications, the PMDA asked the applicant to explain its necessity.

The applicant responded as follows:

In view of the risk such as allergy, determination of residual bovine serum albumin (Specification: [redacted] μg/sheet) and determination of the residual rate of feeder cells (Specification: The percentage of feeder cells in total cells is ≤[redacted]%) will be included in the release testing and the specification limits will be established based on measured values. With respect to antibiotics, it will be stated in the instructions for use that the product should not be used in patients with a history of allergy.

PMDA accepted the above response. In order to check that the final product contains at least a certain level of keratinocytes and that abnormal proliferation of cells other than keratinocytes has not occurred, a test to determine keratinocyte content by [redacted] using [redacted] (Specification: The percentage of keratinocytes in total cells is ≥[redacted]%) was established after regulatory submission.

2.B.(3).2) Cell viability

Although viable cell density is included in the specifications, if the proportion of dead cells is increased due to abnormalities in the manufacturing process, it can not be detected. Therefore, PMDA asked the applicant to explain the necessity of including cell viability in the specifications.

The applicant responded as follows:

It was considered that setting a viable cell density specification would be enough, as dead cells do not contribute to engraftment. However, the safety of the product containing many dead cells

has not been confirmed and the possibility that abnormalities in the manufacturing process leading to increased dead cells remain undetected can not be excluded. Thus, cell viability (Specification: ≥[redacted]%) will be included in the final product specifications.

PMDA accepted the above response.

2.B.(3).3) Colony forming assay

At the time of filing the confirmation application, the applicant said that they would perform colony forming assay with the investigational device for cells at the time of isolation from the skin tissue, cells at passage, and cells in the final product, analyze the results of colony formation rate (%) and abnormal colony formation rate (%) in an exploratory manner, and consider introducing this assay as part of quality control. However, as no relevant information was included in the data for approval application, PMDA asked the applicant to explain it.

The applicant responded as follows:

Colony forming assay was performed with the investigational devices for the 2 patients treated in the clinical study [see 2.A.(3).3) Colony forming assay and [redacted] test]. However, colony forming assay was not set as a specification test, because it takes 14 days for the test results to become available. Another reason is that, although the assay provides a measure of the proliferative capacity for comparison, the number of cells contained in the product or the colony size is not reflected and, therefore, a threshold required for the graft take and wound closure can not be established.

PMDA accepted the above response, taking also account of the following points: Colony formation rate varies among cells at the time of isolation from the skin tissue, cells at passage, and cells in the final product; the results become available after the product is applied to the patient; and a test to check the proliferative capacity of cells at passage has been set as an in-process test [see 2.B.(4) In-process tests].

2.B.(3).4) Physiologically active substances

Regarding the necessity of setting a specification for the types and production of physiologically active substances, the applicant responded as follows:

JACE has been confirmed to have the ability to produce various cytokines [see 2.A.(3).2) Physiologically active substances such as cytokines and their quantities]. However, many different kinds of physiologically active substances are produced and they function compositely. Among physiologically active substances, no specific factors that are concluded as being quantitatively correlated with cellular proliferative capacity or graft survival rate, have been reported. Thus, quantitation of specific physiologically active substances as a product specification test is not justified.

PMDA accepted the above response, taking also account of no clear correlation between the physiologically active substances produced in JACE and cellular proliferative capacity.

2.B.(3).5) Karyological analysis

Since karyological analysis included in the specifications in the data for confirmation application was eliminated, PMDA asked the applicant to provide its justification.

The applicant responded as follows:

A translocation observed in one case, as documented in the data for confirmation application, seems to have preexisted in the skin tissue, the genetic stability of the product in the cell culture process has been confirmed by karyological analysis of the investigational devices for 2 patients treated in the clinical study and 3 samples produced after 5 passages by the manufacturing method proposed in the approval application [see 2.B.(1) Response to the instructions from the Committee regarding the confirmation application], and karyological analysis takes about 3-6 months. In view of these points, it is judged unnecessary to include karyological analysis in the product release specifications.

PMDA accepted the above response.

2.B.(4) In-process tests

Since a test for assuring the proliferative capacity, such as colony forming assay, is not included in the release specifications, PMDA asked the applicant to set a test for the proliferative capacity as an in-process control.

The applicant responded as follows:

With respect to keratinocyte proliferation, the maintenance of cellular proliferative capacity is continuously checked by monitoring [redacted] by cellular morphological observation. However, "[redacted] of keratinocyte" will be newly added to in-process tests at passage and the viable cell density will be calculated from the number of total cells recovered at passage, which will be required to exceed the seeding density of keratinocytes at the previous passage.

PMDA asked the applicant to further explain whether the acceptance criteria, "the viable cell density exceeds the seeding density of keratinocytes at the previous passage" can well assure the proliferative capacity.

The applicant responded as follows:

Based on the measured proliferation rates, Mean−3SD = [redacted] fold/day and the minimum subculture interval is [redacted] days, and therefore, the acceptance criteria will be changed to specifically set the increase in viable cell density at ≥[redacted] fold (established based on

[redacted]).

PMDA accepted this response.

PMDA also asked the applicant to explain the necessity of setting a test to confirm the quality of thawed cells because once cryopreserved keratinocytes may be thawed and cultured.

The applicant responded as follows:
Low cell viability of keratinocytes to be thawed (Specification: ≥[redacted]%) is considered to reflect some abnormalities in the cryopreservation-thawing process of cells. For this reason, determination of cell viability at the time of thawing/seeding will be added as an in-process test.

PMDA accepted the above response.

2.B.(5) Receiving inspection for skin tissue

PMDA asked the applicant to explain the storage stability of skin tissue taken at the medical institution and the necessity of specifying the time from tissue collection to the start of manufacture.

The applicant responded as follows:
After [redacted] samples of skin tissues taken from [redacted] persons were stored at both [redacted]°C and [redacted]°C for [redacted] days_and [redacted] days, the recovered keratinocytes were cultured and morphological observation, physical property test in the operations of sheet detachment/rinsing, and colony forming assay at the time of thawing were performed. Also, appearance inspection, physical property test, and determination of viable cell density were performed on cultured epidermal sheets. The number of cells recovered per skin tissue area tended to decrease over time and the viable cell density of the cultured epidermal sheet produced from keratinocytes derived from skin tissue stored for [redacted] days was low. The colony formation rate also decreased depending on the storage period and [redacted] tended to [redacted]. The above findings suggest that it is desirable to isolate keratinocytes by Day [redacted] of skin tissue collection. Taking account of the safety margin and the results of validation of the heat-insulated transport container, as the receiving inspection for skin tissue, it has been specified that the skin tissue should be received within 62 hours after sending out the heat-insulated transport container to the medical institution.

PMDA also asked the applicant to explain the suitability of the dedicated heat-insulated transport container to transport collected skin tissue and the necessity of temperature control.

The applicant responded as follows:
It has been confirmed that under stressed conditions (ambient temperature: [redacted]°C or –[redacted]°C), the temperature within the heat-insulated transport container is maintained at [redacted]°C-[redacted]°C for [redacted]

hours and [redacted] hours, respectively.

PMDA accepted the above response, taking also into account that the dedicated heat-insulated transport container will be sealed after skin tissue is put into the container and that it will be checked at the time of receiving skin tissue that the container is kept sealed.

2.B.(6) Raw materials of animal origin

PMDA asked the applicant to recheck the use of raw materials of animal origin.

The applicant responded as follows:

In the manufacture of the WCB of human insulin (genetical recombination), peptone (derived from bovine bile, connective tissue, skin, and bones [including skull and vertebrae, but excluding spinal cord] sourced from the US or Canada), beef extract (derived from Australian-sourced bovine muscle), and pepticase (derived from bovine milk sourced from Australia or New Zealand) are used and porcine trypsin is used for cell passage. The culture medium used in the manufacture of amphotericin B which is contained in the culture medium [redacted] etc. contains casein obtained by treatment of bovine milk sourced from the US, Australia, or New Zealand with porcine enzyme, and amphotericin B final product contains sodium desoxycholate derived from bovine bile (sourced from Australia, New Zealand, Argentina, Brazil) and ovine bile (sourced from New Zealand).

The applicant has currently been gathering further information on raw materials of animal origin and PMDA will determine the necessity of providing information to the medical institutions or patients, based on the information that will be submitted in future.

2.B.(7) Washing of fetal bovine serum

Because the residual amount of bovine serum in JACE after supplemented in the medium should be reduced as much as possible in view of allergy etc., PMDA asked the applicant to explain this point.

The applicant responded as follows:

Bovine serum, which is supplemented in the medium etc., is diluted in [redacted] of cultured epidermal sheet and is further reduced in the rinsing process where the sheet is rinsed with [redacted] mL of [redacted] times. As to the number of rinses, the amount of bovine serum albumin per sheet of cultured epidermis was determined to be [redacted] ng, [redacted] ng, and [redacted] ng when unrinsed, rinsed [redacted] times, and rinsed [redacted] times, respectively, which confirmed that bovine serum albumin is removed after [redacted] times of rinsing.

PMDA accepted the response since the additional effect of rinsing [redacted] times or more in the rinsing process seems low and determination of residual bovine serum albumin has been

included in the final product specifications.

Furthermore, PMDA asked the applicant to provide a justification for allowing rinsing of up to [redacted] sheets of cultured epidermis [redacted].

The applicant responded as follows:
When [redacted] sheets of cultured epidermis were rinsed in [redacted], the residual amounts of bovine serum albumin in the [redacted]th sheet and the [redacted]th sheet were comparable. Hence, there should be no problem with rinsing up to [redacted] sheets.

PMDA accepted the above response.

3. Stability

3.A *Summary of the submitted data*

In order to identify the optimum temperature for the storage of JACE (the packaged product), physical property test was performed on the product samples after [redacted] samples each were stored for [redacted] hours at [redacted]°C, [redacted]°C, [redacted]°C, [redacted]°C, [redacted]°C, [redacted]°C, and [redacted]°C. As a result, [redacted] samples at [redacted]°C and [redacted] samples at [redacted]°C failed to meet the acceptance criteria. When [redacted] test was performed on the samples stored under conditions other than [redacted]°C, [redacted] samples each at [redacted]°C and [redacted]°C failed to meet the acceptance criteria. [redacted] samples stored at [redacted]°C for reference also failed to meet the acceptance criteria. [redacted] at [redacted]°C-[redacted]°C, and [redacted] and [redacted] at [redacted]°C-[redacted]°C tended to [redacted] and were lowered markedly at [redacted]°C.

Furthermore, in order to determine expiration period, physical property test and [redacted] test were performed on the product samples after [redacted] samples each were stored at [redacted]°C, [redacted]°C, [redacted]°C and [redacted]°C for [redacted], [redacted] and [redacted] hours. As a result, [redacted] samples each stored at [redacted]°C for [redacted] and [redacted] hours failed to meet the acceptance criteria for [redacted] test. No remarkable decrease in [redacted] was observed at any storage temperature, while [redacted] tended to decrease over time at [redacted]°C and [redacted]°C and [redacted] tended to decrease over time at all storage temperatures.

The following data were added in a revised version of the summary technical documentation submitted as of March 16, 2007:
The specification tests and [redacted] test were performed on [redacted] samples of the product stored at [redacted]°C and [redacted]°C for [redacted] and [redacted] hours. As a result, all samples conformed to the specifications and the acceptance criteria for [redacted] test.

Based on the above, an expiration period of 56 hours under the storage condition of 10°C-25°C has been proposed.

3.B *Outline of the review by PMDA*

According to the data submitted at the time of filing the application for approval of the medical device, physical property test, [redacted] test, [redacted], [redacted], and colony forming assay were performed to assess the stability of the product. PMDA asked the applicant to explain the basis for choosing physical property test and [redacted] test as the primary endpoints and the reason and justification for not assessing all specification attributes.

The applicant responded as follows:

As to physical property test to assess [redacted] for grafting the product, all the criteria need to be met for grafting. Although there are no data on the immediate correlation between [redacted] and [redacted], [redacted] test was chosen because [redacted] in [redacted] test is similar to [redacted] and is considered to serve as an indicator for inferring [redacted]. The assessment criteria were set at ≥ [redacted]% for [redacted] in accordance with [redacted] in the clinical study. Based on the above, physical property test and [redacted] test were chosen as the primary endpoints in order to evaluate the efficacy of the product from grafting to wound closure. Also, the products stored at [redacted]°C and [redacted]°C for [redacted] and [redacted] hours were additionally tested for conformance to the specifications and the acceptance criteria for [redacted] test. As a result, all samples conformed to them. Therefore, it is appropriate to propose an expiration period of 56 hours under the storage condition of 10°C-25°C.

PMDA still had doubts about the appropriateness of the test procedure and the assessment criteria for [redacted] test, but accepted the above response, taking into account that the products stored at [redacted]°C and [redacted]°C for [redacted] and [redacted] hours conformed to the release specifications and the acceptance criteria for the confirmation test.

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

4.A *Summary of the submitted data*

A karyotyping study as well as soft agar colony forming assay and tumorigenicity assay in nude mice were performed due to a translocation in one case identified by karyological analysis as documented in the data for confirmation application.

4.A.(1) Karyotyping study

In a karyotyping study, 3 samples of keratinocytes were obtained from the eyelid skin of a 15-year-old man, the head skin of a 13-year-old man, and the abdominal skin of a 55-year-old woman and subcultured according to the manufacturing method proposed in the approval application. The 5th passage cells at a later stage of cultivation and the 1st passage cells at the initial stage of cultivation were respectively seeded in the feeder cell flasks and cultured, and karyotyping of metaphase cells was performed using Giemsa-staining and quinacrine/hoechst

double staining to determine the number, structure, and banding patterns of the chromosomes. The chromosome counts of about 100 metaphase plates from Giemsa-stained preparations at the initial stage of cultivation and at a later stage of cultivation revealed the modal chromosome number was 46, the same as normal human cells and its percentage was also almost the same. The Q-banding patterns of the chromosomes in 10 metaphase plates by quinacrine/hoechst double staining were compared to those of normal human chromosomes. As a result, the Q-banding patterns from all the 3 samples were consistent with those of the chromosomes of a normal man or normal woman and there were no chromosomal abnormalities such as translocation, deletion, and duplication, either at the initial stage or a late stage of cultivation. These results have confirmed that the genetic properties of keratinocytes remain unchanged even when cultured according to the manufacturing method proposed in the approval application.

4.A.(2) Soft agar colony forming assay

Three samples of keratinocytes at a later stage of cultivation that were used for the karyotyping study were seeded in [redacted] and cultured and the cells were harvested after [redacted] to obtain cell suspension. In a soft agar medium added with [redacted]% agar, this cell suspension and negative control, MRC-5 cells were seeded at [redacted] cells/flask and positive control, HeLa cells ([redacted] strain) were seeded at [redacted]-[redacted] cells/flask and the number of colonies of at least [redacted] cells was counted on Day [redacted] of incubation. Anchorage-dependent proliferation was determined by culturing these cells ([redacted]-[redacted] cells/flask) in liquid media. The colony formation rate in a liquid medium was [redacted]% for HeLa cells, [redacted]%, [redacted]%, and [redacted]% for the 3 samples of keratinocytes, and [redacted]% for MRC-5 cells. The colony formation rate in a soft agar medium was [redacted]% for HeLa cells, whereas no colonies were formed by the 3 samples of keratinocytes or MRC-5 cells. The above results have confirmed that the cells constituting JACE are anchorage-dependent for growth and have not undergone transformation, suggesting that the cells do not acquire tumorigenicity in the manufacturing method proposed in the approval application.

4.A.(3) Tumorigenicity assay in nude mice

Three samples of keratinocytes at a later stage of cultivation that were used for the karyotyping study and positive control, HeLa cells ([redacted] strain) were inoculated subcutaneously into 10 nude mice per group at 1×10^7 cells/0.2 mL/mouse and the development of nodules was observed according to the WHO guideline (WHO Technical Report Series. 1998;878: 41-43). As a result, a nodule developed at the site of inoculation in all mice in both groups. The size of nodules decreased gradually and the nodules were regressive in the test group while the nodules enlarged in all mice of the HeLa group. In accordance with the WHO guideline, on Day 21 of inoculation, all mice were necropsied and histopathologic examination was performed. As a result, all nodules in the test group were histologically confirmed to be epithelioid cysts or clusters of dead cells, necropsy findings revealed no abnormalities in other organs, and no

metastasis of the inoculated cells to the lymph nodes or the lungs was observed histologically. In the positive control group, nodular growth was confirmed by histopathologic examination as well. The above results have confirmed that the cells constituting JACE have no tumorigenic potential following inoculation into nude mice.

4.A.(4) The safety of biological raw materials, antibiotics, etc. that remain in the final product

Assuming that the maximum number of sheets of JACE to be grafted in one operation is 50 and the maximum number of sheets of JACE to be grafted in a series of operations is 200 [see 6.B.(4).2) The quantity of JACE to be grafted (the number of sheets to be grafted)], the safety of biological raw materials, antibiotics, etc. that remain in the final product was evaluated. The residual amount of bovine serum to supplement the media was assessed in terms of the amount of bovine serum albumin. Given the release specification limit of ≤[redacted] μg/sheet, transplantation of 50 or 200 sheets of JACE results in exposure to up to [redacted] μg or [redacted] μg of bovine serum albumin, respectively. The residual amount of bovine serum albumin in 200 sheets of JACE is about 1/[redacted] to 1/[redacted] of the residual amount of bovine serum per sheet of Apligraf, a cultured allogeneic human skin equivalent, which received pre-market approval (PMA) and has been marketed in the US. Although more than 50,000 units (1 unit is 1 product) of Apligraf have been used, no allergy-related adverse events associated with Apligraf have been reported. Therefore, even when JACE is applied in a series of operations, the incidence of allergy-related adverse events should be low, whereas the possibility of the induction of allergy to bovine serum can not be excluded. Therefore, a caution statement will be included in the instructions for use.

Regarding residual feeder cells, [redacted] sheets of JACE were manufactured from each of the skin tissues taken from [redacted] persons and the proportion of cells stained with [redacted] by [redacted] and [redacted] was measured. As a result, the specification limit was set at ≤[redacted]%. However, because the possibility of the induction of allergy can not be excluded, a caution statement will be included in the instructions for use. Feeder cells are prepared by X-ray irradiation of 3T3-J2 cells to render them proliferation-arrested. The survival time of feeder cells prepared by X-ray irradiation was determined and it has been confirmed that the cells decrease over time in culture and all die by Day [redacted]. Thus, even considering a series of operations, the incidence of ectopic growth of residual feeder cells should be low.

Concerning antibiotics, [redacted] sheets of JACE were manufactured from each of the skin tissues taken from [redacted] persons and the residual amounts of benzylpenicillin potassium, kanamycin sulfate, streptomycin sulfate, and amphotericin B to supplement the media, were measured by [redacted] method. As a result, the residual amount per sheet was <[redacted]U, [redacted] μg, [redacted] μg, and <[redacted] μg, respectively and transplantation of up to 200 sheets results in patient exposure to <[redacted]U, [redacted] μg, [redacted] μg, and <[redacted] μg of the antibiotics, respectively. Even assuming that these antibiotics are all absorbed and distributed into the body following grafting, the incidence

of adverse events associated with these antibiotics should be low in terms of the clinical dose. However, as the possibility of the induction of allergy associated with the antibiotics can not be excluded, a caution statement will be included in the instructions for use.

As to cholera toxin, [redacted] sheets of JACE were manufactured from each of the skin tissues from [redacted] persons and the residual amount was measured by [redacted]. As a result, the residual amount of cholera toxin was below the detection limit, i.e., <[redacted] ng/sheet. If 50 sheets of the product containing [redacted] ng/sheet of cholera toxin are grafted onto an adult weighing 60 kg, cholera toxin level will be less than [redacted] μg/kg, which is less than 1/[redacted]-1/[redacted] of the monkey lethal dose and less than 1/[redacted] of the mouse LD_{50}. If up to 200 sheets of the product are grafted, the residual level will be less than [redacted] μg/kg, which is less than 1/[redacted]-1/[redacted] of the monkey lethal dose. The incidence of adverse events associated with cholera toxin should be low. Based on these results, it has been determined that there are no safety concerns about residual cholera toxin levels in the product. However, since cholera toxin is also a protein, the possibility of allergy will be mentioned in the instructions for use to alert the physicians.

With respect to the safety of other medium components etc., the theoretical residual levels in the product were calculated and it has been inferred that there is an adequate safety margin for all of them. Regarding proteins such as human epidermal growth factor (genetical recombination) in the media, caution about the possibility of allergy will be advised in the instructions for use.

4.B *Outline of the review by PMDA*

Among biological safety tests required for filing an application for approval of a medical device, tests results for cytotoxicity, sensitization, etc. were not submitted. Thus, PMDA asked the applicant to explain about the safety evaluation of JACE by such tests.

The applicant responded as follows:

We are aware that since the JACE product is classified as a medical device, biological safety tests in accordance with "Basic Principles on Biological Safety Evaluation Required for Application for Approval for Manufacturing (Import) of Medical Devices" (MHLW/PFSB/ELD [*Iyakushin*] Notification No. 0213001 dated February 13, 2003) are required. However, because JACE is composed of cells only, which do not meet the definition of raw materials as specified in the above notification, those tests are not suitable for JACE and it is difficult to test JACE according to the generally established methods. Therefore, safety evaluation of JACE was performed by conducting karyological analysis, soft agar colony forming assay, and tumorigenicity assay in nude mice in accordance with "Quality and Safety Assurance of Pharmaceuticals etc. Manufactured Using Human or Animal-derived Components as Raw Materials" (MHW/PMSB [*Iyaku*] Notification No. 1314 dated December 26, 2000). Safety evaluation of raw materials was performed in accordance with "Guideline for Xenotransplantation."

PMDA considers as follows:
Concerning non-clinical safety evaluation, there are no generally established testing methods for cell/tissue-based pharmaceuticals or medical devices at present and the applicant's discussion taking account of the relevant guidelines is appropriate. However, since the possibility of tumorigenesis of grafted JACE can not be excluded, a long-term follow-up after grafting is necessary.

5. Performance

5.A *Summary of the submitted data*

For this regulatory submission, evaluation data on the performance of JACE have not been submitted.

The applicant has judged that animal studies to support the indications and basic studies on the mechanism of action are unnecessary for the following reasons and has submitted the reports on clinical studies that have so far been conducted with cultured epidermal autografts generated using Green's technique as reference data.

(a) JACE is proliferative keratinocytes derived from the patient and is expected to achieve wound closure.
(b) Feeder cells to be used for cultivation of JACE grafts (3T3-J2 cell line derived from Swiss mouse embryo), the medium components, and the procedures for keratinocyte cultivation/sheet detachment are similar to those for cultured epidermal autografts generated using Green's technique including "Epicel®" that has already been marketed in the US and these cultured epidermal autografts generated using Green's technique are considered similar products to JACE.

5.B *Outline of the review by PMDA*

5.B.(1) Studies to support indications

PMDA asked the applicant to explain the time course of engraftment of JACE, the mechanism of engraftment, and histological changes after engraftment, etc.

The applicant responded as follows:
Following the application of a cultured epidermal autograft generated using Green's technique, wound engraftment/epithelialization are completed within 1 week at the earliest. Although there is no report on the mechanism of engraftment in this initial phase of transplantation, the process of proliferation and engraftment of the patient's own cells at the graft site within the normal physiological response range is inferred as follows.

(a) Immediately after the transplantation of JACE, the keratinocytes that constitute the JACE

product contact the wound surface due to the immobilization of the graft by surgical technique and a simple adhesive force of wound exudate components.

(b) The keratinocytes that come in contact with the wound surface produce cell adhesion molecules such as cadherin and adhere to the dermal layer.

(c) The keratinocytes start proliferating while desmosomes between the cells are formed by desmogleins belonging to the cadherin family and hemidesmosomes are formed between the keratinocytes and the dermal tissue via integrin, laminin-5, etc., resulting in adhesion of the keratinocytes to the dermal layer.

(d) The keratinocytes proliferate repeatedly and cover the entire wound, adhering to the dermal layer.

Although it is unknown which is earlier, the production of cell adhesion molecules or cellular proliferation, since it is unlikely that just placing JACE on the dermal layer results in engraftment/epithelialization, it is considered that cell-tissue adhesion accompanied by cellular proliferation needs to be completed to achieve wound closure. The long-term course after JACE transplantation should be similar to that described in the reports on histological findings after the transplantation of cultured epidermal autografts generated using Green's technique (Sci Am. 1991;265:96-102, BIO Clinica. 1998;13:48-52). Namely, an epidermal morphology composed of the basal layer, the spinous layer, the granular layer, and the horny layer, will be observed 1 week after the transplantation of a cultured epidermal autograft generated using Green's technique and a basement membrane will be completed 3-4 weeks after grafting. Then, a gentle wavelike border will be formed between the dermal and epidermal layers 1 year after grafting and complicated epidermal projections into the dermis, as with the normal skin morphology, will be constructed within 2-5 years after grafting. A clinical study of JACE was conducted under the protocol with the primary efficacy endpoint of the percentage of epithelialized area to the grafted area and histological assessment of the grafted sites was not performed because an invasive biopsy in subjects was avoided for ethical considerations and it was judged that histological assessment can be performed by a literature review as described above.

According to the proposed "PERFORMANCE, INTENDED USE, INDICTIONS," "It is known that when applied to dermal burn wounds where a dermal layer is present, cultured epidermal autografts will take almost 100% and close the wounds rapidly. For full-thickness burns where a dermal layer is absent, a similar effect can be obtained if JACE is applied after allogeneic skin is grafted first and a dermal layer is reconstructed." PMDA asked the applicant to explain its rationale.

The applicant responded as follows:

It is known that the take rate of cultured epidermal autografts generated using Green's technique is low when applied to full-thickness skin wounds where no dermal layer is present, such as full-thickness burns (Japan Journal of Burn Injuries. 1992;18:59-70). It is understood that this is

because cultured epidermal sheets engraft via the basement membrane and a basement membrane is formed more easily in wounds with preserved dermis compared to wounds without a dermal component. However, Cuono et al. have developed a method of reconstructing a dermal layer by first grafting allogeneic skin such as cadaver skin onto full-thickness burns (Lancet. 1986;8490:1123-1124, Plast Reconstr Surg. 1987;80:623-635). Namely, if allogeneic skin such as cadaver skin is grafted onto a wound where a dermal layer is absent, the cellular component will slough off due to immune rejection, leaving a layer of allodermis. Then, fibroblasts will migrate into the layer and neovascularization will occur, resulting in the reconstruction of a dermal layer. It has been reported that a high take rate of cultured epidermal autografts generated using Green's technique on full-thickness burn wounds can be achieved, as in the case of dermal burn wounds, when transplanted after a dermal layer is reconstructed in full-thickness burn wounds by this method to prepare a dermal burn-like condition (J Burn Care Rehabil. 1992;13:174-180). Also for JACE, the efficacy of the product when applied after cadaver skin is grafted onto full-thickness burns has been confirmed in the clinical study.

PMDA's view on the above two responses is as follows:
JACE is a cultured epidermal autograft generated using Green's technique and it is understandable that the results from clinical studies with cultured epidermal autografts generated using Green's technique are used as reference data because the feeder cells to be used for cultivation, the medium components, and the procedures for keratinocyte cultivation/sheet detachment are similar, and that it was difficult to perform histological assessment in the clinical study of JACE for ethical considerations. However, there is still a concern about the applicant's judgment that the performance of JACE can be evaluated without conducting any animal study to support the performance of JACE or any basic study on the mechanism of action at all.

Therefore, PMDA asked the applicant to explain the necessity of conducting an autografting study in animals or a study of grafting of human cultured epidermis in immunodeficient mice.

The applicant responded as follows:
Unlike the healing process in humans, generally in an animal model of skin defects-wound healing, the contribution of wound contraction to wound closure is great, an accurate epithelialization rate can not be determined, and there is no standardized method for assessing epithelialization in an animal burn model. Also, an animal experiment model where a cultured epidermal autograft is transplanted after dermis is reconstructed by Cuono et al's method of grafting allogeneic skin onto skin defects, has not been established. Furthermore, the use of immunodeficient animals makes an interpretation of the efficacy of allografting and autografting unclear. On the other hand, the clinical usefulness of other cultured epidermal autografts generated using Green's technique in the treatment of wounds, such as dermal burns, where a dermal layer is present and Cuono et al's method for full-thickness wounds such as full-thickness burns have actually been established and the efficacy in humans has been

demonstrated. Moreover, findings on the details of histological course of the graft have also been reported. Therefore, there is no need to develop and standardize a new animal experiment model and carry out a study.

PMDA accepts the above response, but considers that it is necessary to continue to confirm the indications for use of JACE even after the market launch since the performance of JACE was evaluated in only two patients in the clinical study [see 6.B.(6) Post-marketing investigations].

5.B.(2) Studies to support usage method

JACE is a cultured epidermal autograft generated using Green's technique. It is just applied (placed) onto the wound surface, which is covered with a dressing to immobilize the graft for several days. Thus, no specific study to support usage method has been conducted.

6. Clinical data

6.A *Summary of the submitted data*

As evaluation data on efficacy and safety, the results from one Japanese clinical study were submitted. Since the information presented in the data submitted for approval application was very limited, the following statements are based on a revised version of the summary technical documentation submitted as of March 16, 2007.

6.A.(1) Japanese clinical study (Submitted data: (F)-1, Study Number J-TEC003 [Clinical study period: April 2004 to August 2004], No publication)

A multi-center, open-label, uncontrolled clinical study in burn patients aged ≥20 years was conducted at 2 study sites in Japan in order to confirm the efficacy (the percentage of epithelialization) and safety of JACE in the treatment of severe burns.

Eligible patients had to have at least about 400 cm^2 of full-thickness burns to be covered with JACE grafts and a BI (full-thickness burn area [%] + 1/2 × dermal burn area [%]) of around 30-90. The target number of enrollment of 2 patients was set.

The usage method of JACE was as follows: The area of wounds to be grafted had to be at least about 400 cm^2 and the attending physician determined the number of sheets required, based on the size of a sheet of JACE, considering the area and shape of the wounds to be covered. Skin tissue was taken at the initial examination and the manufacture of JACE was started. On the same day or later, eschar excision was performed and allogeneic skin was grafted. About 2-3 weeks later, the engrafted allograft was abraded and JACE was applied.

The efficacy was assessed on a 4-point scale: "Very effective," "Effective," "Slightly effective," and "Ineffective," based on the percentage of epithelialization 4 weeks after grafting, as the results of JACE take onto the dermal layer formed by skin allografting (granulation) and

epithelialization. The safety was assessed on a 4-point scale: “Very safe,” “Safe,” “Not very safe,” and “Unsafe,” based on adverse events occurring within 4 weeks after grafting or by complete epithelialization, whichever is later. At 1 year after the completion of the clinical study, the wound condition (symptoms such as contracture), subjective symptoms such as pain, and adverse events were investigated.

Furthermore, the clinical usefulness was assessed globally based on the results of efficacy and safety assessments (global assessment) on a 5-point scale: “Very useful,” “Useful,” “Slightly useful,” “Neither useful nor useless,” and “Not useful.”

Against the target enrollment of 2 patients, 2 patients were enrolled into the study and both of them were grafted with JACE. The number of effective cases was 2. Since 1 patient died after the end of a follow-up period, the wound condition at 1 year after the completion of the study was investigated for only 1 patient.

As to the efficacy, one patient was rated as “Very effective” (the percentage of epithelialization: 100%) and the other patient was rated as “Effective” (the percentage of epithelialization: 50%).

As to the safety, adverse events occurred in 2 patients, which were assessed as “unrelated to JACE.” As adverse events, acute renal failure only was listed in the clinical study report and the data submitted for regulatory approval. Other adverse events as presented in the section of individual cases were added in the course of a regulatory review. One patient had a serious adverse event of acute renal failure with a fatal outcome, but a causal relationship to JACE was denied. In the clinical usefulness assessment (global assessment), one patient was rated as “Very useful” and the other patient was rated as “Useful.”

Individual cases are presented below.

6.A.(1).1) Case XXHA

The patient was a 34-year-old woman with full-thickness burn involving 25% of the total body surface area and deep dermal burn involving 10% of the total body surface area (BI, 30.0) caused by flames. On the day of burn injury, the patient was enrolled into Study J-TEC003 and cadaver skin was grafted onto full-thickness burn on her right upper arm (the size of the grafted area, 900 cm^2, 30 × 30 cm). At the same time, a piece of full thickness skin tissue (100 × 30 mm) was taken from the patient and 15 sheets of JACE were manufactured using the skin tissue. At the time of grafting JACE 18 days after the burn injury, infection was observed in the wound grafted with cadaver skin. Thus, the graft site was reduced (450 cm^2, 15 × 30 cm) and 12 sheets of JACE were grafted. The percentage of epithelialization 4 weeks after JACE grafting was 50%, which was rated as “Effective.” Also, at this time point, as an additional therapy, split-thickness autografts were applied to the unepithelialized area grafted with JACE of about

200 cm^2 and the epithelialization was completed 33 days later (62 days after JACE grafting).

After being discharged on the following day of the complete epithelialization of the wounds, the patient refused to attend the hospital as an outpatient due to the deterioration of the underlying disease and did not receive post-discharge treatment, and thereafter, no detailed information has been obtained. However, concerning the long-term efficacy, the wound condition was assessed 14 months after JACE grafting as an additional observation after the completion of Study J-TEC003. As a result, hypertrophic scar and scar contracture of the grafted area were observed and ulceration occurred in the area of contracture. Also, hypertrophic scar and scar contracture were observed in the area that was not epithelialized with JACE and additionally treated with split-thickness autografts. The applicant has explained that these occurred partly because adequate post-discharge treatment could not be performed. The patient had no subjective symptoms of the wounds such as itching and pain.

Adverse events observed were methicillin-resistant *staphylococcal aureus* infection, gram-negative rod infection, acute hepatic dysfunction, pyrexia, and inflammatory reaction, all of which were considered associated with the burns and their treatment, and their causal relationship to JACE was denied.

6.A.(1).2) Case LYTO

The patient was a 33-year-old man with full-thickness burn involving 59% of the total body surface area and deep dermal burn involving 3% of the total body surface area (BI, 60.5) caused by flames. The patient was initially not enrolled into Study J-TEC003 because the patient's burns were highly severe and the patient was considered unsuitable for observation and assessment. However, because there was improvement from the initial shock phase and the infection phase, the patient was enrolled into Study J-TEC003 at 44 days after the burn injury. At 46 days after the burn injury, cadaver skin was grafted onto full-thickness burn on both of his front thighs (the size of the grafted area, 322 cm^2, 14 × 23 cm; and 280 cm^2, 14 × 20 cm). At the same time, a piece of full thickness skin tissue (40 × 20 mm) was taken and 11 sheets of JACE were manufactured. At 21 days after the grafting of cadaver skin (67 days after the burn injury), JACE grafts were applied onto the same areas (4 sheets each on both thighs, 8 sheets in total). The percentage of epithelialization 4 weeks after JACE grafting was 100%, which was rated as "Very effective."

The patient developed renal impairment 24 days after JACE grafting (91 days after the burn injury) and was diagnosed with acute renal failure. Then, the patient also had respiratory failure and circulatory failure and died 38 days after the onset of renal failure. This adverse event was not caused by JACE, but occurred due to a composite of infections associated with the primary disease of severe burns and the effects of anti-infectives, leading to a fatal outcome, and was assessed as unrelated to JACE.

Other adverse events reported were fungaemia, acute pancreatitis, acute circulatory failure, acute respiratory failure, disseminated intravascular coagulation, iron deficiency anaemia, infections, hypothermia, pyrexia, haemorrhage, chills, exudates, vomiting, pain, itching, thirst, malaise, cyanosis, acute hepatic dysfunction, inflammatory reaction, anaemia, coagulopathy, amylase decreased, hypocalcaemia, creatine phosphokinase (CK) decreased, and blood iron (Fe) decreased, all of which were considered associated with the burns and their treatment and the aforementioned acute renal failure, and their causal relationship to JACE was denied.

6.B *Outline of the review by PMDA*

The data submitted at the time of filing an application for approval of the medical device lacked specific information on individual patients (treatment before and after grafting and the patient's condition, the anatomic site grafted, adverse events, follow-up data 1 year after grafting, etc.), the safety assessment criteria, and explanation about causality assessment between adverse events and JACE. In addition, the protocol did not provide the specific procedures for collecting necessary safety information adequately and detailed information could not adequately be checked even when traced back to the source documents, e.g. the medical charts. Furthermore, since patients with severe burns requiring skin grafting, i.e., the intended population for JACE, are very limited and no standard therapy has been established so far, taking also account of the feasibility, it is understandable that the target number of subjects for the clinical study was set at 2, but due to the changes that have been made to the manufacturing method, the manufacturing method for the investigational device is different from that proposed in the approval application and any clinical study with the device produced by the manufacturing method proposed in the approval application has not been conducted. However, it has been shown that the investigational device is highly similar to the to-be-marketed device in terms of quality and the manufacturing method changes are unlikely to significantly affect the efficacy and safety of the device [see 2.B.(2) Manufacturing method changes]. Based on the above, although the information obtained from the clinical study is extremely limited, JACE has been evaluated by a detailed review and a discussion of the submitted data from Study J-TEC003 on 2 patients, together with published articles as appropriate.

6.B.(1) Efficacy

Because the protocol-specified objective of the clinical study of the product (as the investigational device) submitted was to obtain information on local epithelialization (the percentage of epithelialization) with JACE in patients with severe burns, although the intended use of JACE is to save the lives of patients with severe burns, no information that allows for an assessment of its contribution to life-saving, is available. However, based on the results of the percentage of epithelialization 4 weeks after grafting in 2 patients, it is well expected that JACE will lower the incidence of burn wound sepsis and can contribute to a higher survival rate. In light of the fact that severe burn is a serious disease for which no standard therapy has been

established, PMDA has concluded that making JACE available in clinical settings is justified at present. However, it is necessary to promptly collect information including the long-term efficacy, e.g. the percentage of epithelialization with JACE (as the to-be-marketed device) and its contribution to life-saving after marketing approval is granted and provide the information to health professionals appropriately.

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

The regulatory review on the efficacy of JACE is detailed below.

6.B.(1).1) The appropriateness of the efficacy endpoint

PMDA asked the applicant to explain the rationale for choosing the percentage of epithelialization 4 weeks after JACE grafting as the efficacy endpoint.

The applicant responded as follows:

The efficacy of JACE refers to the following: When JACE is grafted onto the surface of burn wound where a dermal layer is present, the subject's own keratinocytes promptly adhere to the dermal tissue and proliferate, resulting in the epithelialization of the grafted area and wound closure. Therefore, the percentage of epithelialization as measured by the ratio of the epithelialized area to the total area grafted with JACE was chosen as the endpoint. As with other cultured epidermal autografts generated using Green's technique, epithelialization should be observed 1 week after JACE grafting and a basement membrane should be completed 3-4 weeks after JACE grafting, which attaches the epidermis firmly to the dermis (Sci Am. 1991;265:96-102), and it is necessary to institute an additional therapy beyond 4 weeks if the wound is not healed. Thus, it was decided to observe the wounds for 4 weeks after grafting.

PMDA has judged that it is appropriate to assess the efficacy of JACE based on the percentage of epithelialization from the viewpoint that the wound surface needs to be closed by epithelialization first to achieve the ultimate goal of treatment of extensive burn injuries, i.e., the improvement of the prognosis. With respect to the timing of assessment, while it takes at least 1 year for the area grafted with a cultured epidermal autograft generated using Green's technique to show a structure histologically similar to that of normal skin (Lab Invest. 1989;60:600-612) and it is necessary to confirm that an epithelialized JACE is not rejected or does not slough off over a long period of time, as mentioned in the applicant's response, there is a report that a basement membrane is formed 3-4 weeks after grafting and its connection with the dermal layer is strengthened gradually due to anchoring fibril formation (Lab Invest. 1989;60:600-612). Thus, PMDA has judged that the timing is appropriate also from the standpoint of histological healing process.

6.B.(1).2) Percentage of epithelialization

PMDA asked the applicant to explain why there was a big difference in the percentage of epithelialization between the 2 patients (50% and 100%) in Study J-TEC003.

The applicant responded as follows:
There was no major difference in the proliferation rate of keratinocytes or the colony formation rate of the final product between the 2 patients. Although the time from shipment to graft application was different (Case XXHA, 22 hours 30 minutes; Case LYTO, 6 hours 30 minutes. The time from the detachment from the flasks to graft application was about 2 hours in both cases), the final product produced in parallel with the investigational device was stored under the transport temperature condition and the cell viability (Case XXHA, 90.3%; Case LYTO, 92.4%) and viable cell density (Case XXHA, 20.3×10^4 cells/cm^2; Case LYTO, 28.2×10^4 cells/cm^2) of the final product were measured at the same timing as the actual grafting operation, which showed no major differences. Thus, it is unlikely that the time from shipment to graft application affected the percentage of epithelialization in the 2 cases. The engraftment of cadaver skin was rated as "Neither useful nor useless" for both cases (because bacterial infection was observed in the recipient wound beds and the graft status was not good, the degree of reconstruction of dermis was unassessable). In Case XXHA, wound infection and moderate exudate were observed, which suggests that the recipient wound bed condition was not good, thereby affecting the percentage of epithelialization. In Case XXHA, the grafted area included a joint region (the right upper arm to the right elbow), which could affect the percentage of epithelialization but should not have as much influence as on the recipient wound bed condition. Published articles also indicate that the factors that can affect the percentage of epithelialization (efficacy) of a cultured epidermal autograft generated using Green's technique include (a) the anatomic sites for graft application (Burns. 2000;26:379-387, N Engl J Med. 1984;311:448-451) and (b) the recipient wound bed condition (J Burn Care Rehabil. 1992;13:174-180, Burns. 1989;15:303-309, Jpn J Plast Reconstr Surg. 2000;43:557-562, Transplantation. 2000;70:1588-1598).

PMDA considers as follows:
Generally, the factors affecting the percentage of epithelialization of skin grafting include the movement of the graft, the movement of the recipient wound bed, and the degree of adhesion between the graft and the recipient wound bed. However, patients basically take a complete bed rest by moderate compression or cast or splint immobilization of the graft sites and as the applicant claims, the influence of grafting JACE over the joint region on the percentage of epithelialization is not great. In light of the above points, PMDA has judged that the applicant's response is generally acceptable.

However, in spite of the presence of infections at the time of grafting JACE in Case LYTO, the percentage of epithelialization 4 weeks after grafting was 100%. Thus, PMDA asked for the applicant's view on the necessity of the objective assessment criteria for infected wounds.

The applicant responded as follows:
It is preferable to use the objective assessment criteria for infected wounds, taking into account bacterial species, bacterial count, etc. However, it is difficult to establish criteria even in burn treatment with ordinary autografts.

PMDA considers as follows:
The applicant's view is generally acceptable. However, at present, as long as the applicant responds that there is a concern that the recipient wound bed conditions significantly affect the percentage of epithelialization, objective assessment criteria should be established. Therefore, it is necessary to continue to investigate the factors associated with epithelialization even after the market launch.

6.B.(1).3) Long-term efficacy
Although there is also a report that Prognostic Burn Index (PBI) incorporating age is most useful for predicting the prognosis (Japanese Journal of Acute Medicine. 2003;27:3-6), Study J-TEC003 included one patient with a PBI ≥90 (PBI, 93.5) at which the mortality exceeds 30%. PMDA asked the applicant to explain the association with the improvement of the prognosis, i.e., life-saving, taking account of the proportion of the area grafted with JACE in the total burn wound area in the two cases.

The applicant responded as follows:
The proportion of the grafted area in the total burn area in the two cases was 7.0% and 4.4%, respectively and the contribution to life-saving can not directly be judged. This is attributable to the design of the clinical study protocol and the objective of Study J-TEC003 is not to confirm life-saving of patients with severe burns, but to confirm the percentage of epithelialization and safety of JACE in patients with severe burns. Since Study J-TEC003 has confirmed that JACE is capable of regenerating an epithelium effectively, the product is expected to well contribute to wound closure and life-saving, when grafted extensively in patients with severe burns.

PMDA considers as follows:
As mentioned before, although the true endpoint for JACE is the improvement of the prognosis, i.e., life-saving, the information available at the time of regulatory submission is limited to local epithelialization and it can not be said at the time of regulatory submission and during the regulatory review that the information on JACE's contribution to life-saving is available. However, the applicant's claim that JACE is expected to contribute to the improvement of the prognosis of patients with extensive burns as a result of epithelialization is understandable, in light of the seriousness of the target disease.

Therefore, PMDA has judged that after the market launch, it is necessary to collect information

on the long-term efficacy including the contribution to life-saving, which was not evaluated in the clinical study, and provide the information promptly and appropriately to health professionals.

6.B.(2) Safety

Concerning adverse events in Study J-TEC003, the investigators were not fully informed of the aim of collecting information on adverse events, partly because the study was initiated before the enforcement of the Ministerial Ordinance on Good Clinical Practice for Medical Devices (MHLW Ordinance No. 36 dated March 23, 2005) ("GCP for Medical Devices"). As a result, only 1 event of acute renal failure, which was considered a serious adverse event, was listed in the clinical study report and the data submitted for regulatory approval. However, as defined in Chapter 1, Article 2, Paragraph 18, of the GCP for Medical Devices, an adverse event is "any disease or disorder occurring in a subject who used the investigational device or the device for a post-marketing clinical study, including their signs," and the information on adverse events not judged as serious is also required for safety evaluation. Thus, PMDA instructed the applicant to present all adverse events detected during Study J-TEC003. The applicant submitted the adverse event reports as listed in *Summary of the submitted data* by tracing back to the data from various laboratory tests performed during the clinical study, the medical charts, the nursing records, and the medication history. Regarding the long-term safety of JACE, the applicant has responded that adverse events of hypertrophic scar and contracture and ulceration of the grafted area were observed at the timepoint of 14 months after JACE grafting in Case XXHA and no other safety information from this case is available.

PMDA reviewed all adverse events submitted and confirmed that there were no serious adverse events strongly suggestive of a causal relationship to JACE. According to the submitted data and responses from the applicant, there have been no concerns about serious adverse events associated with JACE so far and there should be no major safety problems with the use of JACE for serious extensive burns. However, the number of patients treated in Study J-TEC003 was 2 in total and of whom, one patient died 62 days after JACE grafting and the other patient who survived refused to attend the hospital as an outpatient after discharge due to the deterioration of the underlying disease and did not receive post-discharge treatment. Consequently, safety information available is extremely limited. It is necessary to collect safety information appropriately after the market launch and issue an alert.

As adverse events relevant to the safety of cultured epidermis, the long-term fragility has been reported: Mechanical stimulation is likely to lead to graft blister formation over several months after grafting (Burns. 2006;32:395-401) and antibody formation against fetal bovine serum after grafting has been reported (J Trauma. 1988;28:1054-1059). Thus, it is necessary to provide such information appropriately.

6.B.(3) Intended population and clinical positioning

6.B.(3).1) Intended population

The proposed indication for JACE at the time of regulatory submission was "severe burns (e.g. extensive burns)." For classification of burn severity, there are Artz's classification (in The treatment of burns, 2nd ed. 1969;89-108), Moylan's classification (in Burns: a team approach. 1979;151-158), American Burn Association's classification (J Burn Care Rehabil. 1990;11:98-104), etc. and furthermore, in Japan, BI incorporating the burn depth and PBI incorporating age are used for severity grading (Japanese Journal of Acute Medicine. 2000;24:476) and the definition of severe burns varies among them. PMDA asked the applicant to explain the definition of severe burns.

The applicant responded as follows:

In Study J-TEC003, JACE was grafted onto the wounds pre-treated with cadaver skin grafts in both patients. Assuming that commonly used allograft skin is currently cadaver skin from a skin bank, which is applied as appropriate, the criteria for the application of cadaver skin (Japanese Society for Burn Injuries' Skin Bank Manual) have been employed for the use of JACE, and it is considered that severe burns are defined as "extensive burns of BI ≥10 or those which are classified as deep dermal or more severe burns covering ≥15% of total body surface area."

PMDA considers as follows:

It is difficult to say that sufficient information on the efficacy and safety of JACE in the patient population proposed by the applicant at the time of regulatory submission has been obtained from the submitted results of Study J-TEC003 because the number of cases was small and the proportion of the grafted area in the total burn area was limited (7.0% and 4.4%). On the other hand, according to multiple published articles or the information on Epicel®, if JACE is indicated for more serious burns for which standard therapy does not exist and of which treatment is difficult, expected benefits should outweigh possible risks in terms of the safety of JACE.

Taking account of the above points, PMDA asked the applicant to reconsider the indication for JACE.

The applicant responded as follows:

The use of JACE will be restricted to life-threatening, severe burn patients. In addition, according to statistic data on burns from the Tokyo Burn Unit Association (TBUA) (Japanese Journal of Acute Medicine. 2003;27:3-6), the case fatality rate is 86.1% at BI ≥50. For example, based on the statistic data, it is assumed that BI ≥50 is set as a criterion for the use of JACE.

Based on the efficacy and safety discussions, PMDA suggests that JACE should be indicated for serious, extensive burns. Although burn patients with a BI ≥50 are considered as "serious," the

appropriateness of the specific intended population will be discussed at the Expert Discussion.

6.B.(3).2) Clinical positioning

PMDA asked the applicant to clearly show the expected usage of JACE according to the depth and seriousness of burn injuries, including its relationship to skin autografts, and then explain the efficacy and safety in its proposed uses.

The applicant responded as follows:

Patients with epidermal burns or superficial dermal burns are not eligible for treatment with JACE, as epithelialization of the burn wounds occurs within 1-2 weeks. Deep dermal burns do not extend into the deepest layers of the dermis, but take a longer period of time to epithelialize. Thus, skin autografting is often performed for early wound closure. Likewise, full-thickness burns where no dermal layer remains are also usually treated with skin autografts. If the area of the wound to be covered with skin autografts is about 2-3 fold the area of donor skin, mesh grafts or patch grafts can be used. However, if the burned area is larger, the wound will be temporarily covered with cadaver skin or fresh allogeneic skin taken from the patient's close relative etc. to prevent infections. The skin allograft will be rejected and slough off after all, but will engraft to the wound for 2-3 weeks. Meanwhile, during this period, JACE can be manufactured, and the thus-manufactured skin autografts can be applied onto the wound after the removal of the allograft, thereby resulting in epithelialization of the wound surface and wound closure. Deep dermal burns can be treated with JACE alone without skin allografts as the patient's own dermis remains, which is a good recipient bed unless it has been damaged by infections etc.

PMDA's view on the clinical positioning of JACE is as follows:

In the treatment of extensive burns, it is important to prevent fluid evaporation and infections by covering and closing the wounds as soon as possible. Usually, necrotic tissue (eschar) is excised early after the burn injury (generally within 1 week) and the wounds are closed with skin autografts as long as donor sites allow (in Total burn care 2nd ed. 2002;170-182, N Engl J Med. 1996;335:1581-1586, JAMA. 2003;290:719-722). Hence, there are limitations to the area that can be covered with skin autografts in one operation. In the case of patients with extensive burns, they have limited donor sites available for autografting to close the excised wound as a result of early excision of eschar. As to such cases, it is necessary to temporarily cover the wound with skin allografts or artificial skin for the prevention of fluid loss and bacterial infiltration until skin autografting can be performed. However, as the reconstructed dermis after skin allografting or artificial skin itself does not contain keratinocytes, spontaneous epithelialization is impossible and skin autografting is required in order to achieve epithelialization. One of the problems with treating extensive burns is that sufficient epithelialization can not be achieved due to a lack of skin autograft donor sites. JACE has clinical significance in terms of supplementing skin autografts, enabling epithelialization of

extensive burn wounds with a small piece of skin taken, and reducing the number of surgical procedures to harvest donor skin. As it takes at least 15 days to manufacture JACE, if there are still wounds that can not be closed due to a lack of skin autograft donor sites when JACE becomes available for grafting, it is expected that the burn wounds will be epithelialized and closed with JACE grafting. Therefore, the results of Study J-TEC003 submitted was intended only to confirm the epithelialization with JACE and at the current stage, the information on the efficacy and safety of JACE is extremely limited, but if JACE is indicated only for serious, extensive burns for which standard therapy does not exist and of which treatment is difficult, JACE is expected to be positioned as a new treatment option.

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

6.B.(3).3) Depth of the burn wound to be grafted

The applicant explained after regulatory submission that the proposed indication for JACE was full-thickness and dermal burns, excluding superficial dermal burns [see "2) Clinical positioning"]. PMDA asked the applicant to provide a justification for setting deep dermal burns as the indication despite that JACE has not been applied onto deep dermal burns in the clinical study.

The applicant responded as follows:

It is envisaged that even deep dermal burns may require JACE in terms of early wound closure. In this case, the burn wounds can be treated with JACE only without skin allografting.

PMDA considers as follows:

As a general rule, JACE should be grafted onto full-thickness burn wounds for which clinical study data are available. However, deep dermal burn wounds take a long period of time to epithelialize (21-60 days) (N Eng J Med. 1996;335:1581-1586) and as mentioned before, the standard care for full-thickness and deep dermal burn wounds is debridement early after the burn injury and wound coverage with allografts etc. if autografting can not be performed due to a lack of skin autograft donor sites. When the standard care is discussed, full-thickness burns are not particularly distinguished from deep dermal burns (in Total burn care 2nd ed. 2002;170-182, N Engl J Med. 1996;335:1581-1586, JAMA. 2003;290:719-722). Moreover, in practice, it may be difficult to graft JACE onto full-thickness burn wounds only, after distinguishing full-thickness burns from deep dermal burns. Based on the above, it is not practicable to exclude the application of JACE to deep dermal burns and such use is not denied, whereas it is appropriate to use JACE by assessing the risks and benefits after being informed that JACE has not been applied onto deep dermal burns in the clinical study. In addition, the efficacy and safety of JACE when applied to deep dermal burn wounds need to be confirmed after the market launch.

6.B.(4) Usage method

6.B.(4).1) A series of JACE grafting operations

In the treatment of patients with serious, extensive burns involving a large percentage of the total body surface area, it is anticipated that not only a single skin grafting operation but also a series of skin grafting operations may be required. PMDA asked the applicant to explain the safety of a series of JACE grafting operations.

The applicant responded as follows:

Since among raw materials that remain in the final product and process-related impurities, the residual amounts of feeder cells, bovine-derived serum, antibiotics, etc. are very small, we have determined that there are no safety problems with a series of skin grafting operations [see 4.A.(4) The safety of biological raw materials, antibiotics, etc. that remain in the final product]. In addition, according to multiple published articles that clearly indicate that cultured epidermal autografts generated using Green's technique, which is considered to be a similar product to JACE, were applied in a series of operations (Lancet. 1981;8211:75-78, N Engl J Med. 1984;311:448-451, Plast Reconst Surg. 1988;82:99-110), there have been no reports of adverse events associated with a series of skin grafting operations, e.g. allergy and infections.

PMDA considers as follows:

No clinical study data on a series of skin grafting operations have been presented and it is difficult to draw a conclusion on the efficacy and safety of a series of skin graft operations, only from published articles and safety concerns about a series of JACE grafting operations in the same patient can not be dispelled. Meanwhile, taking also into consideration that standard therapy in this field has not been established in Japan, such use of the product should not necessarily contraindicated, and it is appropriate to clearly state in the instructions for use etc. that there is no sufficient information on a series of skin grafting operations. However, after the market launch, it is necessary to collect information on a series of skin graft operations and provide the information appropriately and promptly.

6.B.(4).2) The quantity of JACE to be grafted (the number of sheets to be grafted)

PMDA asked the applicant to explain the maximum number of sheets of JACE to be grafted in one operation. PMDA also asked the applicant to explain the maximum number of sheets of JACE to be grafted in a series of operations.

The applicant responded as follows:

In the transplant plan, since there is a limitation on the area of the wound that can be excised in one operation in patients with severe burns involving large areas of the body (up to 20%-30% of the total body surface area), a series of operations are usually performed. Based on this area, it has been established that a maximum of 50 sheets of JACE may be grafted per operation (the average total body surface area of a Japanese adult male: 16,000 cm^2 × 0.25 = 4,000 cm^2 and the

effective area per sheet of JACE = 80 cm^2. 4,000 cm^2/80 cm^2/sheet = 50 sheets). It is estimated that a maximum of 200 sheets of JACE may be grafted in a series of operations in terms of a Japanese adult male with an average total body surface area of 16,000 cm^2.

PMDA considers as follows:
In the results of Study J-TEC003 submitted, the maximum number of sheets used was 12 and no information on the safety and efficacy of the use of more than 12 sheets of JACE is available at present. Furthermore, the area of the wound that can be excised in one operation, as claimed by the applicant, refers to the area to be treated with skin autografts following eschar debridement early after the burn injury, but not the area that can be treated with cultured epidermis. However, when JACE is indicated for very serious, extensive burns of BI ≥50 for which standard therapy has not been established, it is not realistic to limit the number of sheets to be grafted in one operation to 12 sheets as used in the clinical study, and setting the maximum number of sheets to be grafted per operation at 50 is not denied. However, it is necessary to confirm the safety and efficacy of the use of ≥12 sheets after the market launch. For Case XXHA in Study J-TEC003, although grafting onto a burn wound of 900 cm^2 was initially planned, the graft area was reduced to 450 cm^2 due to wound infection detected at the time of grafting, but 12 sheets of JACE manufactured based on the initial plan were applied to largely overlap one another. Originally, JACE is to be placed so that adjacent sheets slightly overlap one another. Regarding the impact of overlapped sheets on safety and efficacy assessment, the applicant has explained that the keratinocytes in cultured epidermal sheets that are in a direct contact with the wound surface are involved in graft take and overlapping sheets grafted on the wound do not affect the efficacy and safety of JACE. However, after the market launch, it is necessary to collect information and then consider the minimum necessary use in order to establish the proper usage method including the procedure for harvesting skin tissue and the area of skin harvested.

6.B.(4).3) Method of pre-treatment of the wound for reconstruction of dermis before JACE grafting

The applicant claims that when JACE is applied onto full-thickness burn wounds where no dermis remains, the wounds need to be pre-treated with allografts etc. to reconstruct a dermal layer 2-3 weeks prior to a planned grafting procedure of JACE. Since the engraftment of skin allograft was rated as “Neither useful nor useless” for both patients in Study J-TEC003, PMDA asked the applicant to explain the degree of reconstruction of a dermal layer in the 2 patients.

The applicant responded that according to the results of an interview with the relevant investigators of the clinical study, the degree of reconstruction of dermis was unassessable in both patients.

Although the applicant stated that artificial skin as well as skin allografts may be chosen at the discretion of the physician for the pre-treatment of the wound, since there is no information on

the use of artificial skin in Study J-TEC003, PMDA asked the applicant to explain a justification for the use of artificial skin for pre-treatment.

The applicant responded as follows:
No publication reporting a systematic comparative study on a take rate etc. of cultured epidermal autografts generated using Green's technique on the wounds pre-treated with allograft or artificial skin, which could justify the use of artificial skin, was found. However, the performance of JACE is that the product grafted onto the wound where dermis is present closes the wound via engraftment/epithelialization. In this sense, JACE is expected to close the wound via engraftment/epithelialization even when applied onto the dermis reconstructed with artificial skin.

PMDA further asked the applicant to explain the use of artificial skin in the treatment of extensive burns in Japan.

The applicant responded as follows:
In the treatment of serious burns for which JACE is indicated, the burn area requiring the reconstruction of dermis is large and it can be difficult to obtain and use skin allografts due to a geographical barrier, poor coordination between the hospitals and other problems. Thus, it is difficult to use allografts only at a single stage and the concurrent use of artificial skin or the use of conservative therapy without early debridement is envisaged.

PMDA considers as follows:
As described before [see 6.B.(3).2) Clinical positioning], the presence of dermis in good conditions, including the infection control of the recipient bed, is first needed for engraftment/epithelialization of JACE. The results from Study J-TEC003 submitted have confirmed engraftment/epithelialization of JACE after the reconstruction of dermis using cadaver skin, though in very limited patients. On the other hand, because JACE has never been used on the wound pre-treated with artificial skin, engraftment/epithelialization of JACE using artificial skin have not been confirmed. Also, there is a textbook stating that the usefulness of cultured epidermis applied to the wounds pre-treated with artificial skin (synthetic dermal analogs) has not been demonstrated (In Total burn care 2nd ed. 2002;212-218). Thus, the use of artificial skin should be avoided wherever possible. Nevertheless, as the applicant mentioned, in the treatment of extensive burns, it is considered that in clinical settings, there would be situations where artificial skin has to be used or conservative therapy is performed, depending on the patient's condition such as infection of the recipient bed or the social environment. Therefore, as a general rule, skin allografts should be used for the pre-treatment of the wound before JACE grafting, whereas the application of JACE to the wounds pre-treated with artificial skin or wounds that received conservative therapy is not denied. It should be informed in the instructions for use etc. that there is no clinical experience with these methods and after the

market launch, efficacy and safety information on each method of dermis reconstruction, including artificial skin, should be collected and taking account of it, the information should be provided appropriately.

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

6.B.(4).4) Postoperative treatment

PMDA asked the applicant to explain JACE grafting procedure and the postoperative treatment.

The applicant responded as follows:

It is recommended that physicians who are familiar with ordinary skin grafting techniques and have knowledge and skills regarding burn treatment should appropriately manage the burn wounds (the graft sites) by debridement etc. for application of JACE. After JACE is grafted, the grafted wound should be covered with a general wound dressing. For several days after grafting, the wound surface should be well protected and attention should be paid to avoiding mechanical damage and infections. These measures are in accordance with the measures recommended for ordinary skin autografting and no special management for cultured epidermal autografts is specifically required.

PMDA considers as follows:

Since it has been reported that cultured epidermal autografts generated using Green's technique are more fragile than split-thickness skin grafts (Burns, 2006;32:395-401), more attention needs to be paid after JACE grafting compared to split-thickness skin grafting so that the wound is covered with a wound dressing to be protected from mechanical damage.

PMDA further asked the applicant to explain how to handle JACE graft failure.

The applicant responded as follows:

JACE can be positioned as an alternative to skin autografts. Thus, any site where JACE fails to engraft may be handled by skin autografting or JACE regrafting. If the cause of primary graft failure can be identified and solved until JACE is manufactured and grafted a second time, regrafting of JACE is recommended. However, if JACE can not be regrafted for some reasons, the patient's condition should be judged carefully and then skin autografting should be performed, wherever possible.

PMDA considers as follows:

The results of the clinical study have not demonstrated the efficacy and safety of JACE as an alternative to skin autografts and it takes at least 15 days to manufacture the product. Therefore, the sites of JACE graft failure should be treated with skin autografts if donor sites are available.

6.B.(5) Qualifications of medical institutions and physicians for using JACE

Since the information on the efficacy and safety of JACE is extremely limited, PMDA asked the applicant about the qualifications of medical institutions and physicians for using JACE after marketing approval is granted.

The applicant responded as follows:

A medical institution (a) which has facilities capable of treating severe burn patients (i.e. the intended population), (b) to which physicians with appropriate skills/experience for treating severe burn patients (i.e. the intended population) belong, and (c) to which physicians with adequate knowledge about the nature of JACE belong, is to be qualified.

PMDA further asked the applicant to explain how many qualified medical institutions there are.

The applicant responded as follows:

At present, there are about 10 qualified medical institutions across Japan. At first, we will provide necessary information for handling JACE to the physicians at these medical institutions to ensure proper use and will try to provide information on the nature of JACE to other medical institutions by distributing a physician's guide describing the product overview and safety (Precautions for use, etc.) and other information and providing a training session on how to handle the product (take-out of the product from the container, transfer of the product to the graft site, etc.), using a simulation material.

PMDA considers that at present, it is appropriate to restrict the use of JACE to the medical institutions satisfying the above qualifications until the efficacy and safety information on the to-be-marketed device is collected and analyzed promptly.

PMDA also considers as follows:

In light of the novelty of JACE, on the premise that JACE will be used by qualified physicians and at the medical institutions satisfying the above qualifications, it is necessary to provide the users with the information such as the nature of the product, the grafting procedure, preoperative and postoperative treatment procedures, and precautions for use, by using explanatory materials etc.

Then, PMDA asked for the applicant's view on the above. The applicant agreed with PMDA and PMDA accepted it.

6.B.(6) Post-marketing investigations

The outline of a post-marketing clinical study and use-results surveys is shown below. Although GCP compliance review/inspection concluded that the clinical study was compliant with GCP, many problems were identified [see IV. Results of GCP Document Compliance Review and

On-site Inspection]. Based on the fact, the applicant has responded that they will enhance the internal system for a post-marketing clinical study and use-results surveys and are considering outsourcing the monitoring, QC activities and other activities for a post-marketing clinical study to a contract research organization (CRO).

6.B.(6).1) A post-marketing clinical study

PMDA considers as follows:

Changes have been made to the manufacturing method for the investigational device and the manufacturing method for the to-be-marketed device is different from the former one. The pre-change and post-change devices have been shown to be highly similar in terms of quality and the manufacturing method changes are unlikely to significantly affect the efficacy and safety of the product. Meanwhile, as efficacy and safety evaluation based on quality data alone has limitations and the information on the clinical positioning of the product obtained from the clinical study is extremely limited, it is necessary to conduct a post-marketing clinical study in order to collect efficacy and safety information on the to-be-marketed device used in accordance with the conditions specified in its approved labeling. The following outline of a post-marketing clinical study plan (draft) has been presented by the applicant. This clinical study should be initiated as early as possible and the information should be provided as soon as the study results become available.

The appropriateness of the post-marketing clinical study plan, including the inclusion criteria and the endpoints, will be discussed at the Expert Discussion.

[Outline of a post-marketing clinical study]

Study objective:	To assess the efficacy and safety of JACE
Study method:	A multi-center, open-label, uncontrolled study
Inclusion criteria:	Patients with life-threatening burns
Primary endpoint:	The percentage of epithelialization 4 weeks after grafting
Secondary endpoints:	Percentages of epithelialization at Weeks 1, 2, 3, and 4 after grafting, the wound conditions (presence or absence of infection, exudate volume and symptoms such as contracture), subjective symptoms (presence or absence of pain etc.), adverse events (any disease or disorder that occur or their signs), survival or death
Planned number of patients:	10 patients (the expected recruitment period is 2 years)
Observation period:	Up to 1 year after discharge for each patient

6.B.(6).2) Use-results surveys

PMDA considers as follows:

JACE (the investigational device) has so far been used in only two patients in total and the safety and efficacy information is extremely limited. Thus, it is necessary to conduct use-results

surveys in all treated patient during the reexamination period, collect the following information, and provide the obtained results appropriately.

1. Percentage of epithelialization with JACE
2. Safety (infections, anaphylactic reactions [allergic reactions], and other adverse events)
3. Factors affecting the transplant results (the recipient wound bed condition, the anatomic site for graft application, etc.)
4. Burn depth
5. The number of grafting operations
6. The number of sheets used
7. Dermis reconstruction method
8. The procedure for harvesting skin tissue and the area of skin harvested

The long-term efficacy (its contribution to life-saving, the functionality of scars etc., aesthetics such as pigmentation, etc.) and safety need to be investigated in at least a certain number of patients, taking the feasibility into account.

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

IV. Results of GCP Document Compliance Review and On-site Inspection

The review on compliance with "Good Clinical Practice for Medical Devices" (MHW/PAB [*yaku*] Notification No. 615 dated July 1, 1992) was conducted in accordance with the provisions of the Pharmaceutical Affairs Law for the data appended to the application for regulatory approval (Study J-TEC003). As a result, inconsistent handling of adverse events attributable to the protocol was found and at some study sites, the contract was incomplete and some source documents could not be found, but there were no major problems. Thus, PMDA concluded that there should be no problem with conducting regulatory review based on the submitted data.

V. Overall Evaluation

In the clinical study, JACE was applied onto a relatively small area of the full-thickness burn wounds pre-treated with allografts in two patients, and there is extremely limited information on the efficacy and safety of JACE under the usage envisaged in clinical practice in Japan, e.g. the application of JACE to deep dermal burns or the wound pre-treated with artificial skin. In addition, the manufacturing method changes have been made between the investigational device and the to-be-marketed device and quality studies have indicated that the manufacturing method changes are unlikely to significantly affect the efficacy and safety of the product, but there is no clinical experience with the to-be-marketed device. However, if JACE is indicated only for serious, extensive burns for which standard therapy has not been established to date, JACE may

offer a new treatment option. Consequently, PMDA has to adopt the following review policy: JACE may be approved if the product is positioned as one of the treatment options for serious, extensive burns that are difficult to treat with existing therapies, on the conditions that the product be used by appropriate physicians at appropriate medical institutions, and that the applicant provide information adequately, collect safety and efficacy information on the to-be-marketed device promptly after approval, and take appropriate actions as needed.

Based on the above review results, PMDA will refer this application for regulatory approval to the Expert Discussion to discuss the following points and the appropriateness of the above review policy and then will make a final decision on the labeling as well as the approvability of the product, taking account of the results of discussions.

- The quality of the product before and after the manufacturing method changes
- Clinical positioning and intended population
- The usage method of JACE (the depth/pre-treatment of the burn wound to be grafted, etc.)
- Information provision
- Post-marketing investigations

Review Report (2)

August 6, 2007

I. Product Submitted for Registration

[Category]	Medical products 4　Orthopedic products
[Generic name]	Other surgical/orthopedic materials (autologous cultured epidermis)
[Brand name]	JACE
[Applicant]	Japan Tissue Engineering Co., Ltd. (J-TEC)
[Date of application]	October 6, 2004

II. Content of the Review

The Pharmaceuticals and Medical Devices Agency (PMDA) sought the expert advisors' opinions based on the Review Report (1). The results of a review taking account of discussions with the expert advisors are reported below.

The expert advisors attending the Expert Discussion have stated that, with respect to the relationship with the product submitted for registration, none of them falls under the category stipulated in Section 1 or 2 (1) of "Regarding immediate measures against problem of conflict of interests involving an external expert for PMDA" (dated May 8, 2007).

(1) Clinical positioning

The following conclusion by PMDA was supported by the expert advisors:

In the clinical study of JACE, the number of patients evaluated was 2 and the product was applied onto a relatively small area of the full-thickness burn wounds pre-treated with allografts, and the information on the efficacy and safety of JACE under the usage envisaged in clinical practice is extremely limited. However, if JACE is indicated only for serious, extensive burns for which standard therapy has not been established and where sufficient donor sites for autologous skin grafts are not available, and if the product is positioned as a new treatment option, then there should be no particular problems.

(2) Intended population and usage method

PMDA discussed with the expert advisors to specify the patient population for which JACE is indicated. The following comments were raised from the expert advisors.

1) Depth of the burn wound to be grafted

PMDA's conclusion that JACE should be grafted onto full-thickness burn wounds, as a general rule, based on the information from the clinical study, was supported by the expert advisors.

There was an opinion that the application of JACE to deep dermal burns should be permitted only when full-thickness and deep dermal burns coexist and it is difficult to treat them separately.

2) Intended population

PMDA's conclusion that JACE should be indicated for "serious, extensive burns where sufficient donor sites for autologous skin grafts are not available" was supported by the expert advisors at the Expert Discussion. On the other hand, the following comments were raised: (a) As the words "serious" or "life-threatening" are ambiguous, the intended population should be described specifically in [PERFORMANCE, INTENDED USE, INDICATIONS]; (b) since patients with a Burn Index (BI) ≥50 are very likely to die during the manufacture of the product, the product can not be used, resulting in no clinical benefits; and (c) as a measure of the severity of extensive burns, the total area of deep dermal and full-thickness burns is more practicable than BI because BI is calculated by incorporating superficial dermal burns as well, for which JACE can not be indicated. Therefore, it is appropriate to specifically define that JACE is indicated for use in patients with the total area of deep dermal and full-thickness burns covering ≥30% of the total body surface area.

3) Method of pre-treatment of the wound for reconstruction of dermis before JACE grafting

PMDA concluded as follows:

Based on the information obtained from the clinical study, skin allografts should be used for the reconstruction of dermis before JACE grafting, as a general rule. However, since in clinical settings, there will be situations where skin allografting can not be performed in the treatment of extensive burns, it is not practicable to prohibit the application of JACE after pre-treatments other than skin allografting. Therefore, it should be informed in the instructions for use etc. that there is no clinical experience with the methods of pre-treatment other than skin allografting and after the market launch, efficacy and safety information on each method of dermis reconstruction, including artificial skin, should be collected and taking account of it, the information should be provided appropriately.

The above conclusion by PMDA was largely supported by the expert advisors. However, the following comments were raised: (a) As JACE graft take on the wound pre-treated with artificial skin may not be well expected according to the literature and experiences, appropriate information provision and post-marketing information collection are essential; (b) since no dermal layer is reconstructed in the full-thickness burn wound that has received conservative therapy, JACE graft take is unlikely to occur; and (c) as to pre-treatment before JACE grafting, the Japanese term corresponding to the term "reconstruct" is should be changed to a more appropriate word when used in the expression "to reconstruct dermis" in Japanese.

Taking account of the above comments from the expert advisors on 1)-3), PMDA instructed the

applicant to reconsider "PERFORMANCE, INTENDED USE, INDICATIONS" and "OPERATION OR USAGE METHOD." The applicant responded that these sections will be amended as follows and PMDA accepted the amendment.

[PERFORMANCE, INTENDED USE, INDICATIONS]

JACE is indicated for use in patients with serious, extensive burns when sufficient donor sites for autologous skin grafts are not available and the total area of deep dermal and full-thickness burns is 30% or more of the total body surface area. JACE is applied onto the reconstructed dermis in a full-thickness burn wound to facilitate the closure of the wound. Dermis should be reconstructed with allografts, as a general rule. JACE should be used for deep dermal burn wounds only when full-thickness and deep dermal burns coexist and it is difficult to treat them separately.

[OPERATION OR USAGE METHOD]

・Pre-treatment of burn wounds where no dermis remains

Burn wounds where no dermis remains should be pre-treated with allografts etc. 2-3 weeks prior to a planned grafting procedure of JACE to reconstruct dermis.

・JACE grafting

Apply JACE onto the reconstructed dermis. If there are necrotic tissues etc. that leave the wound bed unsuitable for grafting, appropriate measures, such as their removal, should be taken before JACE grafting.

JACE should be used on deep dermal burn wounds only when full-thickness and deep dermal burns coexist and it is difficult to treat them separately.

(3) Post-marketing measures

The following conclusion by PMDA was supported by the expert advisors:

The currently available information on the efficacy and safety of the product is extremely limited. Also, the manufacturing method changes have been made between the investigational device and the to-be-marketed device and no clinical study data on the to-be-marketed device are available at present. Meanwhile, based on the results of quality studies, the manufacturing method changes are unlikely to significantly affect the efficacy and safety of the product. However, after the market launch, it is necessary to collect detailed information promptly and provide the information appropriately.

1) Post-marketing clinical study

The applicant explained that there were currently about 10 medical institutions satisfying the qualifying requirements for using JACE [A medical institution (a) which has facilities capable of treating severe burn patients and (b) to which physicians who are familiar with treating severe burn patients and have adequate knowledge about the nature of cultured epidermis belong]. In addition, the applicant stated that they would conduct a post-marketing clinical study

at 3 medical institutions with a recruitment period of 2 years and a target enrollment of 10 patients [see the Review Report (1), 6.B.(6) Post-marketing investigations].

Then the following comments were raised from the expert advisors:
Considering that eligible patients for treatment with JACE vary in terms of the patient background and the characteristics of burns, such as the age, the location of burn injury, and the areas of deep dermal and full-thickness burns, the information on more patients should be collected in a post-marketing clinical study to analyze it. Since the efficacy and safety information obtained from the clinical study (Study J-TEC003) is extremely limited, prompt information collection is necessary and a post-marketing clinical study needs to be initiated immediately after obtaining regulatory approval. Qualified medical institutions for the use of JACE need to be capable of treating severe burn patients and have physicians who are familiar with treating severe burn patients, whereas handling of cultured epidermis does not necessarily require special skills. Furthermore, the addition of medical institutions where JACE is used must be considered carefully. After the efficacy and safety of the product are evaluated in a post-marketing clinical study, the information should be provided appropriately and medical institutions where JACE is used should be added.

Taking account of the above comments raised at the Expert Discussion, PMDA asked the applicant to review the post-marketing clinical study plan.

The applicant responded as follows:
The selection criteria for medical institutions for conducting a post-marketing clinical study are as follows: (a) The medical institution has a critical care center that can accept patients with serious, extensive burns, (b) Board-certified physicians from the Japanese Society for Burn Injuries belong to the medical institution, (c) Allogeneic skin grafting has been performed in the region where the medical institution is located, (d) The medical institution has accepted burn patients who are eligible for the treatment with JACE, (e) The institutional review board (IRB) and the clinical trial management center have been established and a post-marketing clinical study can be conducted in accordance with the GCP for Medical Devices. We will conduct a post-marketing clinical study at 13 medical institutions in Japan. Estimating that 1-3 patients will be included in efficacy analyses at each site per year, the planned number of patients is set at 30, which can be enrolled in a year. The planned observation period will be that from the receipt of order for JACE until 1 year after the last grafting operation. In addition, in order to collect necessary information promptly and provide the information to health professionals, a post-marketing clinical study will be initiated at the same time as the introduction of JACE into the market.

PMDA accepted the response.

PMDA instructed the applicant to submit the results of a post-marketing clinical study as soon as they become available and provide the information on the efficacy and safety of JACE appropriately and promptly. The applicant responded that they will handle the instruction appropriately.

2) Use-results surveys

PMDA's conclusion that as a general rule, it is necessary to conduct use-results surveys in all treated patient during the re-examination period, was supported by the expert advisors.

The following comments were raised:

For conducting the surveys, the central registration system is needed in order to register all patients treated with JACE and check the prognosis. As to the information to be collected, in addition to the data items proposed by PMDA [see the Review Report (1), 6.B.(6) Post-marketing investigations], the percentages of epithelialization 1, 2, and 4 weeks after JACE grafting, the wound contraction rate at the graft site, and the condition of the re-epithelialized epidermis 3-6 months after grafting (blister formation, erosion, etc.) need to be documented.

Taking account of the above discussions, PMDA asked the applicant to review the use-results survey plan.

The applicant responded as follows:

Use-results surveys will be conducted in all patients treated with JACE through the central registration system at all medical institutions where JACE will be used during the re-examination period, as a rule. The information obtained from the survey as well as the information obtained from a post-marketing clinical study, will be provided appropriately and promptly, via the instructions for use, a product guide for healthcare professionals, a patient information leaflet, the J-TEC website and other means. After the survey is conducted in all patients treated with JACE over a certain period of time, the data will be summarized and reviewed and then the survey plan for the remaining portion of the re-examination period will be considered. As to the data items to be collected, the procedure for harvesting skin tissue and the area of skin harvested, the wound contraction rate, and the mid-term wound condition at about 3-6 months after grafting will be documented, in addition to the percentages of epithelialization at Weeks 1, 2, 3, and 4 after JACE grafting and the long-term efficacy, the factors affecting the transplant results, the depth of the burn injury, the number of skin grafting operations, the number of sheets used, and the dermis reconstruction method.

PMDA accepted the above response.

(4) Proper use of JACE

The following comment was raised from the expert advisors:

JACE sheets were applied to overlap one another in Case XXHA and it is necessary to check its impact on the efficacy and safety of JACE.

PMDA asked the applicant about the above point and the applicant responded as follows:
Only keratinocytes in the cultured epidermal sheets that are in a direct contact with the wound surface are involved in graft take and the upper cultured epidermal sheets are considered to slough off along with keratinization and desquamation of keratinocytes in the cultured epidermal sheets that are in a direct contact with the wound surface. The investigator did not make a comment suggesting that overlapped sheets had affected the efficacy and safety of JACE. Based on the above, it is considered overlapping sheets grafted on the wound has little impact on the efficacy and safety of the product.

PMDA instructed the applicant to promote the proper use of JACE after the market launch. The applicant responded that they will ensure that the physicians are informed of the proper use via an autologous cultured epidermis "JACE" guide for healthcare professionals. PMDA accepted it.

(5) Manufacturing method changes

PMDA considered that the manufacturing method changes are unlikely to significantly affect the efficacy and safety of the product as the results of release testing/in-process tests/confirmation tests/characterization studies were comparable between the investigational device and the to-be-marketed device. This conclusion by PMDA was supported by the expert advisors. However, there was a comment that it is necessary to continue to investigate the relationship between quality and clinical efficacy after the market launch, because this issue could not be evaluated in Study J-TEC003.

Taking account of the above comment, PMDA asked the applicant to take an action. The applicant responded that they will perform analysis of the cellular proliferative capacity, cytokine production, and cell marker of the final product, in addition to in-process tests, release testing, etc. in a post-marketing clinical study, continue to investigate the relationship between these quality data and clinical efficacy and safety, and take an appropriate action as needed.

PMDA accepted it.

(6) Test items and acceptance criteria for in-process tests etc.

PMDA asked the applicant to review the acceptance criteria for receiving inspection for skin tissue and some of in-process tests and release testing so as to make the criteria more specific, and the applicant handled it appropriately. In-process test items were also reviewed and checking the storage time of skin tissue as an in-process test at the start of manufacture and the number of cells recovered as an in-process test at 3T3-J2 cell passage were added.

(7) Use of bovine-derived raw materials

PMDA asked the applicant to recheck the information on raw materials of animal origin, but the applicant responded that more detailed information than what is described in the Review Report (1) is not available [see the Review Report (1), 2.B.(6) Raw materials of animal origin].

PMDA asked the applicant to perform a risk assessment of bovine-derived peptone sourced from the US or Canada, which is used in the manufacture of the WCB of human insulin, in accordance with the Attachment of the PFSB/ELD Notification No. 0801001 and PFSB/SD Notification No. 0801001 jointly issued by the Director of Evaluation and Licensing Division and the Director of Safety Division, Pharmaceutical and Food Safety Bureau, MHLW, dated August 1, 2003.

The applicant responded as follows:

Peptone has a risk assessment score of +2 (bile, connective tissue, skin, and bones other than vertebrae, –2; vertebrae, +2), which does not meet a threshold to ensure a certain level of safety, i.e., <–3 as specified in the above notification (relative risk, giving no consideration to the usage method/processing during the manufacture, for raw materials conforming to the regulations on the specific bovine body parts used and the country of origin). However, because the dilution factor of raw materials in the manufacturing method for human insulin (genetical recombination) is unknown, the risk assessment score has been calculated assuming no dilution, while peptone is a medium component to produce a WCB and is expected to be diluted considerably in the cultivation process. Also, the risk assessment score has been calculated assuming that there is no risk reduction, such as by inactivation/removal, and the process step capable of inactivating/removing prions in the manufacturing method for human insulin (genetical recombination) has not been taken into consideration. In addition, peptone used to produce a WCB conforms to the European Pharmacopoeia, which assures a certain level of its safety as a raw material. Therefore, although the risk assessment score has been determined to be +2, the risk may be overestimated.

PMDA concludes as follows:

Since the detailed information on the manufacturing method for human insulin (genetical recombination) is not available, the risk assessment score has been determined to be +2. However, as the applicant responded, it is inferred that the actual risk assessment score is even lower. While this human insulin (genetical recombination) has so far been used extensively as a pharmaceutical product, there have been no reports of TSE transmission. Based on the above, in view of the seriousness of the target disease, the expected benefits of JACE should outweigh the risk of TSE transmission associated with peptone and at present, it is unavoidable to use this human insulin (genetical recombination). However, it should be stated in the instructions for use that the risk of TSE transmission can not completely be excluded and that it is necessary to

obtain fully informed consent from the patient before the use of JACE, and the applicant was instructed accordingly. In addition, since in future, it should be preferable to switch to a raw material that conforms to the Standards for Ruminant Animal Derived Materials, PMDA asked the applicant about its necessity. The applicant responded that they would continue to consider it and PMDA accepted it.

(8) Manufacture system for JACE

Since JACE is composed of autologous cells from the patient, donor screening or receiving inspection for the harvested skin tissue for infections etc. is not performed. The following comment was raised from the expert advisors: Eligible patients for the treatment with JACE are in serious condition and the request for performing tests such as viral testing in advance is not reasonable, whereas it is necessary to take appropriate precautionary measures against contamination of cultured cells during the manufacture and contamination of the surrounding environment such as the manufacturing facility from cultured cells, in terms of reducing the risk of cross-contamination and ensuring the safety of the production personnel.

PMDA asked the applicant about the suitability of the measures to prevent contamination.

The applicant responded as follows:

Although the information on the patient's test results for infections will be obtained from the medical institution in advance if available and used as a reference for ensuring the safety of the personnel, regardless of availability of the test results and whether the patient is tested positive or negative, all patient-derived cells and tissue will be handled as those containing infectious agents. The production personnel will wear appropriate protection and a health management program will be provided to ensure their safety. With respect to cross-contamination, measures to prevent contamination via the facility/equipment and apparatus and the production personnel will be implemented appropriately.

PMDA accepted this response, on the premise that appropriate measures to prevent contamination will be taken.

(9) Internal system after the market launch

There were problems with the applicant's response in the course from the filing of the confirmation application through the approval application review, as described in the Review Report (1) [see the Review Report (1), 1. Origin or history of development and usage conditions in foreign countries, *Outline of the review by PMDA, the applicant's response, and the quality of the data and responses submitted*]. In addition, although it was concluded that Study J-TEC003 was compliant with "the GCP for Medical Devices" (MHW/PAB Notification No. 615 dated July 1, 1992), the following problems were identified: (a) The definition of an adverse event and other items were interpreted differently among the medical institutions due to unclear statements

in the protocol; (b) one of the investigators had not concluded a contract; (c) some of the source documents concerning the efficacy endpoint etc. could not be found; (d) a subject's consent document was lost and could not be located; and (e) because a subject was transferred to another medical institution during the clinical study, the information after the transfer could not be obtained. Taking also into account that the Ministerial Ordinance on Good Post-marketing Study Practice (GPSP) for Medical Devices (MHLW Ordinance No. 38 dated March 23, 2005) is applied to post-marketing surveillance and clinical study, PMDA asked the applicant about appropriate post-marketing measures.

The applicant responded as follows:
In order to appropriately perform a post-marketing clinical study, use-results surveys, and post-marketing safety management activities, the standard operating procedures will be prepared, manpower/system will be put in place, and an external organization such as CRO will be utilized. In order to enhance the internal system, an increase of the personnel is planned and adequate education and training will be provided, and preparations will be made so as to always set up a suitable system and respond appropriately.

PMDA accepted the above response.

III. Overall Evaluation

PMDA concludes that JACE may be approved for "PERFORMANCE, INTENDED USE, INDICATIONS" as described below, with the following conditions, provided that the product will be properly used under the supervision of physicians with adequate knowledge/experience at appropriate medical institutions and sufficient information will be provided, and post-marketing safety and efficacy information will be collected promptly and appropriate actions will be taken as needed.

JACE falls under the category of medical devices with a novel structure and it is appropriate to set the re-examination period of 7 years in view of the properties of the product and the number of eligible patients. Although JACE is a manipulated autologous cell-based product, it should be classified as a specified biological product, taking account of raw materials etc. to be used in its manufacturing process.

[PERFORMANCE, INTENDED USE, INDICATIONS]

JACE is indicated for use in patients with serious, extensive burns when sufficient donor sites for autologous skin grafts are not available and the total area of deep dermal and full-thickness burns is 30% or more of the total body surface area. JACE is applied onto the reconstructed dermis in a full-thickness burn wound to facilitate the closure of the wound. Dermis should be reconstructed with allografts, as a general rule. JACE should be used for deep dermal burn wounds only when full-thickness and deep dermal burns coexist and it is difficult to treat them separately.

[CONDITIONS FOR APPROVAL]

1. Appropriate measures should be taken to ensure that the product will be used, with an understanding of its efficacy and safety, by physicians with adequate knowledge/experience for treating severe burns, at medical institutions capable of treating the intended population appropriately.
2. Due to the very limited number of patients treated in the clinical study, a post-marketing clinical study should be conducted to confirm the efficacy and safety of the product and the results should be reported promptly.
3. Due to the very limited number of patients treated in the clinical study, use-results surveys in all patients treated with JACE should be conducted until the end of the re-examination period as a rule, efficacy and safety information on the product should be collected early after market launch, and the results should be reported periodically.
4. The results etc. of the post-marketing clinical study and use-results surveys should be disclosed promptly. Also, the information should be provided to relevant physicians and medical institutions adequately and incorporated into a patient information leaflet appropriately.