Report on the Deliberation Results

February 26, 2020
Medical Device Evaluation Division
Pharmaceutical Safety and Environmental Health Bureau
Ministry of Health, Labour and Welfare

Classification	Human cellular/tissue-based products, 2. Human somatic stem cell-processed products
Non-proprietary Name	Human (autologous) corneal limbus-derived corneal epithelial cell sheet
Brand Name	Nepic
Applicant	Japan Tissue Engineering Co., Ltd.
Date of Application	March 20, 2019 (Application for marketing approval)

Results of Deliberation

In its meeting held on February 26, 2020, the Committee on Regenerative Medical Products and Biotechnology made the following decision and concluded that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product may be approved. The approval is not classified as a conditional and time-limited approval. The re-examination period is 10 years.

Approval Conditions

1. The applicant is required to take necessary measures such as dissemination of the guideline for proper use prepared in cooperation with relevant academic societies and conducting seminars to ensure that physicians with adequate knowledge and experience in limbal stem cell deficiency acquire full skills of the product usage and knowledge in complications associated with the procedures and that the physicians use the product in compliance with the "Indication or Performance" as well as "Dosage and Administration or Method of Use" at medical institutions with an established system for treatment of limbal stem cell deficiency.
2. Since only a limited number of patients participated in clinical studies of the product, the applicant is required to conduct a use-results survey covering all patients treated with the product in principle until the end of the re-examination period in order to understand the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure proper use of the product.
3. The applicant is required to take necessary measures such as storage of reserve samples of the final product and retention of use records for 30 years to ensure appropriate handling in view of a risk

of xenogeneic transplantation related to mouse embryonic 3T3-J2 cells used as feeder cells in the manufacturing process of the product.

Review Report

February 7, 2020
Pharmaceuticals and Medical Devices Agency

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

Brand Name	Nepic
Classification	Human cellular/tissue-based products, 2. Human somatic stem cell-processed products
Non-proprietary Name	Human (autologous) corneal limbus-derived corneal epithelial cell sheet
Applicant	Japan Tissue Engineering Co., Ltd.
Date of Application	March 20, 2019

Shape, Structure, Active Ingredient, Quantities, or Definition

The product is a regenerative medical product consisting of a cultured corneal epithelium package including a corneal epithelial cell sheet, the primary component, and a tissue transport set and pre-treatment fluid bottle, the secondary components. The primary component is a cultured corneal epithelium package produced from corneal epithelial cells, which are derived from the patient's own corneal limbal tissue and cultured in sheet form. The secondary components are the tissue transport set consisting of tissue transport tubes for transport of the corneal limbal tissue collected at a medical institution and blood storage tubes for transport of blood for storage as well as the pre-treatment fluid bottle for detachment of the corneal epithelial cell sheet from a culture dish.

Application Classification	(1-1) New regenerative medical products
Items Warranting Special Mention	Orphan regenerative medical product (Orphan Regenerative Medical Product Designation No. 2 of 2015 [*27 sai*]; PFSB/MDRMPE Notification No. 0325-8 dated March 25, 2015, issued by the Office of Medical Device and Regenerative Medicine Product Evaluation Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare)
Reviewing Office	Office of Cellular and Tissue-based Products

Results of Review

On the basis of the data submitted, PMDA has concluded that the product has a certain level of efficacy in the treatment of limbal stem cell deficiency, and that the product has acceptable safety in view of its benefits (see Attachment).

As a result of its review, PMDA has concluded that the product may be approved for the indication or performance and dosage and administration or method of use shown below, with the following conditions.

Indication or Performance

Limbal stem cell deficiency. The following patients, however, will be excluded:

- Patients with Stevens-Johnson syndrome
- Patients with ocular pemphigoid
- Patients with graft versus host disease
- Patients with aniridia or other congenital corneal epithelial stem cell dysplasia
- Patients with recurrent pterygium
- Patients with idiopathic limbal stem cell deficiency

Dosage and Administration or Method of Use

Operations in manufacture of corneal epithelial cell sheet

1. An area in the patient's donor eye (contralateral eye of the eye planned to receive the product as a graft or the recipient eye) is confirmed to be free from inflammation and infection and approximately 2 × 3 mm piece of the corneal limbal tissue in area that have no sign of conjunctivalization is collected. The collected corneal limbal tissue is placed in a tissue transport tube and sent to the manufacturer.
2. Blood is collected in accordance with a conventional procedure. The collected blood is placed in a blood storage tube and sent to the manufacturer. This blood specimen is used as the reserve sample.

Operations in transplantation of corneal epithelial cell sheet

The corneal epithelial cell sheet is rinsed with a pre-treatment fluid and immersed in this fluid, and detached with a ring-shaped culture disk from the corneal epithelium culture dish. Conjunctival scar tissue is removed from the eye surface of the patient wherever possible and the corneal epithelial cell sheet is transplanted onto the eye surface including the corneal limbus. The rim of the corneal epithelial cell sheet is sutured where necessary. After the transplantation, the therapeutic contact lens is applied and tarsorrhaphy is performed where necessary.

Approval Conditions

1. The applicant is required to take necessary measures such as dissemination of the guideline for proper use prepared in cooperation with relevant academic societies and conducting seminars to ensure that physicians with adequate knowledge and experience in limbal stem cell deficiency acquire full skills of the product usage and knowledge in complications associated with the procedures and that the physicians use the product in compliance with the "Indication or Performance" as well as "Dosage and Administration or Method of Use" at medical institutions with an established system for treatment of limbal stem cell deficiency.
2. Since only a limited number of patients participated in clinical studies of the product, the applicant is required to conduct a use-results survey covering all patients treated with the product in principle until the end of the re-examination period in order to understand the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure proper use of the product.
3. The applicant is required to take necessary measures such as storage of reserve samples of the final product and retention of use records for 30 years to ensure appropriate handling in view of a risk of xenogeneic transplantation related to mouse embryonic 3T3-J2 cells used as feeder cells in the manufacturing process of the product.

Attachment

Review Report (1)

November 1, 2019

The following is an outline of the data submitted by the applicant and content of the review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Product Submitted for Approval

Brand Name	Nepic
Classification	Human cellular/tissue-based products, 2. Human somatic stem cell-processed products
Non-proprietary Name	Human (autologous) corneal limbus-derived corneal epithelial cell sheet
Applicant	Japan Tissue Engineering Co., Ltd.
Date of Application	March 20, 2019

Shape, Structure, Active Ingredient, Quantities, or Definition

The product is a regenerative medical product consisting of a cultured corneal epithelium package including a corneal epithelial cell sheet, the primary component, and a tissue transport set and pre-treatment fluid bottle, the secondary components. The primary component is a cultured corneal epithelium package produced from corneal epithelial cells, which are derived from the patient's own corneal limbal tissue and cultured in sheet form. The secondary components are the tissue transport set consisting of tissue transport tubes for transport of the corneal limbal tissue collected at a medical institution and blood storage tubes for transport of blood for storage as well as the pre-treatment fluid bottle for detachment of the corneal epithelial cell sheet from a culture dish.

Proposed Indication or Performance

Severe limbal stem cell deficiency is subjected to be the indication. The product is intended for use in corneal epithelium reconstruction by supplying corneal epithelial cells containing corneal epithelial stem cells.

Proposed Dosage and Administration or Method of Use

Operations performed before manufacture of corneal epithelial cell sheet

1. Corneal limbal tissue is collected from the patient. An area appropriate for the tissue collection is selected in view of the corneal limbus condition. The collected corneal limbal tissue is placed in a tissue transport tube and sent to Japan Tissue Engineering Co., Ltd.
2. Blood is collected in accordance with a conventional procedure. The collected blood is placed in a blood storage tube and sent to Japan Tissue Engineering Co., Ltd. This blood specimen is used as the reserve sample.

Operations in transplantation of corneal epithelial cell sheet

The corneal epithelial cell sheet is rinsed with a pre-treatment fluid filled in a pre-treatment fluid bottle, immersed in this fluid, detached with a ring-shaped culture disk from the corneal epithelium culture dish, and transplanted onto the eye surface including the corneal limbus region.

Table of Contents

List of Abbreviations

See Appendix.

1. Origin or History of Discovery, Use in Foreign Countries, and Other Information

1.1 Outline of the proposed product

Nepic is a human somatic stem cell-processed product manufactured from corneal epithelial cells, which are derived from the patient's own corneal limbal tissue and cultured in sheet form. Nepic is intended for use in corneal epithelium reconstruction and, more specifically, to be transplanted onto the eye surface of the patient with severe limbal stem cell deficiency (LSCD) with the expectation that the corneal epithelial cells will be engrafted and epithelized. It is to be handled as a combination product consisting of the following primary component and secondary components:

Primary component: Cultured corneal epithelium package produced from corneal epithelial cells, which are derived from the patient's own corneal limbal tissue and cultured in sheet form

Secondary components: Tissue transport set consisting of tissue transport tubes for transport of the corneal limbal tissue collected at a medical institution and blood storage tubes for transport of blood for storage as well as the pre-treatment fluid bottle for detachment of the corneal epithelial cell sheet from a culture dish.

Nepic is designated as the orphan regenerative medical product with the intended indication or performance of "limbal stem cell deficiency" dated March 25, 2015 (Orphan Regenerative Medical Product Designation No. 2 of 2015 [*27 sai*]).

1.2 Development history etc.

LSCD is a disease group characterized by a congenital or acquired deficiency or loss of corneal epithelial stem cells in the corneal limbus at the border between the cornea and conjunctiva, which would allow conjunctival epithelium to migrate onto the cornea and cover the surface, resulting in corneal opacity and reduced vision. LSCD can be caused by extrinsic factors such as thermal and chemical injuries as well as intrinsic factors such as Stevens-Johnson Syndrome (SJS), ocular pemphigoid, and aniridia, a developmental defect.

The fundamental treatment of LSCD is corneal epithelium reconstruction by supplying corneal epithelial stem cells. Although autologous and allogeneic corneal limbal transplantation procedures are already available for the treatment, these procedures have the following issues, and thus a new option for treatment of LSCD is needed. Amniotic membrane transplantation is occasionally performed on an area with the conjunctival scar tissue removed from the eye surface, but it is positioned as an adjunctive procedure performed with the corneal limbal transplantation because the recipient eye must have corneal epithelial stem cells left for corneal epithelium reconstruction.

- Autologous corneal limbal transplantation involves a highly invasive procedure because the corneal limbal tissue has to be extensively collected from the patient's eye as a graft and is not indicated for bilateral LSCD.
- Allogeneic corneal limbal transplantation requires post-transplant continuous treatment with immunosuppressants and involves a risk of rejection leading to graft failure, and the lack of donors has limited the operation.

In 1997, Pellegrini et al., Italian researchers, isolated corneal epithelial cells from corneal limbal tissue in a patient with LSCD, cultured the corneal epithelium using fibrin gel preparation as anchorage,

transplanted the cultured corneal epithelium in the same patient as an autologous graft, and demonstrated that the transplantation improved the corneal transparency and visual acuity (*Lancet*. 1997;349:990-3).

The applicant developed Nepic by [redacted] to [redacted] based on the techniques developed by Pellegrini et al.

A Japanese clinical study in patients with LSCD (EYE-01M study) was initiated in [redacted] 20[redacted], and the application of Nepic has been submitted, using data from EYE-01M study as the pivotal study results.

As of September 2019, Nepic has not been approved or marketed in any country or region. In Europe, on the other hand, the product developed by Pellegrini et al. was approved as "Holoclar" in 2014.

2. Data Relating to Manufacturing Process and Specifications and Outline of the Review Conducted by PMDA

The primary component of Nepic is the cultured corneal epithelium package containing corneal epithelial cell sheet produced from corneal epithelial cells, for which corneal epithelial cells derived from the patient's own corneal limbal tissue were co-cultured with mouse embryonic 3T3-J2 cells as feeder cells and proliferated, and the obtained cells were cultured in sheet form. The secondary components of Nepic are the tissue transport set consisting of tissue transport tubes and blood storage tubes used for transport of the collected corneal limbal tissue and blood for storage to the manufacturing site as well as the pre-treatment fluid bottle used to detach the corneal epithelial cell sheet from a culture dish.

2.1 Manufacturing process

2.1.1 Manufacturing process

The manufacturing process of Nepic consists of manufacture of the cultured corneal epithelium package, the primary component, and manufacture of the secondary components.

2.1.1.1 Manufacturing process of primary component

The manufacturing process of the cultured corneal epithelium package, the primary component, consists of manufacture of feeder cells and that of the corneal epithelial cell sheet.

2.1.1.1.1 Preparation and control of 3T3-J2 cells

As feeder cells, mouse embryonic 3T3-J2 cells are used. Using 3T3-J2 cells provided by H. Green in 20[redacted] (clone isolate from mouse total fetus established in 1963 by H. Green) as the source, the master cell bank (MCB), master working cell bank (MWCB), and working cell bank (WCB) were prepared.

Characterization and a purity test were performed on the MCB, WCB, and cells cultured beyond the upper limit of the passage generations or cells at the limit of *in vitro* cell age (CAL) from the step of MCB thawing and seeding in accordance with the ICH Q5A (R1) guideline ("Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin" [PMSB/ELD Notification No. 329 dated February 22, 2000]) and Q5D guideline ("Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products" [PMSB/ELD Notification No. 873 dated July 14, 2000]). Table 1 shows tests performed for adventitious agents.

Results from these tests demonstrated the genetic stability during the manufacturing period and neither viral nor non-viral adventitious agents were detected within the extent of the test items performed.

MCB, MWCB, and WCB are stored at −[redacted]℃ or lower. Although new MCB will be not prepared, new MWCB and WCB will be prepared where necessary.

Table 1. Tests for adventitious agents

Sterility test
Mycoplasma test
Extended $S^{+}L^{-}$ assay
Extended XC plaque assay
Electron microscopy
Reverse transcriptase activity test
In vitro tests (MRC-5 cells, Vero cells, and NIH-3T3 cells)
In vivo tests (suckling mice, post-weaning mice, guinea pigs, and embryonated eggs)
Mouse antibody production test
Bovine aberrant virus test (bovine testis cells, bovine turbinate cells, and Vero cells)

2.1.1.1.2 Manufacturing process of feeder cells

The manufacturing process of feeder cells consists of processes for [redacted], [redacted], and [redacted].

A critical step includes [redacted].

2.1.1.1.3 Manufacturing process of corneal epithelial cell sheet

The manufacturing process of corneal epithelial cell sheet consists of processes for receipt of corneal limbal tissue, [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], packaging and labeling, inspection, and packing and shipment.

Critical steps identified include processes for [redacted].

2.1.1.2 Manufacturing process of secondary components

The manufacturing process of a tissue transport set consists of processes for [redacted] ([redacted]), packaging and labeling, and packing and shipping of the tissue transport set. The manufacturing process of a pre-treatment fluid bottle consists of processes for [redacted] ([redacted]), packaging, labeling, and storage, and packing and shipment of the pre-treatment fluid bottle.

2.1.2 In-process control tests

Table 2 shows in-process control tests in the manufacturing process of feeder cells.

Table 2. In-process control tests in manufacturing process of feeder cells

Process	Test item
[redacted]	[redacted]

Table 3 shows in-process control tests in the manufacturing process of the cultured corneal epithelium package, the primary component.

Table 3. In-process control tests in manufacturing process of cultured corneal epithelium package

Process	Test item
[redacted]	[redacted]

Tables 4 and 5 show in-process control tests in the manufacturing process of the tissue transport set and pre-treatment fluid bottle, the secondary components.

Table 4. In-process control tests in manufacturing process of tissue transport set

Process	Test item
[redacted]	[redacted]
	Sterility test
	Bacterial endotoxins test

Table 5. In-process control test in manufacturing process of pre-treatment fluid bottle

Process	Test item
[redacted]	[redacted]

2.2 Safety evaluation of adventitious agents

2.2.1 Corneal limbal tissue

The corneal limbal tissue used as a raw material of Nepic conforms to the Standards for Biological Ingredients (MHLW Ministerial Notification No. 210, 2003).

2.2.2 Biological ingredients other than corneal limbal tissue

All of the 3T3-J2 cells, porcine trypsin, fetal bovine serum, calf serum, and bovine serum used in the manufacturing process of Nepic conform to the Standards for Biological Ingredients (MHLW Ministerial Notification No. 210, 2003).

2.3 Manufacturing process development (comparability)

Main changes from the manufacturing process of the primary component at the clinical study[1)] to the proposed commercial process are as shown below:

1) EYE-01M study

- Addition of [redacted] and [redacted] used as [redacted] and [redacted] in [redacted]
- Change of [redacted] in the final product

For either change, comparability evaluation on quality attributes was performed and demonstrated comparability between the pre- and post-change products.

2.4 Characterization

Characterization was performed on the primary component as shown in Table 6.

Table 6. Characterization items

Cell type	immunostaining*1 ([redacted])
Immunohistological analysis	immunostaining*2 ([redacted])
Cytokine secretion capability	ELISA*3 ([redacted])
Colony-forming activity	*4

*1 [redacted]

*2

*3

*4

2.5 Evaluation of manufacturing process

2.5.1 Removal of process-related impurities

Process-related impurities include bovine serum, feeder cells, antibiotics (benzylpenicillin potassium, streptomycin sulfate, amphotericin B, and kanamycin sulfate), cholera toxin, and Impurity A.

Benzylpenicillin potassium, streptomycin sulfate, amphotericin B, kanamycin sulfate, cholera toxin, and Impurity A were considered unlikely to raise a safety concern in humans based on their measured residual values in the final product or [redacted] calculated from their estimated residual values, and thus no control items are specified for these substances. Residues of bovine serum and feeder cells, on the other hand, are controlled by the product specifications (residual bovine serum albumin and residual rate of feeder cells).

2.5.2 Verification

Quality attributes required for Nepic include viable cell count, cell viability, [redacted], [redacted], and sterility.

At present, any source of variation has not been identified in the manufacturing process of the primary component, but to ensure the target quality attributes for each manufacture session, a verification-based quality control strategy has been constructed in light of quality risks that may be raised by variations in the quality attributes of corneal limbal tissue. More specifically, the strategy consists of manufacturing process parameters and in-process control tests as well as the specifications for the primary component presented in Table 7.

2.6 Control of Nepic

Tables 7 and 8 show specifications for the primary component and secondary components. Because the shelf life of the primary component is limited to 60 hours [see Section 3], the sterility test is specified to be performed using [redacted] days before the release as a specimen. In addition to the specifications, the sterility confirmatory test (Sterility Test [membrane filtration method] in the Japanese Pharmacopoeia) is to be performed on [redacted] collected at the release. The result of the sterility confirmatory test is to be obtained after transplantation in the patient.

Table 7. Specifications for primary component

Test item	Test method
[redacted]	[redacted]
Viable cell density	[redacted]
Cell viability	[redacted]
Percentage of [redacted] cells	Immunostaining [redacted]
Percentage of [redacted] cells	Immunostaining [redacted]
Residual rate of feeder cells	Immunostaining [redacted]
Residual bovine serum albumin	ELISA
Sterility test*	Membrane Filtration Method (Japanese Pharmacopoeia) (incubation time, [redacted] days)
Mycoplasma test	Nucleic amplification test (General Information in the Japanese Pharmacopoeia)
Bacterial endotoxins test	Gel-clot techniques or turbidimetric techniques (Japanese Pharmacopoeia)
Physical property test	[redacted]

* Use [redacted] days before the release.

Table 8. Specifications for secondary components

Secondary component	Test item
Tissue transport set	[redacted]
Pre-treatment fluid bottle	[redacted] Sterility test [redacted]

2.R Outline of the review conducted by PMDA

2.R.1 Control of primary component

2.R.1.1 Sterility test

The proposed sterility test in the specifications for the primary component was specified to be performed using [redacted] days before the release as a specimen by the Membrane Filtration Method under Sterility Test in the Japanese Pharmacopoeia (JP) Seventeenth Edition with the incubation time reduced to [redacted] days [see Section 2.6].

The concerned test was specified in accordance with the JP, but the incubation time was reduced. Concerning the reduced time, PMDA asked the applicant to present investigation results on detectability of slow growing microorganisms such as [redacted] and explain the appropriateness of the specified test method based on results from comparison with the other rapid detection methods for microorganisms.

The applicant's explanation:
The applicant presented data on detectability of slow growing microorganisms in the concerned test method and justified the specified test method based on the results from comparison with the other rapid detection methods for microorganisms, which were shown to have lower detectability than that of the concerned test method.

PMDA's view:
It is inevitable to make a release judgement based on the result from the sterility test using [redacted] in the manufacturing process as a specimen, which is specified as a part of the strategy to ensure the sterility of Nepic of which the shelf life is limited to 60 hours [see Section 3]. On the basis of results from the additional investigation, the test method proposed by the applicant may be included in the specifications for the primary component, and results from the test may be used in the release judgment.

2.R.1.2 [redacted]
Corneal epithelium formed by corneal epithelial cells is [redacted], and the proposed in-process control tests and specifications for the primary component did not include a test to evaluate [redacted].

PMDA's view:
Because Nepic is a product of cultured corneal epithelial cells in sheet form and intended to be transplanted onto the eye surface, [redacted] should be specified as the critical quality attribute and [redacted] should be included in the release specifications. The applicant's response and PMDA's conclusion are reported in the Review Report (2).

2.R.1.3 Verification
The proposed verification items included in-process control tests and specifications as well as a process parameter of [redacted] in the [redacted] process.

PMDA concluded that the verification items should additionally include manufacturing parameters such as culture conditions, [redacted] in [redacted], [redacted], and [redacted] in [redacted], which are considered critical in ensuring the product quality. The applicant's response and PMDA's conclusion are reported in the Review Report (2).

2.R.2 Control of secondary components
At the time of application, a container integrity test of [redacted] had not been performed for the tissue transport tube and pre-treatment fluid bottle, the secondary components.

PMDA concluded that container integrity should be controlled for the tissue transport tube and pre-treatment fluid bottle.

The applicant's response and PMDA's conclusion are reported in the Review Report (2).

3. Data Relating to Stability and Outline of the Review Conducted by PMDA

Table 9 shows outline of the stability study of the primary component.

Table 9. Stability study of primary component

Number of batches	Process	Storage condition	Study period	Storage form
3	Process for clinical study	20°C	60, [redacted] hours	Primary container (polystyrene container, polyethylene lid, polyethylene dish holder, polystyrene pick-up handle, polyethylene terephthalate/[redacted] ring-shaped culture disk, polystyrene/[redacted] culture dish)
		28°C		

No clear changes were observed in quality attributes under either storage condition in the stability study. Taking account of the above, a shelf life of 60 hours has been proposed for the primary component when stored at 20°C to 28°C.

3.R Outline of the review conducted by PMDA

PMDA accepted the proposed storage condition and shelf life of the primary component on the basis of the submitted data.

4. Data Relating to Indication or Performance and Outline of the Review Conducted by PMDA

The applicant submitted the following data relating to the indication or performance of Nepic: A report (Attached document 4-1), which evaluated the performance of Nepic by extracting the efficacy data from "Study of autologous corneal epithelial cell sheet transplantation in LSCD model rabbits (Attached document 6-1), conducted as a non-clinical safety study.

4.1 Performance evaluation in study of autologous corneal epithelial cell sheet transplantation in LSCD model rabbits (Attached documents 4-1 and 6-1)

4.1.1 Study procedure and group structure

From the left eye in a male rabbit (Slc:NZW), the corneal epithelium including corneal limbus was surgically removed followed by alkali treatment to create the LSCD model[2)] (the right eye was not treated and handled as the untreated eye). Using the corneal limbal tissue removed to create the LSCD model, a rabbit autologous corneal epithelial cell sheet ("Nepic analogue") was prepared from the same materials and manufacturing processes as Nepic. The Nepic analogue was transplanted to the LSCD model rabbits after the scar tissue infiltrating the cornea had been surgically removed ("transplantation group"). LSCD model rabbits with the scar tissue removed only were included in the control group ("non-transplantation group"). The quality of the Nepic analogue was confirmed to be similar to that of Nepic.

Table 10 shows the structure of the transplantation and non-transplantation groups.

2) Approximately 3 weeks after the surgical procedure and alkali treatment, impression cytology (technique to collect the surface cells by impressing a membrane filter against the eye surface covering the cornea, corneal limbus, and conjunctiva) and periodic acid-Schiff (PAS) staining were performed. Animals in which goblet cells, originally present in the conjunctiva, were observed in the corneal area were used in the study as the LSCD model animal.

Table 10. Group structure

Group	Procedure	
	First session	Second session (6 weeks after the first transplantation)
First transplantation	Transplantation	—
First non-transplantation	Scar removal only	—
Second transplantation	Transplantation	Transplantation
Second non-transplantation	Transplantation	Scar removal only

Of animals with the Nepic analogue transplanted, animals in which ≥50% of the transplanted cells are estimated to survive at Week 4 of transplantation by meeting the following condition were subjected to evaluation (first transplantation group): According to the Draize's criteria (Table 11), "the score on corneal opacity is 0 or 1" or "the score on corneal opacity is 2, and the score on percentage of corneal opaque area is ≤2."

Animals in which <50% of the transplanted cells were estimated to survive at Week 4 of transplantation of the Nepic analogue underwent a procedure for removal of the scar tissue again and were allocated to either the transplantation or non-transplantation group. Of animals in the transplantation group, animals meeting the following conditions were subjected to evaluation (second transplantation group) (Figure 1): The Nepic analogue was transplanted; and ≥50% of the transplanted cells are estimated to survive at Week 4 of second transplantation according to the Draize's criteria as done with the first transplantation.

Table 11. Draize's criteria

Item	Assessment	Score
Corneal opacity (most dense region to be assessed)	Clear and no opacity	0
	Scattered or diffuse opacity and clearly visible iris	1
	Easily discernible translucent areas and slightly obscured iris	2
	Opalescent areas, no details of iris visible, and size of pupil barely discernible	3
	Opaque and iris invisible	4
Area of corneal opacity involved	0	0
	0-1/4	1
	1/4-1/2	2
	1/2-3/4	3
	3/4-1	4

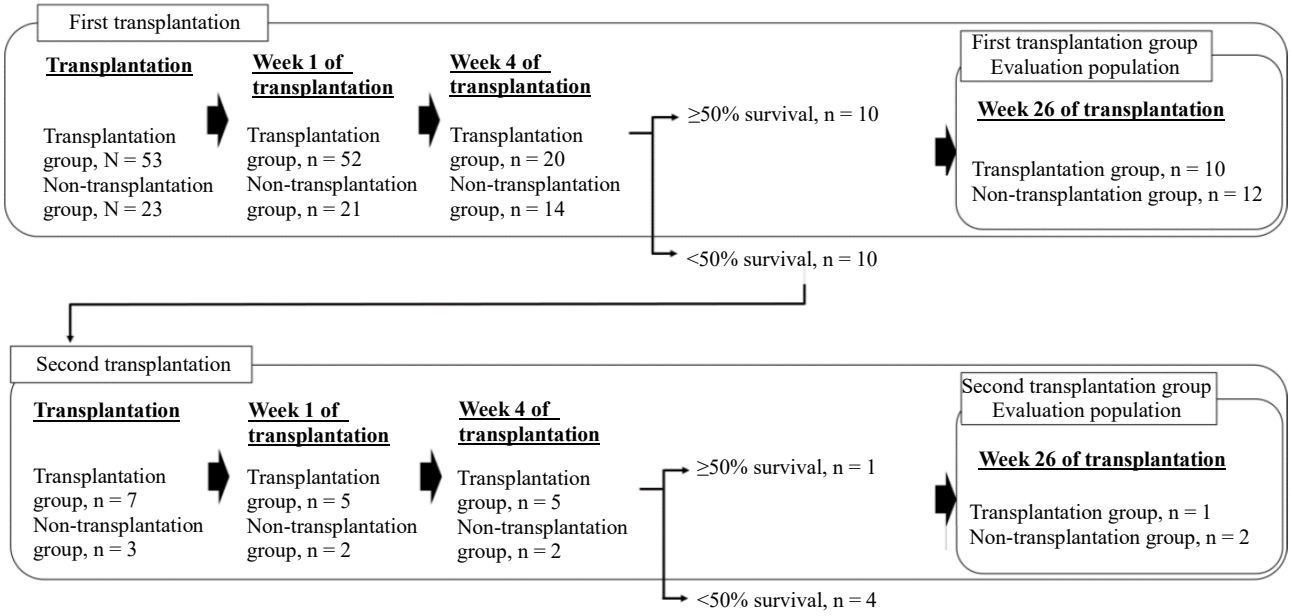


Figure 1. Changes in animal sample size at transplantation and each evaluation point

4.1.2 Corneal clearing rate

At Weeks 1, 4, 8, 12, 16, 20, and 26 of Nepic analogue transplantation, corneal opacity and extent of corneal epithelium lesion were assessed by observation of the anterior segment and fluorescein staining. The area with corneal opacity or fluorescein-stained was identified as the non-clearing corneal area, and the corneal clearing rate[3)] was calculated. Table 12 show the results.

Table 12. Corneal clearing rate in transplantation and non-transplantation groups

Group (n, number of animals)	Evaluation point (after transplantation*)						
	Week 1	Week 4	Week 8	Week 12	Week 16	Week 20	Week 26
First transplantation (n = 10) (mean ± standard deviation)	23.62 ± 26.11	71.16 ± 14.24	78.94 ± 15.63	64.48 ± 37.58	76.30 ± 28.21	79.70 ± 29.39	79.97 ± 29.59
First non-transplantation (n = 12) (mean ± standard deviation)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.99 ± 23.63	14.76 ± 28.83	11.36 ± 26.80
Second transplantation (n = 1)	21.79	67.28	78.30	69.31	68.85	75.55	78.89
Second non-transplantation (n = 2) (mean)	18.71	25.04	43.87	34.34	36.56	33.33	41.44

* For the second transplantation and second non-transplantation groups, weeks were counted from the second transplantation of the Nepic analogue.

4.1.3 Optical media and fundus examinations

Optical media and fundus examinations were performed by indirect ophthalmoscopy at Week −7 (2 weeks before the surgical procedure and alkali treatment), Week −1 (4 weeks after the surgical procedure and alkali treatment), and Weeks 1, 4, 8, 12, 16, 20, and 26 of transplantation. Table 13 shows change in the number of animals with observable optical media and fundus.

3) (Total corneal area − Non-optical clearing corneal area) / Total corneal area × 100

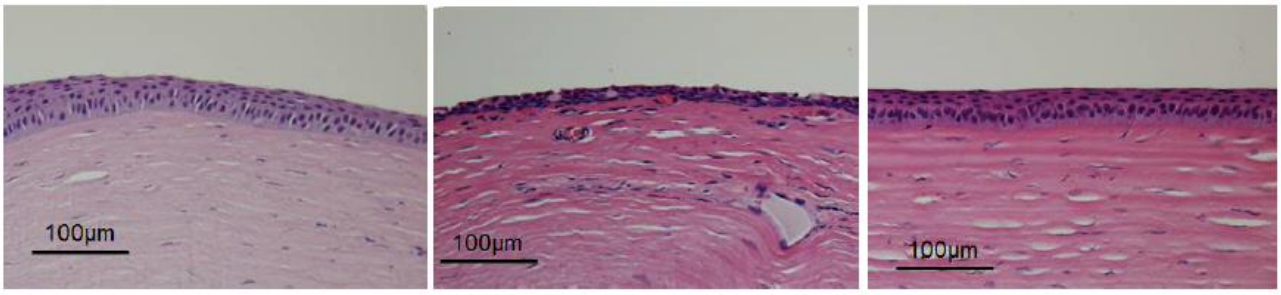
Table 13. Number of animals with observable optical media and fundus

Group (n, number of animals)	Evaluation point (before transplantation*)		Evaluation point (after transplantation*)						
	Week −7	Week −1	Week 1	Week 4	Week 8	Week 12	Week 16	Week 20	Week 26
First transplantation (n = 10)	10	0	9	10	9	9	9	9	9
First non-transplantation (n = 12)	12	0	0	0	0	2	3	3	4
Second transplantation (n = 1)	—	1	1	1	1	1	1	1	1
Second non-transplantation (n = 2)	—	2	2	2	2	2	2	2	2

* For the second transplantation and second non-transplantation groups, weeks were counted from the second transplantation of the Nepic analogue.

4.1.4 Histopathologic examination

Eyeballs were removed at Week 26 of transplantation, stained with hematoxylin-eosin (HE) solution, and subjected to histopathologic examination. In stained images, the untreated eye typically had squamous cells on the cortical layer of the corneal epithelium and aligned cubical cells on the basal layer, while the non-transplanted eye had a thin epithelial layer, goblet cells, originally present in the conjunctiva, on the cornea, and vascular invasion into the corneal stroma. The transplanted eye, on the other hand, had morphological features similar to those in the corneal epithelium layer in the untreated eye (Figure 2).



Untreated eye (right eye) | **Non-recipient eye (left eye in the non-transplantation group)** | **Recipient eye (left eye in the transplantation group)**

Figure 2. HE stained images in histopathologic examination

Table 14 shows results on the extent of corneal epithelium formation assessed based on the stained images in the histopathologic examination.

Table 14. Results from histopathologic examination

Group (n, number of animals)	Score*				
	-	±	+	2+	3+
First transplantation (n = 10)	0	1	0	0	9
First non-transplantation (n = 12)	3	0	6	2	1
Second transplantation (n = 1)	0	0	0	0	1
Second non-transplantation (n = 2)	0	0	1	1	0

* Percentage of the epithelium morphologically differentiated into cornea with respect to the cornea area observed
± <50%
\+ Approximately 50%
2+ Approximately 75%
3+ Approximately 100%

4.R Outline of the review conducted by PMDA

The applicant's explanation about performance of Nepic:

Because the LSCD model was created by surgical removal of the corneal epithelium including the corneal limbus and furthermore alkali treatment, the corneal epithelium is unlikely to be reconstructed unless corneal epithelial stem cells are externally supplied. The Nepic analogue was transplanted to LSCD model rabbits. The corneal clearing rate in the first transplantation group was kept higher than that in the first non-transplantation group throughout a period from Weeks 1 to 26 of transplantation. In addition, the optical media and fundus examinations showed that the percentage of animals with observable optical media and fundus in the first transplantation group tended to be higher than that in the first non-transplantation group, suggesting that transplantation of Nepic would make the cornea transparent. In addition, the histopathologic examination suggests that transplantation of Nepic would lead to corneal epithelium formation.

The above results indicate that transplantation of Nepic onto the eye surface in a patient with LSCD is expected to lead to the corneal epithelium formation, preventing conjunctival tissues and blood vessels from invading into the corneal region, and thereby making the cornea transparent.

PMDA's view:

In the study, not all the animals with the Nepic analogue transplanted were subjected to evaluation, but only the animals in which the extent of corneal opacity and area based on the score given according to the Draize's criteria at Week 4 of transplantation were at certain levels or below were evaluated as animals in the transplantation group. This evaluation procedure raises a concern about overestimation of the performance of Nepic. PMDA asked the applicant to explain the performance of Nepic based on results from direct comparison between animals with the Nepic analogue transplanted and non-transplantation animals without selecting the animals based on the score assessed according to the Draize's criteria at Week 4 of transplantation.

The applicant's explanation:

Tables 15 and 16 show the corneal clearing rate as well as results on optical media and fundus examinations in animals with the Nepic analogue transplanted and animals without transplantation that were not screened based on the score assessed according to the Draize's criteria at Week 4 of transplantation.

Table 15. Corneal clearing rate in animals with and without transplantation

Group (n, number of animals)	Evaluation point (after the first transplantation)	
	Week 1	Week 4
With transplantation	13.3 ± 22.1 (n = 52)	35.6 ± 37.8 (n = 20)
Without transplantation	0.00±0.00 (n = 21)	1.9±7.0 (n = 14)

(mean ± standard deviation)

Table 16. Number of animals with observable optical media and fundus in animals with and without transplantation

Group (n, number of animals)	Evaluation point (after the first transplantation)	
	Week 1	Week 4
With transplantation	24 (n = 52)	11 (n = 20)
Without transplantation	1 (n = 21)	0 (n = 13*)

* No data available in 1 of 14 animals

Of animals with the Nepic analogue transplanted, animals not included in the first transplantation group based on the score assessed according to the Draize's criteria at Week 4 of transplantation underwent a procedure for removal of the scar tissue 6 weeks after the first transplantation and used as animals for evaluation of the second transplantation of the Nepic analogue. Performance comparison between animals with the Nepic analogue transplanted and animals without transplantation is possible only on corneal clearing rates at Weeks 1 and 4 of transplantation as well as results from optical media and fundus examinations, but for the following reasons, performance of Nepic can be explained:

- At Weeks 1 and 4 of transplantation, corneal clearing was hardly observed in animals without transplantation, while corneal clearing was observed in animals with transplantation at a higher rate than that in animals without transplantation.
- Although direct comparison is difficult owing to differences in animal condition between these groups, the corneal clearing rate in animals without transplantation remained low at Week 4 and thereafter, and optical media and fundus were observed only in a limited number of the animals, while in animals with transplantation, the corneal clearing rate was high, and optical media and fundus were observed in many of animals (Tables 12 and 13).

PMDA accepted the applicant's explanation about performance of Nepic.

5. Data Relating to Biodistribution and Outline of the Review Conducted by PMDA

The biodistribution of Nepic is discussed based on the results from "Study of autologous corneal epithelial cell sheet transplantation in LSCD model rabbits" (Attached document 6-1), conducted as a non-clinical safety study as shown below.

5.1 Biodistribution evaluation in study of autologous corneal epithelial cell sheet transplantation in LSCD model rabbits (Attached document 6-1)

The Nepic analogue was transplanted to the LSCD model rabbits after the scar tissue forming on the cornea had been surgically removed. Animals only with the scar tissue removed were included in the non-transplantation group [see Section 4.1].

The applicant's explanation about the biodistribution of Nepic:

There are no results from direct evaluation on survival and maintenance period of transplanted cells, but cells contained in the Nepic analogue were determined to have survived and been maintained until Week 26 of transplantation because the corneal clearing rate remained higher in the transplantation group than in the non-transplantation group throughout a period from Week 1 to Week 26; and the corneal epithelium was histopathologically confirmed at Week 26 [see Section 4.1]. In addition, cells in Nepic are considered unlikely to be distributed in tissues other than the cornea for the following reasons: Nepic is transplanted on the eye surface; the transplanted cells, if falling off by eyeblink, etc., would be

eliminated with tear fluid; and no invasion of the graft into the corneal stroma was observed at least in the histopathologic examination.

5.R Outline of the review conducted by PMDA

PMDA's view:

On the basis of anatomical characteristics of the site where Nepic is to be transplanted and results from the histopathologic examination, the applicant explained that cells in Nepic are unlikely to be widely distributed into tissues other than the cornea. The applicant's explanation is understandable to some extent. Because there are no results from direct evaluation on survival and maintenance period of transplanted cells, it has limitations to explain the survival and maintenance period of Nepic based on the submitted data. However, based on the findings in the LSCD model with the corneal epithelium including the corneal limbus removed in which the transplantation led to corneal clearing and formation of a tissue morphologically similar to the corneal epithelium, cells in the transplanted Nepic are suggested to survive at the transplantation site for a certain period.

6. Data Relating to Non-clinical Safety and Outline of the Review Conducted by PMDA

The applicant submitted the following data relating to the non-clinical safety of Nepic: The study of rabbit autologous corneal epithelial cell sheet (Nepic analogue) transplantation in the LSCD model, tumorigenicity tests of Nepic (karyology test, soft agar colony formation assay, and tumorigenicity test with nude mice), and safety of the impurities.

6.1 Study of autologous corneal epithelial cell sheet transplantation in LSCD model rabbits (Attached document 6-1)

The following study was conducted: In the LSCD model rabbits with the scar tissue forming on the cornea surgically removed, the Nepic analogue was transplanted once or re-transplanted 6 weeks after the first transplantation, and necropsy was performed 26 weeks after the last transplantation. Compared with the non-transplantation group, the transplantation group indicated no toxicological changes attributable to the Nepic analogue in the whole body or at the transplantation site (eye) (Table 17). In addition, the applicant determined that the test product was prepared from the same materials and manufacturing method as those for the concerned autologous cell sheet and confirmed to be similar to the cell sheet in terms of performance, properties related to process-related impurities, and others, and the safety of Nepic in humans was evaluable in this study.

Table 17. Transplantation study using rabbit autologous corneal epithelial cell sheet

Test system	Transplantation route	Observation period	Test product	Dose	Major findings
LSCD model rabbit	Corneal epithelium	26 weeks	Rabbit autologous corneal epithelial cell sheet	1 sheet/eye/ body	Whole body: No toxicological changes Transplantation site (eye): No toxicological changes in the ophthalmologic examination (anterior segment irritation sign, optical media and ophthalmoscopy, corneal epithelium lesion, and corneal clearing rate) or histopathologic examination

6.2 Other safety

6.2.1 Tumorigenicity test

In vitro tests (karyology test and soft agar colony formation assay) as well as the *in vivo* tumorigenicity test with nude mice were performed, and the applicant explained that the tumorigenicity risk of Nepic is low.

6.2.1.1 Karyology test (Attached document 6-2)

Chromosomal aberrations were observed in a part of the specimens in the karyology test (Table 18), but the applicant explained that Nepic has no concerns in terms of the genetic stability by referring to the following definitions in International System for Human Cytogenetic Nomenclature (ISCN) 2009 (11.1.1 Definition of a Clone): "Loss of a single chromosome must be detected in at least three such cells for listing in the karyotype" and "at least two cells with identical excess of one or more chromosomes or the same structural aberration(s) must be detected for listing in the karyotype."

Table 18. Karyology test

Human cornea tissue	Results
BWXA	No chromosomal aberration in corneal epithelial cells in the initial[*1] or overage culture[*2]
JEKV	Marker chromosome of unknown origin in 1 cell and trisomy 18 in another cell were observed when 20 corneal epithelial cells in the initial culture[*1] were examined. Loss of Y chromosome was observed in 1 cell when 20 corneal epithelial cells in the overage culture[*2] were examined.
UEHD	Trisomy 18 in 1 cell as well as trisomy 5, translocation of chromosomes 15 and 16, and 2 marker chromosomes of unknown origin in another cell were observed when 20 corneal epithelial cells in the initial culture[*1] were examined. Loss of short arm in chromosome 3 was observed in 1 cell when 20 corneal epithelial cells in the overage culture[*2] were examined.

*1 Corneal epithelial cells manufactured by culture with [redacted] feeder cells ([redacted]th generation)
*2 Corneal epithelial cells passaged by culture with [redacted] feeder cells ([redacted]th generation)

6.2.1.2 Soft agar colony formation assay (Attached document 6-3)

Three specimens of human cornea tissue (BWXA, JEKV, and UEHD)-derived [redacted], and corneal epithelial cells ([redacted]th generation) passaged by culture with feeder cells were seeded on the soft agar layer followed by incubation for [redacted] days. No anchorage-independent colony formation was observed.

6.2.1.3 Tumorigenicity test in nude mice (Attached document 6-4)

Three specimens of human cornea tissue (BWXA, JEKV, and UEHD)-derived [redacted], and corneal epithelial cells ([redacted]th generation) passaged by culture with feeder cells were subcutaneously transplanted in nude mice. No increase in tumorigenesis related to the transplantation was observed (Table 19).

Table 19. Tumorigenicity test in nude mice

Test system	Route of administration	Observation period	Test cells	Dose (cell count)	Major findings
Nude mice	Subcutaneous	23 days[*1]	3 specimens (BWXA, JEKV, and UEHD)-derived [redacted], corneal epithelial cells passaged by culture with feeder cells ([redacted]th generation)	1×10^7 cells/body[*2]	Whole body and implantation site (subcutaneous): No increase in incidence of tumor lesion attributable to the corneal epithelial cell sheet

*1 Defined based on [redacted].
*2 Defined based on World Health Organization (WHO) guideline (TRS878) B.2.3.7 Tests for tumorigenicity.

6.2.2 Safety evaluation of impurities (Attached documents 2-8, 2-10, 6-5, 6-6, and 6-7)

Impurities potentially remaining in the final product are bovine serum, feeder cells, antibiotics (benzylpenicillin potassium, streptomycin sulfate, amphotericin B, and kanamycin sulfate), cholera toxin, and Impurity A. The safety of these impurities were evaluated based on their residual amounts in Nepic. The applicant explained that these results indicated that these impurities did not pose any safety risk in humans.

6.R Outline of the review conducted by PMDA

PMDA asked the applicant to explain the following matters: Reason why no tumorigenicity test was performed with Nepic transplanted on the eye surface of the clinical application site; and a risk of local malignant transformation of Nepic on the eye.

The applicant's explanation:
It is technically difficult to transplant Nepic on the eye surface of immunodeficiency animals (mice and rats). In addition, conducting an *in vivo* tumorigenicity test in which Nepic was transplanted on the eye surface of rabbits was determined to be difficult because many of the rabbits treated with immunosuppressants would die, and even the surviving rabbits would damage the graft by scratching themselves. Although it is difficult to evaluate a risk of local malignant transformation in animals with Nepic transplanted on the eye surface, the concerned risk is considered low for Nepic for the following reasons:

- Starting material of Nepic is corneal epithelium containing corneal epithelial stem cells that do not have pluripotency.
- No genetic modification is involved in the manufacturing process of Nepic.
- Results from *in vitro* tumorigenicity tests were negative.
- No increase in tumorigenesis was observed in the tumorigenicity test with nude mice subjected to subcutaneous implantation.
- In clinical studies of Nepic, no adverse events related to corneal tumorigenesis have been reported.

PMDA accepted the applicant's explanation on the feasibility of the tumorigenicity test with Nepic transplanted on the eye surface and the risk of tumorigenicity of Nepic on the eye, but considers post-marketing information about the tumorigenesis on the human eye needs to be collected.

On the basis of the submitted data, PMDA has concluded that Nepic has no particular concerns in terms of the non-clinical safety.

7. Data Relating to Clinical Study Results and Outline of the Review Conducted by PMDA

The applicant submitted evaluation data on the efficacy and safety from 2 Japanese clinical studies shown in Table 20.

Table 20. List of clinical studies for efficacy and safety

Data category	Geographical location	Study identifier	Phase	Study population	No. of patients enrolled	Dosage regimen	Main endpoints
Evaluation	Japan	EYE-01M	III	Patients with LSCD	12	Single transplantation with 1 sheet of Nepic	Efficacy Safety
		EYE-01M-FU	III	Patients with LSCD	10	— (Follow-up study in patients who completed EYE-01M study)	Efficacy Safety

7.1 Evaluation data

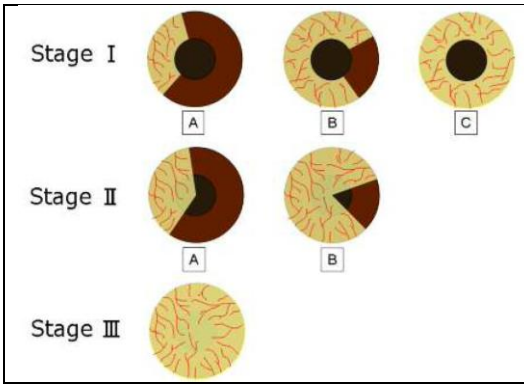
7.1.1 Japanese clinical study

7.1.1.1 EYE-01M study (Attached document 7-1; study period, ■ 20■ to ■ 20■)

An open-label, uncontrolled, Japanese phase III study was conducted at 5 study centers to evaluate the efficacy and safety of Nepic transplanted in patients with LSCD[4)] (target sample size, 10 patients[5)]) who were assessed as Stage IIB[6)] or III according to the severity classification in Figure 3 by the investigator and eligibility assessment committee.[7)]

In this study, the period from obtaining informed consent to transplantation of Nepic was referred to as the "run-in period," and that from the transplantation to Week 52 of transplantation was referred to as the "treatment period."

In light of effects on the efficacy and safety evaluation of Nepic, corneal transplantation, conjunctival epitheliectomy, amniotic membrane transplantation, and concomitant autologous serum eye-drops were prohibited.



Stage I: No conjunctivalization involving the central cornea (5 mm in diameter) with the limbus in a condition of A to C A: Conjunctivalization <50% B: Conjunctivalization ≥50% and <100% C: 100% conjunctivalization
Stage II: Conjunctivalization involving the central cornea (5 mm in diameter) with the limbus in a condition of A or B A: Conjunctivalization <50% B: Conjunctivalization ≥50% and <100%
Stage III: Corneal surface totally covered with conjunctival tissue

Figure 3. Severity classification of LSCD

4) LSCD of any cause was accepted.
5) Enrollment of ≥1 to ≤4 patients at Stage IIB was planned.
6) Of patients at Stage IIB, patients in whom conjunctivalization was not alleviated in the past 3 months were enrolled.
7) The eligibility assessment committee, which consisted of third-party members to confirm that subjects selected by the investigators or sub-investigators were eligible, examined the eligibility using the anterior segment image of the eye to be transplanted, and only those determined to be eligible by the committee were enrolled.

The corneal limbal tissue to be used in manufacture of Nepic should be approximately 2 × 3 mm piece collected from the contralateral eye (donor eye) of the recipient eye that was free from inflammation and infection under slit-lamp examination. The conjunctival scar tissue on the cornea of the recipient eye was removed wherever possible before transplantation of Nepic. After the single transplantation with 1 sheet, a therapeutic soft contact lens was applied followed by tarsorrhaphy where necessary to close the eyelids.

A total of 12 patients were enrolled, and except for 2 patients who discontinued the study before collection of the limbal tissue (Subject number A-2 and D-2),[8)] 10 patients who had Nepic transplanted were included in the full analysis set (FAS)[9)] and also safety analysis set. The FAS was used as the efficacy primary analysis set. Table 21 shows patient characteristics (age, sex, causative etiology of LSCD, ophthalmologic findings at the enrollment, medical history, and previous ophthalmic surgery).

8) A-2: Study continuation was determined impossible owing to adverse events (right pneumothorax and central line infection) that occurred before tissue collection.
D-2: The investigator rated the disease as Stage III in severity and enrolled the patient but the eligibility assessment committee rated it as Stage IIB, and thus the final rating was Stage IIB. By the time of the final rating, 4 patients at Stage IIB were enrolled, and enrollment of the patient was stopped.

9) FAS was defined as a population of patients who provided informed consent excluding patients applicable to (a) to (c) below:
(a) Patients who underwent tissue collection or transplantation of Nepic outside of the contract period with the study center and thereby violated the GCP in a narrow sense
(b) Patients who did not undergo transplantation of Nepic
(c) Patients who underwent transplantation of Nepic but were never subjected to observation for the efficacy endpoints

Table 21. Patient characteristics

Subject number	Age	Sex	Causative etiology of LSCD	Ophthalmologic findings at enrollment		Medical history	Previous ophthalmic surgery
A-1	20	Female	Chemical injury (alkali)	Recipient eye	Corneal stromal opacity (moderate), anterior subcapsular cataract (moderate)	—	—
				Donor eye	—	—	—
				Both eyes	—	—	—
A-3	79	Male	Unknown cause	Recipient eye	Superficial punctate keratopathy (mild), corneal stromal opacity (severe), trichiasis (mild), symblepharon (mild), cataract (moderate)	Pterygium	Pterygium surgery
				Donor eye	Pterygium (mild)	Cataract	Phacoemulsification cataract surgery and intraocular lens implantation
				Both eyes	Dry eye (mild), meibomian gland dysfunction (moderate)	—	—
B-1	23	Female	Long-term HCL use	Recipient eye	Aphakia, corneal endothelial cell loss (mild), bullous keratopathy (mild), microcornea (mild), corneal stromal opacity (mild), superficial punctate keratopathy (mild)	—	Lensectomy
				Donor eye	Cataract (mild), keratic precipitates (mild)	—	—
				Both eyes	—	Iritis	—
B-2	52	Female	Vernal kerato-conjunctivitis and other treatment (soft contact lens, corneal limbal cryopexy, etc.)	Recipient eye	Eyelid ptosis (mild)	Corneal epithelium erosion	—
				Donor eye	—	—	—
				Both eyes	Vernal keratoconjunctivitis (mild), meibomian gland obstruction (mild), glaucoma (mild), cataract (mild), dry eye (mild)	—	Corneal limbal cryopexy
B-3	83	Male	Ocular pemphigoid	Recipient eye	Ocular pemphigoid (moderate), corneal stromal opacity (moderate), symblepharon (mild), cataract (moderate), irregular astigmatism (mild), corneal thinning (moderate)	Herpetic keratitis Herpetic uveitis	—
				Donor eye	Pseudophakia, meibomian gland dysfunction (mild)	Conjunctivitis	Intraocular lens implantation
				Both eyes	Glaucoma (moderate)	—	—
B-4	38	Male	Chemical injury (acid)	Recipient eye	Corneal stromal opacity (moderate), irregular astigmatism (moderate)	—	—
				Donor eye	Myopic astigmatism (mild)	—	—
				Both eyes	Dry eye (mild), allergic conjunctivitis (mild)	—	—
C-1	37	Male	Chemical injury (alkali)	Recipient eye	Herpetic keratitis (mild), superficial punctate keratopathy (mild), corneal epithelium defect (mild), corneal stromal opacity (mild)	—	Amniotic membrane graft transplantation
				Donor eye	—	—	—
				Both eyes	Suspected dry eye (mild)	—	—
C-2	67	Male	Chemical injury (alkali)	Recipient eye	—	—	Corneal epithelium transplantation
				Donor eye	—	Narrow angle	Laser iridotomy
				Both eyes	Cataract (mild)	—	—
E-1	42	Male	Chemical injury (alkali)	Recipient eye	Prolonged corneal epithelium erosion (mild), secondary glaucoma (mild), corneal stromal opacity (severe)	—	—
				Donor eye	—	—	—
				Both eyes	—	—	—
E-2	70	Male	Chemical injury (alkali)	Recipient eye	Corneal opacity (moderate), irregular astigmatism (mild)	Pseudopterygium Meibomian gland dysfunction	Pseudopterygium excision
				Donor eye	—	—	—
				Both eyes	Cataract (moderate), glaucoma (moderate)	—	—

Table 22 shows the donor eye and collection site of corneal limbal tissue for each subject. The corneal limbal tissue was collected once from all subjects, and no re-collection procedure was performed. Unless there was a specific reason, in light of the esthetic outcome, the tissue was collected from the 12 o'clock position where the surgical wound would be covered by the upper eyelid and thus unlikely to be noticeable.

Table 22. Donor eye and collection site of corneal limbal tissue for each subject

Subject number	Donor eye	Collection site
A-1	Right	12 o'clock position
A-3	Right	12 o'clock position
B-1	Right	12 o'clock position
B-2	Left	7 o'clock position*1
B-3	Right	1 o'clock position*2
B-4	Left	1 o'clock position*3
C-1	Left	12 o'clock position
C-2	Right	12 o'clock position
E-1	Left	12 o'clock position
E-2	Left	12 o'clock position

*1 Limbal deficiency was partially observed at the 12 o'clock position.
*2 Glaucoma surgery may be performed in the future.
*3 Collection at the 12 o'clock position had been intended, but the tissue was finally collected at the 1 o'clock position.

The primary efficacy endpoint was the success rate (%)[10] of corneal epithelium reconstruction at Week 52 of transplantation of Nepic. Successful corneal epithelium reconstruction[11] was defined as change to Stage IA to IC in LSCD severity at Week 52. In addition, the LSCD severity was rated by the data monitoring committee using randomized image records with information about the subject and evaluation point (including the screening point) masked to ensure the objectivity and independence of the evaluation.

The success rate (%) of corneal epithelium reconstruction at Week 52 of transplantation of Nepic, the primary efficacy endpoint, was 60.0% (6 of 10) of patients (95% confidence interval [CI] [26.2, 87.8]), of which the lower limit of the 95% CI exceeded the pre-determined threshold of 15%.[12]

Table 23 shows change in LSCD severity and outcome on corneal epithelium reconstruction in each subject.

[10] The statistical analysis plan specified that the analysis should be performed using the rating results made by the eligibility assessment committee at screening and those made by the data monitoring committee after the transplantation.
[11] Because conjunctivalization involving the central cornea would affect the visual acuity, successful corneal epithelium reconstruction was defined as Stage IA to IC, which indicates a condition free from conjunctivalization in the central cornea.
[12] The success rate (%) of corneal epithelium reconstruction in eyes which underwent allogeneic corneal limbal transplantation in Japan was estimated to be 14.3% (2 of 14 eyes) based on one (*N Eng J Med*. 1999;340:1697-703) of the literatures on clinical outcome of allogeneic corneal limbal transplantation in treatment of LSCD that met the following requirements: The efficacy of single transplantation could be compared; the follow-up period was ≥1 year; and successful corneal epithelium reconstruction was defined, and evaluation of corneal epithelium reconstruction was possible using the LSCD severity classification, which would be used in EYE-01M study. The success rate in the literature, however, was from the study in which only patients with LSCD caused by chemical or thermal injury were included, and "failure of corneal epithelium reconstruction" was defined as repeated allogeneic corneal limbal transplantations during a period of 3 years, the mean follow-up period and thus is not the success rate (%) of corneal epithelium reconstruction at Year 1 of transplantation.

Table 23. Change in LSCD severity in each subject

Subject number	Recipient eye Severity rating	Severity						Outcome on corneal epithelium reconstruction at Week 52
		At screening	After transplantation					
			Week 2	Week 4	Week 12	Week 24	Week 52	
A-1	Rated by investigator	III	IA	IA	IA	IA	IA	○
	Centrally rated	IIB/IA	IA	IA	IIA	IA	IA	
A-3	Rated by investigator	III	IA	IA	IA	IIB	IIB	
	Centrally rated	III/III	IIB	IA	IB	IIB	IIB	
B-1	Rated by investigator	IIB	IA	IA	IA	IA	IA	○
	Centrally rated	IIB/IA	IA	IA	IA	IA	IA	
B-2	Rated by investigator	IIB	IA	IA	IA	IA	IA	○
	Centrally rated	IIB/IA	IA	IA	IA	IA	IA	
B-3	Rated by investigator	III	IA	IA	IA	IIB	IIB	
	Centrally rated	III/IB	IA	IB	IB	IIB	IIB	
B-4	Rated by investigator	III	IA	IA	IA	IB	IIB	
	Centrally rated	III/III	IA	IA	IB	IB	IIB	
C-1	Rated by investigator	III	IA	IA	IA	IA	IA	
	Centrally rated	IIB/IIB	IA	IA	IA	IB	IIA	
C-2	Rated by investigator	III	IA	IA	IA	IB	IB	○
	Centrally rated	III/III	IA	IA	IA	IA	IA	
E-1	Rated by investigator	III	IA	IA	IA	IA	IA	○
	Centrally rated	III/III	IA	IA	IA	IA	IA	
E-2	Rated by investigator	III	IA	IA	IA	IA	IA	○
	Centrally rated	III/IIA	IA	IA	IA	IA	IA	

Central rating: Result rated by the eligibility assessment committee only at screening (left) and that rated by the data monitoring committee at screening (right) and all the points after transplantation

Tables 24 and 25 show results on corrected visual acuity, the secondary efficacy endpoint.

Table 24. Change in visual acuity (visual acuity test with Landolt rings)

Subject number		At screening	Week 2	Week 4	Week 12	Week 24	Week 52
A-1	Decimal visual acuity	0.01	0.01	0.02	0.01	0.06	0.04
	Converted LogMAR value*	+2.00	+2.00	+1.70	+2.00	+1.22	+1.40
A-3	Decimal visual acuity	0.01	0.03	0.01	0.02	0.03	0.02
	Converted LogMAR value	+2.00	+1.52	+2.00	+1.70	+1.52	+1.70
B-1	Decimal visual acuity	0.20	0.15	0.15	0.15	0.15	0.20
	Converted LogMAR value	+0.70	+0.82	+0.82	+0.82	+0.82	+0.70
B-2	Decimal visual acuity	0.40	0.15	0.50	0.50	0.20	0.10
	Converted LogMAR value	+0.40	+0.82	+0.30	+0.30	+0.70	+1.00
B-3	Decimal visual acuity	0.06	0.01	0.02	0.04	0.06	0.03
	Converted LogMAR value	+1.22	+2.00	+1.70	+1.40	+1.22	+1.52
B-4	Decimal visual acuity	0.08	0.10	0.10	0.08	0.15	0.15
	Converted LogMAR value	+1.10	+1.00	+1.00	+1.10	+0.82	+0.82
C-1	Decimal visual acuity	0.01	0.05	0.05	0.07	0.09	0.08
	Converted LogMAR value	+2.00	+1.30	+1.30	+1.15	+1.05	+1.10
C-2	Decimal visual acuity	0.01	0.10	0.15	0.10	0.06	0.07
	Converted LogMAR value	+2.00	+1.00	+0.82	+1.00	+1.22	+1.15
E-1	Decimal visual acuity	0.01	0.02	0.02	0.02	0.02	0.01
	Converted LogMAR value	+2.00	+1.70	+1.70	+1.70	+1.70	+2.00
E-2	Decimal visual acuity	0.01	0.01	0.01	0.01	0.04	0.05
	Converted LogMAR value	+2.00	+2.00	+2.00	+2.00	+1.40	+1.30

* Angle (θ) formed by 2 rays from the eye to 2 barely visible points is referred to as minimum visual angle of resolution (MAR) (°), of which logarithm value is LogMAR. The smaller target perceived means better visual acuity, which leads to a smaller LogMAR value.

Table 25. Change in visual acuity (ETDRS visual acuity test)

Subject number		At screening	Week 2	Week 4	Week 12	Week 24	Week 52
A-1	Number of letters	Counting fingers[*2]	Counting fingers	Counting fingers	Counting fingers	17	16
	Converted LogMAR[*1] value	+2.00	+2.00	+2.00	+2.00	+1.36	+1.38
A-3	Number of letters	Hand motion[*3]	Counting fingers	Counting fingers	Counting fingers	Counting fingers	Counting fingers
	Converted LogMAR value	+3.00	+2.00	+2.00	+2.00	+2.00	+2.00
B-1	Number of letters	45	37	44	47	52	52
	Converted LogMAR value	+0.80	+0.96	+0.82	+0.76	+0.66	+0.66
B-2	Number of letters	52	33	54	57	29	28
	Converted LogMAR value	+0.66	+1.04	+0.62	+0.56	+1.12	+1.14
B-3	Number of letters	32	Counting fingers	Counting fingers	8	21	4
	Converted LogMAR value	+1.06	+2.00	+2.00	+1.54	+1.28	+1.62
B-4	Number of letters	18	15	28	22	42	24
	Converted LogMAR value	+1.34	+1.40	+1.14	+1.26	+0.86	+1.22
C-1	Number of letters	Counting fingers	22	15	23	25	28
	Converted LogMAR value	+2.00	+1.26	+1.40	+1.24	+1.20	+1.14
C-2	Number of letters	Counting fingers	44	33	24	28	27
	Converted LogMAR value	+2.00	+0.82	+1.04	+1.22	+1.14	+1.16
E-1	Number of letters	1	Counting fingers	29	19	17	10
	Converted LogMAR value	+1.68	+2.00	+1.12	+1.32	+1.36	+1.50
E-2	Number of letters	Counting fingers	Counting fingers	Counting fingers	Counting fingers	27	28
	Converted LogMAR value	+2.00	+2.00	+2.00	+2.00	+1.16	+1.14

*1 In the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart, 5 letters correspond to LogMAR value of 0.1. Counting fingers and hand motion were handled as converted LogMAR values +2.00 and +3.00, respectively.
*2 Visual acuity of the eye that cannot recognize any letter at a distance 1 m apart but can count fingers of the examiner standing at a specified distance apart
*3 Visual acuity of the eye that cannot identify fingers of the examiner but can recognize his or her hand motion

Tables 26 to 29 show results on the secondary endpoints, severities of corneal opacity, corneal neovascularisation, and symblepharon as well as the appropriateness or necessity of indicating additional treatment to improve visual acuity at Week 52 of transplantation of Nepic.

Table 26. Change in severity of corneal opacity

Subject number	Severity of corneal opacity (Grade)*					
	Day of screening	After transplantation				
		Week 2	Week 4	Week 12	Week 24	Week 52
A-1	3	2	2	2	2	2
A-3	3	3	2	2	2	3
B-1	2	1	1	1	1	1
B-2	1	1	1	1	1	1
B-3	2	1	1	1	1	1
B-4	3	1	1	1	1	1
C-1	2	1	1	1	1	1
C-2	2	0	1	0	1	1
E-1	2	1	1	1	1	1
E-2	2	1	1	1	1	1

* Grade 0: Iris details observable
Grade 1: Iris details observable but partially opaque
Grade 2: Iris details not observable but pupil margin slightly perceivable
Grade 3: Neither iris nor pupil margin observable

Table 27. Change in severity of corneal neovascularisation

Subject number	Severity of corneal neovascularisation (Grade)*					
	Day of screening	After transplantation				
		Week 2	Week 4	Week 12	Week 24	Week 52
A-1	3	3	3	3	3	3
A-3	3	2	2	2	2	2
B-1	0	0	0	0	0	0
B-2	1	1	1	1	1	1
B-3	3	1	1	1	1	2
B-4	3	2	2	2	2	2
C-1	3	0	1	1	1	2
C-2	3	0	0	1	1	2
E-1	3	0	0	0	0	1
E-2	0	0	0	0	0	0

* Grade 0: No neovascularisation
Grade 1: Neovascularisation around cornea
Grade 2: Neovascularisation to pupil margin
Grade 3: Neovascularisation beyond pupil margin

Table 28. Change in severity of symblepharon

Subject number	Severity of symblepharon (Grade)*					
	Day of screening	After transplantation				
		Week 2	Week 4	Week 12	Week 24	Week 52
A-1	0	0	0	0	0	0
A-3	1	1	1	2	2	2
B-1	0	0	0	0	0	0
B-2	0	0	0	0	0	0
B-3	2	1	1	1	1	1
B-4	0	0	0	0	0	0
C-1	0	0	0	0	0	0
C-2	0	0	0	0	0	0
E-1	0	0	0	0	0	0
E-2	0	0	0	0	0	0

* Grade 0: No symblepharon
Grade 1: Symblepharon only involving the conjunctival surface
Grade 2: Symblepharon involving <50% of the corneal surface
Grade 3: Symblepharon involving ≥50% of the corneal surface

Table 29. Appropriateness or necessity of indicating additional treatment to improve visual acuity at Week 52 of transplantation of Nepic

Subject number	Additional treatment to improve visual acuity[*1]	Appropriateness or necessity of indicating additional treatment
A-1	Corneal transplant to resolve corneal stromal opacity	Appropriate
	Conjunctival epitheliectomy	Not necessary
A-3	Corneal transplant to resolve corneal stromal opacity	Appropriate
	Conjunctival epitheliectomy	Appropriate
	Amniotic membrane transplantation to resolve symblepharon	Appropriate
	Cataract surgery	Appropriate
B-1	Corneal transplant to resolve corneal stromal opacity	Not necessary
	Conjunctival epitheliectomy	Not necessary
B-2	Corneal transplant to resolve corneal stromal opacity	Not necessary
	Conjunctival epitheliectomy	Not necessary
	Cataract surgery	Appropriate
B-3	Corneal transplant to resolve corneal stromal opacity	Not appropriate[*2]
	Conjunctival epitheliectomy	Appropriate
	Cataract surgery	Appropriate
B-4	Corneal transplant to resolve corneal stromal opacity	Appropriate
	Conjunctival epitheliectomy	Appropriate
C-1	Corneal transplant to resolve corneal stromal opacity	Appropriate
	Conjunctival epitheliectomy	Not necessary
C-2	Corneal transplant to resolve corneal stromal opacity	Not necessary
	Conjunctival epitheliectomy	Not necessary
E-1	Corneal transplant to resolve corneal stromal opacity	Appropriate
	Conjunctival epitheliectomy	Not necessary
E-2	Corneal transplant to resolve corneal stromal opacity	Appropriate
	Conjunctival epitheliectomy	Not necessary
	Cataract surgery	Appropriate

*1 Corneal transplant to resolve corneal stromal opacity and conjunctival epitheliectomy were commonly considered for all the subjects. For a subject requiring consideration of the other additional treatment, appropriateness or necessity of the concerned treatment was also considered.

*2 "Not appropriate" was determined because corneal transplant would pose a high risk of rejection owing to chronic conjunctivitis.

For the safety during the run-in period, adverse events for which a causal relationship to tissue collection could not be ruled out occurred in the donor eye of 6 subjects (60.0%) and were eye pain and foreign body sensation in eyes in 3 subjects each. For the safety during the treatment period, there were no adverse events for which a causal relationship to tissue collection could not be ruled out, but adverse events for which a causal relationship to Nepic could not be ruled out occurred in the recipient eye of 9 subjects (90.0%) and were corneal epithelium defect in 7 subjects, punctate keratitis in 6 subjects, corneal neovascularisation in 2 subjects, cellulitis orbital, conjunctivitis, conjunctival erosion, dry eye, eye pain, visual acuity reduced, foreign body sensation in eyes, intraocular pressure increased, and procedural pain in 1 subject each. Serious adverse events occurred in 1 subject (Subject number A-2) before tissue collection during the run-in period and were right pneumothorax, port infection, and central line infection, for any of which causal relationships to the tissue collection and Nepic were denied. No death occurred during either run-in or treatment period.

7.1.1.2 EYE-01M-FU study (Attached documents 7-2 and 7-3; study period, [redacted] to [redacted])

An open-label, uncontrolled study was conducted at 4 study centers to evaluate the long-term efficacy and safety of Nepic in patients who completed EYE-01M study. The follow-up period was 52 weeks from Weeks 53 to 104 of transplantation of Nepic. This study enrolled 10 subjects, all of whom were included in the efficacy and safety analysis populations. Neither prohibited concomitant drugs nor therapies were specified, and information about additional treatment (corneal transplant, conjunctival

epithelial curettage, and cataract surgery) on the recipient eye of Nepic transplanted in EYE-01M study was to be collected.

The efficacy primary endpoint was specified as the LSCD severity at Week 104 of transplantation of Nepic, and for severity rating, the same criteria (Figure 3) as those used in EYE-01M study were applied. As with EYE-01M study, the LSCD severity was rated by the data monitoring committee using randomized image records with information about the subject and evaluation point masked to ensure the objectivity and independence of the evaluation.

Table 30 shows changes in LSCD severity.

Table 30. Changes in LSCD severity from the end of the follow-up period of EYE-01M study

Subject number	Severity rating on recipient eye	Severity		
		At screening for EYE-01M study	After transplantation	
			Week 78	Week 104
A-1	Rated by investigator	III	IB	IB
	Centrally rated	IIB/IA	IA	IA
A-3	Rated by investigator	III	IIB	IIB
	Centrally rated	III/III	IIB	IIB
B-1	Rated by investigator	IIB	IA	IA
	Centrally rated	IIB/IA	IA	IA
B-2	Rated by investigator	IIB	IA	IA
	Centrally rated	IIB/IA	IA	IA
B-3	Rated by investigator	III	IIB	IIB
	Centrally rated	III/IB	IIB	IIB
B-4	Rated by investigator	III	IIB	IIB
	Centrally rated	III/III	IIB	IIB
C-1	Rated by investigator	III	IA	IA
	Centrally rated	IIB/IIB	IB	IB
C-2	Rated by investigator	III	IB	IB
	Centrally rated	III/III	IA	IA
E-1	Rated by investigator	III	IA	IA
	Centrally rated	III/III	IA	IA
E-2	Rated by investigator	III	IA	IA
	Centrally rated	III/IIA	IA	IA

Central rating: Result rated by the eligibility assessment committee only at screening for EYE-01M study (left) and that rated by the data monitoring committee at screening (right) and at all the points after transplantation

Tables 31 and 32 show results on corrected visual acuity, the secondary endpoint.

Table 31. Change in visual acuity (visual acuity test with Landolt rings)

Subject number		At screening for EYE-01M study	Week 78	Week 104
A-1	Decimal visual acuity	0.01	0.06	0.04
	Converted LogMAR value	+2.00	+1.22	+1.40
A-3	Decimal visual acuity	0.01	0.01	0.01
	Converted LogMAR value	+2.00	+2.00	+2.00
B-1	Decimal visual acuity	0.20	0.30	0.20
	Converted LogMAR value	+0.70	+0.52	+0.70
B-2*1	Decimal visual acuity	0.40	0.02	0.40
	Converted LogMAR value	+0.40	+1.70	+0.40
B-3*2	Decimal visual acuity	0.06	0.01	0.04
	Converted LogMAR value	+1.22	+2.00	+1.40
B-4	Decimal visual acuity	0.08	0.15	0.15
	Converted LogMAR value	+1.10	+0.82	+0.82
C-1	Decimal visual acuity	0.01	0.05	0.06
	Converted LogMAR value	+2.00	+1.30	+1.22
C-2	Decimal visual acuity	0.01	0.09	0.05
	Converted LogMAR value	+2.00	+1.05	+1.30
E-1*3	Decimal visual acuity	0.01	0.02	0.01
	Converted LogMAR value	+2.00	+1.70	+2.00
E-2*4	Decimal visual acuity	0.01	0.40	0.03
	Converted LogMAR value	+2.00	+0.40	+1.52

*1 Cataract surgery at Week 79
*2 Cataract surgery at Week 80
*3 Corneal transplant at Week 84
*4 Cataract surgery at Week 59

Table 32. Change in visual acuity (ETDRS visual acuity test)

Subject number		At screening for EYE-01M study	Week 78	Week 104
A-1	Number of letters	Counting fingers	10	4
	Converted LogMAR value	+2.00	+1.50	+1.62
A-3	Number of letters	Hand motion	Counting fingers	Counting fingers
	Converted LogMAR value	+3.00	+2.00	+2.00
B-1	Number of letters	45	53	53
	Converted LogMAR value	+0.80	+0.64	+0.64
B-2*1	Number of letters	52	Counting fingers	56
	Converted LogMAR value	+0.66	+2.00	+0.58
B-3*2	Number of letters	32	Counting fingers	16
	Converted LogMAR value	+1.06	+2.00	+1.38
B-4	Number of letters	18	47	39
	Converted LogMAR value	+1.34	+0.76	+0.92
C-1	Number of letters	Counting fingers	19	28
	Converted LogMAR value	+2.00	+1.32	+1.14
C-2	Number of letters	Counting fingers	24	26
	Converted LogMAR value	+2.00	+1.22	+1.18
E-1*3	Number of letters	1	26	8
	Converted LogMAR value	+1.68	+1.18	+1.54
E-2*4	Number of letters	Counting fingers	40	18
	Converted LogMAR value	+2.00	+0.90	+1.34

*1 Cataract surgery at Week 79
*2 Cataract surgery at Week 80
*3 Corneal transplant at Week 84
*4 Cataract surgery at Week 59

Tables 33 to 36 show results on the secondary endpoints, severities of corneal opacity, corneal neovascularisation, and symblepharon as well as practice of additional treatment to improve visual acuity and time of the practice.

Table 33. Change in severity of corneal opacity

Subject number	Severity of corneal opacity* (Grade)		
	At screening for EYE-01M study	Week 78	Week 104
A-1	3	3	2
A-3	3	2	3
B-1	2	1	1
B-2	1	1	1
B-3	2	1	1
B-4	3	1	1
C-1	2	2	1
C-2	2	1	1
E-1	2	1	1
E-2	2	1	1

* Grade 0: Iris details observable
Grade 1: Iris details observable but partially opaque
Grade 2: Iris details not observable but pupil margin slightly perceivable
Grade 3: Neither iris nor pupil margin observable

Table 34. Change in severity of corneal neovascularisation

Subject number	Severity of corneal neovascularisation* (Grade)		
	At screening for EYE-01M study	Week 78	Week 104
A-1	3	3	3
A-3	3	3	3
B-1	0	0	0
B-2	1	1	1
B-3	3	3	3
B-4	3	1	1
C-1	3	2	2
C-2	3	1	2
E-1	3	1	1
E-2	0	0	0

* Grade 0: No neovascularisation
Grade 1: Neovascularisation around cornea
Grade 2: Neovascularisation to pupil margin
Grade 3: Neovascularisation beyond pupil margin

Table 35. Change in severity of symblepharon

Subject number	Severity of symblepharon* (Grade)		
	At screening for EYE-01M study	Week 78	Week 104
A-1	0	0	0
A-3	1	2	2
B-1	0	0	0
B-2	0	0	0
B-3	2	1	1
B-4	0	0	0
C-1	0	0	0
C-2	0	0	0
E-1	0	0	0
E-2	0	0	0

* Grade 0: No symblepharon
Grade 1: Symblepharon only involving the conjunctival surface
Grade 2: Symblepharon involving <50% of the corneal surface
Grade 3: Symblepharon involving ≥50% of the corneal surface

Table 36. Practice of additional treatment to improve visual acuity and time of the practice

Subject number	Additional treatment to improve visual acuity (reason for omission of the additional treatment)	Time of additional treatment
A-1	—* (the corneal epithelium condition was determined to be unstable)	—
A-3	— (hospitalization schedule could not be arranged owing to subject's convenience)	—
B-1	— (additional treatment was determined to be unnecessary)	—
B-2	Cataract surgery	Week 79
B-3	Cataract surgery	Week 80
B-4	— (hospitalization schedule could not be arranged owing to subject's convenience)	—
C-1	— (hospitalization schedule could not be arranged owing to subject's convenience)	—
C-2	— (additional treatment was determined to be unnecessary)	—
E-1	Corneal transplant	Week 84
E-2	Cataract surgery	Week 59

* No additional treatment

There were no adverse events for which a causal relationship to tissue collection could not be ruled out, but adverse events for which a causal relationship to Nepic could not be ruled out occurred in the recipient eye of 3 subjects (30.0%) and these events were corneal epithelium defect in 2 subjects and punctate keratitis in 2 subjects. Neither deaths nor serious adverse events occurred.

7.R Outline of the review conducted by PMDA

7.R.1 Data for review

EYE-01M study and EYE-01M-FU study, from which results were submitted as evaluation data in this application, were conducted in an open-label uncontrolled manner, and the evaluation method of the efficacy in clinical studies in patients with LSCD has not been established. PMDA reviews the efficacy of Nepic in Section 7.R.2 in view of the following investigations:

Investigations

- Whether it is possible to evaluate the efficacy of Nepic based on results from EYE-01M study and EYE-01M-FU study, which are open-label uncontrolled studies
- Appropriateness of the evaluation method of the efficacy (endpoints, evaluation points, and threshold)
- Effects of previous ophthalmic surgery on the efficacy of Nepic
- Some of the severity rating results at screening differed between the investigator and data monitoring committee

7.R.2 Efficacy

7.R.2.1 Reason why EYE-01M study was conducted in an open-label uncontrolled manner

The applicant's explanation on reasons why EYE-01M study was conducted in an open-label uncontrolled manner:

Reason why it was designed as an uncontrolled study

For the following reasons, it was difficult to specify the allogeneic or autologous corneal limbal transplantation conventionally used in treatment of LSCD as the comparator:

- For allogeneic corneal limbal transplantation, the shortage of donors was serious, requiring patients to wait for an eye donated for transplantation, and thus the enrollment is difficult.

- Because the corneal limbal tissue collected for autologous corneal limbal transplantation is large in size, requiring a procedure highly invasive in the donor eye, such a transplantation has not been positively chosen and has been rarely performed.

For the following reasons, only removal of the conjunctival scar tissue would not lead to corneal epithelium reconstruction in the patient population of EYE-01M study, and thus the applicant considers it possible to evaluate the efficacy of Nepic by confirming corneal epithelium reconstruction even in an uncontrolled study:

- Patients at Stage III have no normal corneal limbus left, and only removal of the conjunctival scar tissue does not enable corneal epithelium reconstruction.
- In patients at Stage IIB, a possibility of spontaneous corneal epithelium reconstruction is not ruled out, and 1 report (*Tohoku Medical Journal*. 2006;118:117-122) indicates that the corneal epithelium in a normal eye is replaced with new corneal epithelial cells in approximately 2 weeks. The following inclusion criterion, therefore, was specified for patients at Stage IIB: Conjunctivalization has not been alleviated during a period of 3 months before enrollment, which is longer than the above 2 weeks.

Reason why it was designed as an open-label study
In light of medical care and monitoring required for the transplantation of Nepic, it is difficult to blind the physician and subject to information about whether the transplantation has been performed.

Considering the above applicant explanation understandable, PMDA concluded that it is acceptable for the applicant to have conducted EYE-01M study as an open-label uncontrolled study.

7.R.2.2 Efficacy endpoints, evaluation points, and threshold in EYE-01M study

The applicant's explanation about reasons for establishing the primary endpoint as the success rate (%) of corneal epithelium reconstruction at Week 52 of transplantation of Nepic, the secondary endpoints as corrected visual acuity, etc., and the efficacy threshold for the primary endpoint as 15%:
The fundamental treatment of LSCD is corneal epithelium reconstruction [see Section 1.2], but such reconstruction with the conventional treatment is difficult in the patient population of EYE-01M study. The efficacy of Nepic, therefore, is evaluable based on the success rate (%) of corneal epithelium reconstruction.

The evaluation points were specified through the following considerations. On the basis of a report (*Eye.* 2004;18:241-8) showing that it took 35.6 ± 60.2 days for the autologous corneal limbus graft to achieve corneal epithelialization, a post-transplantation period of approximately 3 months might be enough for the cultured corneal epithelium to complete epithelialization. On the other hand, some reports (*Invest Ophthalmol Vis Sci*. 1981;21:434-41, *Surv Ophthalmol*. 1997;41:275-313) indicated that it would take up to 1 year for the corneal epithelium graft to reconstruct and stabilize the recipient site. On the basis of these reports, to confirm normal reconstruction of the corneal epithelium with Nepic, the follow-up period of approximately 1 year was considered necessary. The applicant planned to evaluate the success rate (%) of corneal epithelium reconstruction at Week 52 of transplantation.

In addition to the above, corrected visual acuity was included in the secondary endpoints to evaluate improvement of the clinical conditions.

Of reports in Japan or overseas on long-term results of allogeneic corneal limbal transplantation, 4 reports (*N Engl J Med.* 1999;340:1697-703, *Ophthalmology.* 2002;109:1159-66, *Ophthalmology.* 2002;109:1278-84, *Ophthalmology.* 2004;111:38-44) were found to present transplantation results with the follow-up period of ≥1 year in treatment of LSCD caused by thermal or chemical injury, which was presumed as the major cause of the disease in the patient population of EYE-01M study. Of these, 3 reports (*Ophthalmology.* 2002;109:1159-66, *Ophthalmology.* 2002;109:1278-84, *Ophthalmology.* 2004;111:38-44) included results from patients who had undergone multiple transplantations and patients with the follow-up period <1 year, and the remaining report (*N Engl J Med*. 1999;340:1697-703) by Tsubota, et al. was used in setting the threshold. On the basis of this report, the success rate (%) of corneal epithelium reconstruction in patients who underwent allogeneic corneal limbal transplantation was estimated to be 14.3%, and the efficacy threshold was established at 15% for EYE-01M study.

PMDA's view:
The efficacy endpoints and evaluation points are acceptable. On the other hand, setting the efficacy threshold at 15% is inappropriate for the following reasons:

- The applicant estimated the success rate (%) of corneal epithelium reconstruction in patients who underwent allogeneic corneal limbal transplantation as 14.3% based on the report by Tsubota et al. (*N Engl J Med*. 1999;340:1697-703) (2 of 14 eyes, in patients with LSCD caused by chemical or thermal injury, recipient eyes that did not undergo multiple transplantations were deemed as ones with successful corneal epithelium reconstruction), but individual patient information about time of transplantation, etc. remained unclear, and eyes which underwent multiple transplantations were deemed as ones with "failed corneal epithelium reconstruction." It is therefore unclear whether the rationale for setting the efficacy threshold at Year 1 of transplantation is appropriate. In addition, the concerned report provided results from a study with the mean follow-up period of 3 years, and thus the threshold was not established based on results at Year 1 of allogeneic corneal limbal transplantation.
- In the other multiple literatures presented by the applicant (*Ophthalmology.* 2002;109:1159-66, *Ophthalmology*. 2002;109:1278-84, *Ophthalmology*. 2004;111:38-44), the treatment results at Year 1 of transplantation were approximately 40% to 75%.

Considering the importance of not only the success rate (%) of corneal epithelium reconstruction in EYE-01M study but also results at Year 2 of transplantation in EYE-01M-FU study, PMDA decided to evaluate the both results.

7.R.2.3 Effects of previous ophthalmic surgery on the efficacy evaluation of Nepic

PMDA asked the applicant to explain effects of previous ophthalmic surgery in each subject on the efficacy evaluation of Nepic.

The applicant's response:

Table 37 shows details of previous ophthalmic surgery in each of the subjects enrolled in EYE-01M study and reasons for the previous ophthalmic surgery having no effects on the efficacy of Nepic. The previous ophthalmic surgery did not affect the efficacy evaluation of Nepic.

Table 37. Details of previous ophthalmic surgery in each subject and reasons for ruling out effects on efficacy evaluation of Nepic

Subject number	Previous ophthalmic surgery	Site	Purpose	Reasons for previous ophthalmic surgery having no effects on efficacy evaluation of Nepic
A-3	Pterygium surgery	Recipient eye	Treatment of pterygium	Operation was performed ≥30 years before screening.
	Phacoemulsification cataract surgery Intraocular lens implantation	Donor eye	Treatment of cataract	Operation was performed on the donor eye.
B-1	Lensectomy	Recipient eye	Treatment of cataract	Operation was performed just after birth (23 years at the enrollment), and the long-term use of HCL caused LSCD.
B-2	Corneal limbal cryopexy	Both eyes	Treatment of vernal keratoconjunctivitis	Operation was performed approximately 36 years before screening.
B-3	Intraocular lens implantation	Donor eye	Treatment of cataract	Operation was performed on the donor eye.
C-1	Amniotic membrane graft transplantation	Recipient eye	Treatment of LSCD	A total of 3 transplantations with an amniotic membrane patch were performed for treatment of LSCD over a period of 3 months approximately 4 years before screening, but conjunctivalization of the corneal epithelium was not alleviated.
C-2	Corneal epithelium transplantation	Recipient eye	Treatment of LSCD	A total of 2 corneal epithelium transplantations were performed approximately 7 and 6 years before screening, but conjunctivalization of the corneal epithelium was not alleviated.
	Laser iridotomy	Donor eye	Treatment of glaucoma	Operation was performed on the donor eye.
E-2	Pseudopterygium excision	Recipient eye	Treatment of pseudopterygium	Operation was performed approximately 4 years and 6 months before screening.

PMDA concluded that the above applicant's explanation is acceptable.

7.R.2.4 Reason for the differences in LSCD severity rating results at screening between the investigator and data monitoring committee

PMDA asked the applicant to explain reason for some of the differences in LSCD severity rating results at screening between the investigator and data monitoring committee.

The applicant's response:

LSCD severity rating was made based on (a) epithelial opacity, (b) neovascularisation, and (c) roughness of the corneal surface, which are characteristics of conjunctivalization.

(a) EYE-01M study included patients with corneal stromal opacity, and it was difficult to assess the epithelial opacity in the presence of corneal stromal opacity.

(b) For neovascularisation, the investigator was able to obtain information about the depth by applying slit light from various angles. The data monitoring committee, on the other hand, rated the severity using photographs of the anterior segment and had limited information about the depth compared with the investigator. Furthermore, the investigator was able to obtain information about neovascularisation over time starting the pre-transplantation point from the medical record, but the data monitoring committee rated the severity without such information.

(c) For roughness of the corneal surface, the data monitoring committee, which rated the severity using photographs of the anterior segment, had difficulty in determining whether the roughness was the corneal epithelium associated with conjunctivalization or the corneal stroma being rough.

The above different information availabilities were considered to have led to differences in rating results between the investigator and data monitoring committee.

PMDA's view:
The above applicant's explanation is understandable to some extent, but clear reasons for different rating results being given to the same subject remain unknown. For the subjects in whom different rating results were given at screening, PMDA reviews the efficacy evaluation of Nepic, also taking the rating results of the data monitoring committee into account.

For EYE-01M study, the statistical analysis plan prepared after the start of the study (version 1.0 prepared on [redacted], 20[redacted]) included a statement to the effect that the rating results of the eligibility assessment committee would be used at screening, but the protocol did not include this. Because the concerned statement was important, considerably affecting results on the primary endpoint of Nepic, it should have been included in the protocol before the start of the study.

7.R.2.5 Efficacy

The applicant's explanation about the efficacy of Nepic in treatment of LSCD:
In EYE-01M study to confirm the efficacy and safety of Nepic in treatment of LSCD, success of corneal epithelium reconstruction was defined as a change from Stage IIB or III, severity classification of the patient population, to Stages IA to IC.

Patient population
In treatment of LSCD, the clinical condition is determined by a physician based on the residual area of the normal corneal limbus, without clear criteria. In general, the condition at Stage II or III in which conjunctivalization involves the central cornea would affect the visual acuity, requiring aggressive treatment. If ≥50% of the corneal limbus remains normal (corresponding to Stage IIA in severity classification applied to EYE-01M study), removal of conjunctival scar tissue from the cornea is considered to have a possibility of corneal epithelium reconstruction, but if <50% of the corneal limbus remains normal (corresponding to Stage IIB or III in severity classification applied to EYE-01M study), only such removal is unlikely to achieve corneal epithelium reconstruction, requiring additional supply of corneal epithelial stem cells. For patients with LSCD at Stage IIB or III, treatment achieving satisfactory outcome is not currently available.

Definition of successful corneal epithelium reconstruction
Because conjunctivalization involving the central cornea would affect the visual acuity, "successful corneal epithelium reconstruction" was defined as the outcome rated as Stages IA to IC, which indicates a condition free from conjunctivalization in the central cornea.

EYE-01M study and EYE-01M-FU study where the above definition was applied presented the following results, and the applicant considered that the efficacy of Nepic was demonstrated:

- The success rate (%) of corneal epithelium reconstruction at Week 52 of transplantation of Nepic, the primary endpoint in EYE-01M study, was 60% (6 of 10) of subjects (95% CI [26.2, 87.8]), and the lower limit of the 95% CI exceeded the threshold of 15%, pre-determined based on the treatment results of allogeneic corneal limbal transplantation. Furthermore, all of the 6 subjects who had achieved successful corneal epithelium reconstruction in EYE-01M study were confirmed to maintain the reconstructed state even at Week 104 of transplantation, and 1 subject (Subject number C-1) who had not achieved the reconstruction at Week 52 of transplantation was found to achieve the successful reconstruction at Week 104 of transplantation. As a final result, 70% (7 of 10) of subjects achieved the successful reconstruction. In addition, a report on transplantation of autologous cultured corneal epithelium in patients with LSCD (*N Engl J Med*. 2010;363:147-55) shows that if the corneal epithelium remains stable for 1 year after transplantation, the effectiveness will be continued thereafter. On the basis of this report, the long-term effectiveness of Nepic can be expected.
- For corrected visual acuity, a change in converted logarithmic minimum angle of resolution (LogMAR) value ≥0.2 (corresponding to 10 letters) in the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity test lead to reproducible detection of a clinical change in visual acuity (*Invest Ophthalmol Vis Sci*. 2003;44:3278-81). In the studies, changes in converted LogMAR value ≥0.2 in ETDRS visual acuity test occurred in 5 of 10 subjects at Week 52 of transplantation and 6 of 10 subjects at Week 104. These results are considered to have clinical significance. In the visual acuity test with Landolt rings, changes in converted LogMAR value ≥0.2 occurred in 6 of 10 subjects at Week 52 of transplantation and 5 of 10 subjects at Week 104, and the concerned results are also considered to have clinical significance.

PMDA's view:
The efficacy of Nepic can be evaluated based on data from EYE-01M study, conducted as an open-label, uncontrolled study [see Section 7.R.2.1]. However, the efficacy threshold is not appropriately established in EYE-01M study [see Section 7.R.2.2], and PMDA cannot determine that the efficacy of Nepic is demonstrated by the success rate (%) of corneal epithelium reconstruction at Week 52 of transplantation, the primary endpoint in EYE-01M study, exceeding the threshold.

Allogeneic corneal limbal transplantation, a conventional treatment of LSCD, on the other hand, leads to poor long-term prognosis due to immunological rejections, requiring multiple transplantations owing to graft failure. Autologous corneal limbal transplantation has a drawback of being highly invasive in the donor eye. Taking account of these points, PMDA recognizes clinical significance in the study results showing that single transplantation of Nepic in patients with LSCD, in whom supply of corneal epithelial stem cells is the only possible solution to enable corneal epithelium reconstruction, led to successful corneal epithelium reconstruction and long-term maintenance of the reconstructed state.

Although the consensus has not been established for clinical significance of an improvement in converted LogMAR value ≥0.2 in the ETDRS visual acuity test, the change ≥0.2 is reported to lead to reproducible detection of a clinical improvement in visual acuity. Taking this report into account, improvements in converted LogMAR value ≥0.2 can be used as data supporting the efficacy of Nepic.

Taking account of the above points, PMDA reviewed the efficacy of Nepic in EYE-01M study and EYE-01M-FU study as described below and has considered that the efficacy can be expected. The review

covered not only the FAS (n = 10) but also subjects in whom the severity at screening was rated as Stage IIB or III by the data monitoring committee (Subject numbers A-3, B-4, C-1, C-2, and E-1 [data monitoring committee population]) because the LSCD severity rating at screening differed between the investigator and data monitoring committee.

FAS

- The success rate (%) of corneal epithelium reconstruction at Week 52 of transplantation of Nepic, the primary endpoint, was 60% (6 of 10) of subjects (95% CI [26.2, 87.8]). The results of the single transplantation of Nepic are comparable to the treatment results (approximately 40%-75%) at Year 1 of allogeneic corneal limbal transplantation presented in the reports on the long-term transplantation results (*Ophthalmology*. 2002;109:1159-66, *Ophthalmology*. 2002;109:1278-84, *Ophthalmology*. 2004;111:38-44).
- Of the FAS in EYE-01M study, all of 6 subjects who achieved successful corneal epithelium reconstruction maintained the reconstructed state even at Week 104 of transplantation. In addition, an additional 1 subject (Subject number C-1) achieved the successful corneal epithelium reconstruction after Week 52 of transplantation of Nepic. As a final result, 70% (7 of 10) of subjects achieved the successful reconstruction.
- Improvements in converted LogMAR value ≥0.2 in ETDRS visual acuity test were observed in 5 of 10 subjects (50%) at Week 52 of transplantation of Nepic and 6 of 10 subjects (60%) at Week 104. Changes in visual acuity test with Landolt rings showed a similar improving trend.

Data monitoring committee population

- The success rate (%) of corneal epithelium reconstruction at Week 52 of transplantation of Nepic, the primary endpoint, was 40% (2 of 5) of subjects, and an additional 1 subject achieved the successful reconstruction by Week 104 of transplantation. As a final result, the success rate (%) was 60% (3 of 5) of subjects.
- Of 2 subjects who had achieved successful corneal epithelium reconstruction at Week 52 of transplantation of Nepic, both were found to maintain the reconstructed state at Week 104 of transplantation.
- Improvements in converted LogMAR value ≥0.2 in ETDRS visual acuity test were observed in 3 of 5 subjects (60%) at Week 52 of transplantation of Nepic and 4 of 5 subjects (80%) at Week 104. Changes in visual acuity test with Landolt rings showed a similar improving trend.

7.R.3 Safety

7.R.3.1 Adverse events in EYE-01M study and EYE-01M-FU study

The applicant's explanation on the safety of Nepic:

Tables 38 to 41 show adverse events during the run-in and treatment periods in EYE-01M study and in EYE-01M-FU study. Table 42 shows adverse events by time to onset after transplantation of Nepic. Local adverse events in the eye were rated into one of 3 stages (mild, readily tolerable sign or symptom; moderate, sign or symptom interfering with activity of daily living; severe, sign or symptom precluding working or activity of daily living), and the other adverse events were rated in accordance with Common Terminology Criteria for Adverse Events (CTCAE) v4.0 (Japanese version).

Table 38. Local adverse events in the eye reported during run-in period (after tissue collection) in EYE-01M study

Adverse events (N = 10)					
Term	Total	Severity			Site
		Mild	Moderate	Severe	
All adverse events	6 (60.0)	6 (60.0)	0	0	
Serious adverse events	0	0	0	0	
Infections and infestations	1 (10.0)	1 (10.0)	0	0	
Conjunctivitis	1 (10.0)	1 (10.0)	0	0	Recipient eye
Eye disorders	6 (60.0)	6 (60.0)	0	0	
Eye pain	3 (30.0)	3 (30.0)	0	0	Donor eye for all
Foreign body sensation in eyes	3 (30.0)	3 (30.0)	0	0	Donor eye for all

Number of subjects with the event (%), Medical Dictionary for Regulatory Activities Japanese version (MedDRA/J) ver21.0

A causal relationship to tissue collection could not be ruled out for eye pain and foreign body sensation in eyes in 3 subjects each in Table 38.

Table 39. Adverse events other than local ones in the eye reported during run-in period (after tissue collection) in EYE-01M study

Adverse events (N = 10)				
Term	Total	Severity		
		Grade 1	Grade 2	Grade ≥3
All adverse events	1 (10.0)	1 (10.0)	0	0
Serious adverse events	0	0	0	0
General disorders and administration site conditions	1 (10.0)	1 (10.0)	0	0
Pyrexia	1 (10.0)	1 (10.0)	0	0

Number of subjects with the event (%), MedDRA/J ver21.0

A causal relationship to tissue collection was denied for pyrexia in Table 39.

Table 40. Local adverse events in the eye reported during the treatment period in EYE-01M study and in EYE-01M-FU study

Adverse events (N = 10)					
Term	Total	Severity			Site
		Mild	Moderate	Severe	
All adverse events	10 (100)	10 (100)	5 (50.0)	0	
Serious adverse events	0	0	0	0	
Infections and infestations	2 (20.0)	0	2 (20.0)	0	
Cellulitis orbital	1 (10.0)	0	1 (10.0)	0	Recipient eye
Conjunctivitis	1 (10.0)	0	1 (10.0)	0	Recipient eye
Eye disorders	10 (100)	10 (100)	2 (20.0)	0	
Corneal epithelium defect	7 (70.0)	7 (70.0)	1 (10.0)	0	Recipient eye for all
Punctate keratitis	6 (60.0)	6 (60.0)	0	0	Donor eye, n = 1; recipient eye, n = 6
Eye pain	5 (50.0)	5 (50.0)	0	0	Recipient eye for all
Dry eye	2 (20.0)	2 (20.0)	1 (10.0)	0	Donor eye, n = 2; recipient eye, n = 2
Corneal neovascularisation	2 (20.0)	2 (20.0)	0	0	Recipient eye for all
Cataract	1 (10.0)	1 (10.0)	0	0	Recipient eye
Conjunctival erosion	1 (10.0)	1 (10.0)	0	0	Recipient eye
Eyelid oedema	1 (10.0)	1 (10.0)	0	0	Recipient eye
Glaucoma	1 (10.0)	0	1 (10.0)	0	Donor eye
Lacrimation increased	1 (10.0)	1 (10.0)	0	0	Recipient eye
Ocular hypertension	1 (10.0)	1 (10.0)	0	0	Recipient eye
Visual acuity reduced	1 (10.0)	1 (10.0)	0	0	Recipient eye
Foreign body sensation in eyes	1 (10.0)	1 (10.0)	0	0	Recipient eye
Investigations	2 (20.0)	1 (10.0)	2 (20.0)	0	
Intraocular pressure increased	2 (20.0)	1 (10.0)	2 (20.0)	0	Recipient eye for all
Injury, poisoning and procedural complications	1 (10.0)	0	1 (10.0)	0	
Procedural pain	1 (10.0)	0	1 (10.0)	0	Recipient eye

Number of subjects with the event (%), MedDRA/J ver21.0

A causal relationship to Nepic could not be ruled out for corneal epithelium defect in 7 subjects (70.0%), punctate keratitis in 6 subjects (60.0%), corneal neovascularisation in 2 subjects (20.0%), and cellulitis orbital, conjunctivitis, conjunctival erosion, dry eye, eye pain, visual acuity reduced, foreign body sensation in eyes, intraocular pressure increased, and procedural pain in 1 subject (10.0%) each in Table 40.

Table 41. Adverse events other than local ones in the eye reported during the treatment period in EYE-01M study and in EYE-01M-FU study

Adverse events (N = 10)				
Term	Total	Severity		
		Grade 1	Grade 2	Grade ≥3
All adverse events	6 (60.0)	3 (30.0)	5 (50.0)	0
Serious adverse events	0	0	0	0
Infections and infestations	2 (20.0)	0	2 (20.0)	0
Influenza	1 (10.0)	0	1 (10.0)	0
Tinea pedis	1 (10.0)	0	1 (10.0)	0
Metabolism and nutrition disorders	1 (10.0)	0	1 (10.0)	0
Diabetes mellitus	1 (10.0)	0	1 (10.0)	0
Respiratory, thoracic and mediastinal disorders	1 (10.0)	0	1 (10.0)	0
Upper respiratory tract inflammation	1 (10.0)	0	1 (10.0)	0
Gastrointestinal disorders	2 (20.0)	2 (20.0)	0	0
Constipation	1 (10.0)	1 (10.0)	0	0
Diarrhoea	1 (10.0)	1 (10.0)	0	0
Gastric polyps	1 (10.0)	1 (10.0)	0	0
Vomiting	1 (10.0)	1 (10.0)	0	0
Skin and subcutaneous tissue disorders	2 (20.0)	2 (20.0)	0	0
Rash	2 (20.0)	2 (20.0)	0	0
Musculoskeletal and connective tissue disorders	2 (20.0)	0	2 (20.0)	0
Rheumatoid arthritis	1 (10.0)	0	1 (10.0)	0
Intervertebral disc protrusion	1 (10.0)	0	1 (10.0)	0
Renal and urinary disorders	1 (10.0)	0	1 (10.0)	0
Renal impairment	1 (10.0)	0	1 (10.0)	0
Injury, poisoning and procedural complications	2 (20.0)	1 (10.0)	1 (10.0)	0
Animal bite	1 (10.0)	0	1 (10.0)	0
Limb injury	1 (10.0)	1 (10.0)	0	0

Number of subjects with the event (%), MedDRA/J ver21.0

A causal relationship to Nepic was denied for all of the adverse events other than local events in the eye in Table 41.

Table 42. Adverse events during the treatment period in EYE-01M study and in EYE-01M-FU study by time to onset

Adverse events (N = 10)			
	Time to onset		
	Day of transplantation to Week 24	Weeks 25 to 52	Week 53 and thereafter
Local adverse events in the eye	10 (100)	5 (50.0)	5 (50.0)
Infections and infestations	1 (10.0)	1 (10.0)	0
Cellulitis orbital	1 (10.0)	0	0
Conjunctivitis	0	1 (10.0)	0
Eye disorders	10 (100)	4 (40.0)	5 (50.0)
Corneal epithelium defect	6 (60.0)	1 (10.0)	2 (20.0)
Punctate keratitis	4 (40.0)	2 (20.0)	3 (30.0)
Eye pain	5 (50.0)	0	1 (10.0)
Dry eye	2 (20.0)	0	1 (10.0)
Corneal neovascularisation	2 (20.0)	0	0
Cataract	0	1 (10.0)	0
Conjunctival erosion	1 (10.0)	0	0
Eyelid oedema	1 (10.0)	0	0
Glaucoma	1 (10.0)	0	0
Lacrimation increased	1 (10.0)	0	0
Ocular hypertension	1 (10.0)	0	0
Visual acuity reduced	1 (10.0)	0	0
Foreign body sensation in eyes	1 (10.0)	0	0
Investigations	1 (10.0)	1 (10.0)	1 (10.0)
Intraocular pressure increased	1 (10.0)	1 (10.0)	1 (10.0)
Injury, poisoning and procedural complications	1 (10.0)	0	0
Procedural pain	1 (10.0)	0	0
Adverse events other than local ones in the eye	4 (40.0)	2 (20.0)	6 (60.0)
Infections and infestations	0	1 (10.0)	1 (10.0)
Influenza	0	0	1 (10.0)
Tinea pedis	0	1 (10.0)	0
Metabolism and nutrition disorders	0	0	1 (10.0)
Diabetes mellitus	0	0	1 (10.0)
Respiratory, thoracic and mediastinal disorders	1 (10.0)	0	0
Upper respiratory tract inflammation	1 (10.0)	0	0
Gastrointestinal disorders	2 (20.0)	0	2 (20.0)
Constipation	0	0	1 (10.0)
Diarrhoea	1 (10.0)	0	0
Gastric polyps	0	0	1 (10.0)
Vomiting	1 (10.0)	0	0
Skin and subcutaneous tissue disorders	1 (10.0)	1 (10.0)	0
Rash	1 (10.0)	1 (10.0)	0
Musculoskeletal and connective tissue disorders	0	0	2 (20.0)
Rheumatoid arthritis	0	0	1 (10.0)
Intervertebral disc protrusion	0	0	1 (10.0)
Renal and urinary disorders	0	0	1 (10.0)
Renal impairment	0	0	1 (10.0)
Injury, poisoning and procedural complications	2 (20.0)	0	0
Animal bite	1 (10.0)	0	0
Limb injury	1 (10.0)	0	0

Number of subjects with the event (%), MedDRA/J ver21.0

7.R.3.2 Adverse events requiring attention

PMDA's view:

Eye pain and foreign body sensation in eyes for which a causal relationship to tissue collection could not be ruled out occurred in the donor eye. Therefore, attention should be paid to the condition in the donor eye after tissue collection. The following information should be provided to healthcare professionals using a package insert, etc.: Tissue collection for manufacture of Nepic is associated with a risk of eye pain and foreign body sensation in eyes.

PMDA also reviewed risks of "intraocular pressure increased, ocular hypertension, and glaucoma," "corneal epithelium defect and punctate keratitis," and "corneal neovascularisation" as shown below. The time to onset (Day) described in the following sections represents the number of days counted from the day of transplantation of Nepic.

7.R.3.2.1 Intraocular pressure increased, ocular hypertension, and glaucoma

Table 43 shows details of the subjects who experienced intraocular pressure increased, ocular hypertension, and glaucoma in EYE-01M study and EYE-01M-FU study.

Table 43. List of subjects with intraocular pressure increased, ocular hypertension, and glaucoma

Subject number	Age	Sex	MedDRA Preferred term	Severity	Intraocular pressure (mmHg)	Site	History of glaucoma	Time to onset (Day)	Relationship to causative etiology	Causal relationship to Nepic	Cause	Outcome
B-2	52	Female	Ocular hypertension	Mild	26.0	Recipient eye	Yes	1	No	No	Straining oneself associated with postoperative pain during intraocular pressure measurement and increased orbital pressure associated with anesthetization for operation	Resolved
			Intraocular pressure increased	Mild	31.8	Recipient eye	Yes	289	No	No	Corticosteroid eye drop	Resolved
			Intraocular pressure increased	Mild	25.5	Recipient eye	Yes	506	No	No	Corticosteroid eye drop	Resolved
			Intraocular pressure increased	Moderate	39.0	Recipient eye	Yes	549	No	No	Cataract surgery	Resolved
			Intraocular pressure increased	Mild	28.4	Recipient eye	Yes	716	No	No	Comorbidity (glaucoma)	Not resolved
B-3	83	Male	Glaucoma	Moderate	24.0	Donor eye	Yes	109	No	No	Corticosteroid eye drop	Not resolved
E-2	70	Male	Intraocular pressure increased	Moderate	51.0	Recipient eye	Yes	3	No	Yes	—	Resolved

In EYE-01M study, there was intraocular pressure increased in 1 subject for which a causal relationship to Nepic could not be ruled out. Taking into account that corticosteroid may be administered to suppress post-operative inflammation during transplantation of Nepic, attention should be paid to the onset of intraocular pressure increased, ocular hypertension, and glaucoma when the transplantation is performed. The following information should be provided to healthcare professionals using a package insert, etc.: Transplantation of Nepic is associated with a risk of intraocular pressure increased, etc.

7.R.3.2.2 Corneal epithelium defect and punctate keratitis

Tables 44 and 45 show details of the subjects who experienced corneal epithelium defect and punctate keratitis in EYE-01M study and EYE-01M-FU study.

Table 44. List of subjects with corneal epithelium defect

Subject number	Age	Sex	Severity	Site	Time to onset (Day)	Relationship to causative etiology	Causal relationship to Nepic	Outcome
A-1	20	Female	Mild	Recipient eye	7	No	Yes	Resolved
			Mild	Recipient eye	84	No	Yes	Resolved
A-3	79	Male	Mild	Recipient eye	14	No	Yes	Resolved
B-3	83	Male	Mild	Recipient eye	30	No	Yes	Resolved
C-1	37	Male	Mild	Recipient eye	26	No	Yes	Resolving
			Mild	Recipient eye	712	No	Yes	Resolved
C-2	67	Male	Mild	Recipient eye	12	No	Yes	Resolved
E-1	42	Male	Mild	Recipient eye	198	No	Yes	Resolved
			Mild	Recipient eye	338	No	Yes	Not resolved
			Moderate	Recipient eye	398	No	Yes	Resolved
E-2	70	Male	Mild	Recipient eye	30	No	Yes	Resolved
			Mild	Recipient eye	100	No	Yes	Resolved

Table 45. List of subjects with punctate keratitis

Subject number	Age	Sex	Severity	Site	Time to onset (Day)	Relationship to causative etiology	Causal relationship to Nepic	Outcome
A-1	20	Female	Mild	Recipient eye	13	No	Yes	Resolved
			Mild	Recipient eye	182	No	Yes	Resolved
			Mild	Recipient eye	559	No	Yes	Resolved
B-2	52	Female	Mild	Recipient eye	18	No	Yes	Resolving
B-3	83	Male	Mild	Recipient eye	16	No	Yes	Resolved
			Mild	Donor eye	527	No	No	Resolving
B-4	38	Male	Mild	Recipient eye	16	No	Yes	Resolved
C-2	67	Male	Mild	Recipient eye	243	No	Yes	Resolving
E-1	42	Male	Mild	Recipient eye	370	No	Yes	Not resolved

Many patients experienced corneal epithelium defect and punctate keratitis in EYE-01M study and EYE-01M-FU study, and attention should be paid to the onset of corneal epithelium defect and punctate keratitis when Nepic is transplanted. The following information should be provided to healthcare professionals using a package insert, etc.: Transplantation of Nepic is associated with a risk of corneal epithelium defect and punctate keratitis.

7.R.3.2.3 Corneal neovascularisation

Table 46 shows details of the subjects who experienced corneal neovascularisation in EYE-01M study and EYE-01M-FU study.

Table 46. List of subjects with corneal neovascularisation

Subject number	Age	Sex	Severity	Site	Time to onset (Day)	Relationship to causative etiology	Causal relationship to Nepic	Outcome
C-1	37	Male	Mild	Recipient eye	30	No	Yes	Not resolved
C-2	67	Male	Mild	Recipient eye	54	No	Yes	Not resolved

Corneal neovascularisation occurred in EYE-01M study, and relevant information should be continuously collected even after marketing of Nepic.

7.R.4 Clinical positioning

The applicant's explanation about clinical positioning of Nepic in treatment of LSCD:

The conventional procedures for corneal epithelium reconstruction in treatment of LSCD, allogeneic and autologous corneal limbal transplantations have the following problems:

- The allogeneic corneal limbal transplantation is frequently associated with immunological rejections, leading to poor prognosis, and the lack of donors limits the patients who can undergo transplantation.
- The autologous corneal limbal transplantation requires collection of a large corneal limbal tissue piece from the contralateral eye of the recipient eye and thus is highly invasive in the donor eye, and adverse events such as local corneal opacity and pseudopterygium occurred (*Cornea.* 2008;27:730-3). The concerned procedure lead to exhaustion of the corneal limbal tissue in the donor eye, potentially causing LSCD (*Invest Ophthalmol Vis Sci.* 1990;31:1301-14). Its application to a severe case is difficult. In addition, it is not applicable to bilateral LSCD.

Transplantation of Nepic, on the other hand, is superior to the allogeneic and autologous corneal limbal transplantations in terms of the following points and thus is positioned as a new option in treatment of LSCD:

- Because the patient's cells are used in the treatment, it has no risk of immunological rejections, which is a problem associated with the allogeneic corneal limbal transplantation.
- Although the procedure is invasive involving the normal corneal limbal tissue, the corneal limbal tissue piece (approximately 2 × 3 mm) collected is smaller than that required for the autologous corneal limbal transplantation and potentially achieves corneal epithelium reconstruction. Therefore, the treatment with Nepic is potentially applicable even in patients with LSCD to whom application of the autologous corneal limbal transplantation is difficult.

PMDA accepted the above applicant's explanation.

7.R.5 Indication or performance

The proposed "Indication or Performance" of Nepic was "Severe limbal stem cell deficiency is subjected to be the indication. The product is intended for use in corneal epithelium reconstruction by supplying corneal epithelial cells containing corneal epithelial stem cells."

PMDA asked the applicant to explain (a) rationale for setting the indication or performance; (b) applicability to patients with bilateral LSCD who have an affected site on the contralateral eye as well; and (c) causative etiologies in patients with LSCD potentially indicated for the proposed product.

The applicant's explanation:

(a) Rationale for setting the indication or performance

In EYE-01M study, the target success rate (%) of corneal epithelium reconstruction was achieved in patients at Stages IIB or III in severity classification, and in light of the issues in the conventional treatment [see Section 7.R.4], the applicant considered it appropriate to indicate these patients for Nepic.

The EYE-01M study included patients at Stages IIB or III in the severity classification in whom conjunctivalization involved the central cornea and affected ≥50% of the corneal limbus, but even for the following patients at Stage IIA, Nepic is potentially used as a therapeutic option: The patients

conventionally eligible for removal of conjunctival scar tissue (and amniotic membrane transplantation where necessary) due to conjunctivalization involving the central cornea but affecting <50% of the corneal limbus and have initially undergone the conventional procedure but failed corneal epithelium reconstruction.

(b) Applicability to patients with bilateral LSCD who have an affected site on the contralateral eye as well

EYE-01M study included 2 subjects (Subject numbers A-3 and B-2) in whom the corneal limbus of the contralateral eye was partially involved in conjunctivalization but had an area in a stable condition that was enough for manufacture of Nepic. Figure 4 shows representative photos of the donor eye at screening and investigator's evaluation. In the concerned 2 subjects, conjunctivalization did not extend from the collection site of the donor eye, indicating that the corneal limbal tissue can be safely collected from patients with bilateral LSCD. Therefore, such patients are potentially eligible for manufacture and transplantation of Nepic.

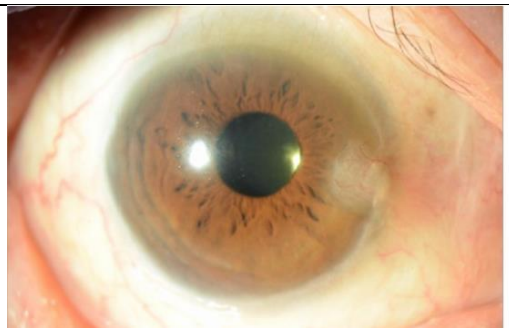
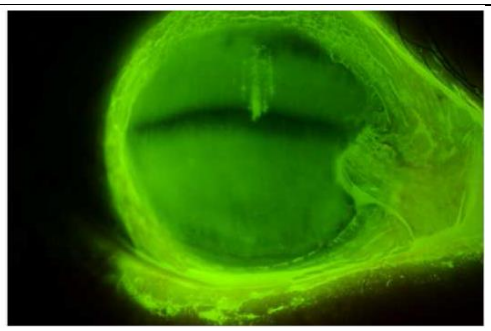
Subject number	Left, Photo taken with diffuser; Right, Photo taken with blue free filter
A-3	  (Investigator's evaluation) Pterygium partially invaded the cornea on the nasal side, but the conjunctival tissue did not involve the other area including the tissue collection site.

Figure 4. Photos of the donor eye at screening and investigator's evaluation.

A risk of additional onset of LSCD caused by extension of conjunctivalization from the tissue collection site is not completely denied. In addition, collection of the corneal limbal tissue corresponding to ≥50% of the whole corneal limbal circumference should be avoided owing to a risk of inducing LSCD (*Indian J Ophthalmol.* 2004;52:5-22). To reduce the safety risk associated with tissue collection, the following guide for appropriate tissue collection necessary should be specified: ≥50% of the corneal limbus area should remain normal (the corneal limbal tissue piece collected for manufacture of Nepic is approximately 2 × 3 mm in size, of which the area corresponds to approximately 8% of the whole corneal limbal circumference). The information materials to be distributed to medical institutions will include the risk of additional LSCD associated with tissue collection and the above guide.

(c) Causative etiologies in eligible patients

Causative etiologies of LSCD are largely classified into extrinsic (chemical injury, thermal injury, etc.) and intrinsic (SJS, ocular pemphigoid, etc.) etiology. The causative etiologies are considered to affect the appropriateness of tissue collection from the corneal limbus and manufacture of Nepic as well as the efficacy. The success rate (%) of corneal epithelium reconstruction by causative etiology at Week 52 of transplantation of Nepic in EYE-01M study (FAS) was 66.7% for chemical injury (6 patients) and 100%

each for long-term hard contact lens (HCL) use (1 patient) and "vernal keratoconjunctivitis and the treatment" (1 patient), but patients with LSCD induced by ocular pemphigoid (1 patient) and an unknown cause (1 patient) did not achieve corneal epithelium reconstruction. On the other hand, all the patients with LSCD of any cause including ones not achieving corneal epithelium reconstruction showed an improving trend in LSCD severity, and any collected tissue led to successful manufacture of Nepic, and no problematic adverse events occurred at the collection site. Thus, patients with LSCD of chronic causative etiology in which inflammation and diseases are appropriately controlled are capable of providing the tissue that leads to manufacture of Nepic, and thus such patients are considered eligible.

For the following reasons, the applicant considers patients with 1) to 3) below are eligible for manufacture and transplantation of Nepic:

1) Thermal injury

Pathological conditions of LSCD caused by thermal injury are generally similar to those of LSCD caused by chemical injury (*Today's Therapy in Ophthalmology.* Ver. 3. Igaku-Shoin Ltd; 2016:364-5). In addition, patients in whom the injury progression is stopped by infection prophylaxis and anti-inflammatory measures after removal of the heat source are capable of providing the tissue that leads to manufacture of Nepic. The efficacy is therefore expected in such patients.

2) SJS of known cause

SJS can be treated at an early phase if the cause such as drug or infection is identified, and thus the damage on the corneal limbus associated with SJS is transient. Furthermore, outcomes of treatment such as cataract surgery are favorable even in patients with SJS (*Ophthalmology*. 2000;107:1518-23, *J Cataract Refract Surg*. 2005;31:860-2, etc.). Invasion associated with the tissue collection and transplantation is therefore considered controllable. Patients with chronic SJS of known cause are capable of providing the tissue that leads to manufacture of Nepic. The efficacy is therefore expected in such patients.

3) Traumatic injury and infection

In patients with LSCD induced by extrinsic causes such as traumatic injury due to long-term use of contact lens and infection such as Acanthamoeba keratitis in whom the cause is removed by treatment and the corneal limbus and affected eye are in a stable condition, collection of the corneal limbal tissue and transplantation of Nepic are considered possible.

The applicant, on the other hand, considers patients with causative injury of 4) to 7) below are ineligible for manufacture and transplantation of Nepic:

4) Ocular pemphigoid

EYE-01M study included 1 patient with LSCD caused by ocular pemphigoid. Although corneal epithelium reconstruction was not achieved, the autologous cell sheet was expected to be effective in terms of LSCD severity and extent of corneal opacity, and the safety was confirmed. The cause of ocular pemphigoid, however, remained unknown, and a possibility of the damage on the corneal limbus being continued by an unknown cause could not be denied, even if appropriate treatment was provided. Patients with LSCD caused by ocular pemphigoid therefore may not provide the quality corneal limbal tissue, a raw material of Nepic, and thus these are deemed ineligible.

5) Aniridia

Aniridia is a genetic disease caused by *pax6* gene mutation and characterized by bilaterally dysfunctional corneal limbus and consequent loss of corneal epithelial stem cells. Accordingly, it is considered difficult to manufacture Nepic from the patient's tissue, and thus patients with aniridia are deemed ineligible.

6) SJS of unknown cause

A possibility of the damage on the corneal limbus being continued by an unknown cause cannot be ruled out, and patients with LSCD caused by SJS of an unknown cause therefore may not provide the quality corneal limbal tissue, a raw material of the Nepic, and thus these are deemed ineligible.

7) Recurrent pterygium

Recurrent pterygium is reported to develop in association with ultraviolet rays and also deemed as an abnormally grown subconjunctival tissue in response to a lesion at the border between the cornea and conjunctiva induced by dust or long-term mechanical stimulation. Growth activity of the pterygium is considered to be caused by intrinsic factors in the Tenon's capsule (*Practical Ophthalmology 3 Ocular Surfaces.* Bunkodo Co., Ltd.; 2005, etc.). In light of this report, transplantation of Nepic in such patients is unlikely to have favorable outcome because a risk of recurrent pterygium would remain. Patients with recurrent pterygium are therefore deemed ineligible.

PMDA's view:

PMDA accepts the above applicant's explanation to the effect that Nepic is recommended for patients with LSCD at Stage IIB or III in the severity classification in EYE-01M study. In addition, it is meaningful to provide Nepic as a new therapeutic option to patients at Stage IIA in the severity classification who have undergone removal of conjunctival scar tissue (amniotic membrane transplantation where necessary) but failed corneal epithelium reconstruction, in light of the issues in the conventional treatment [see Section 7.R.4] and the nature of LSCD, which is a serious disease adversely affecting activity of daily living (ADL).

In patients with bilateral LSCD, experience with the transplantation of Nepic is limited, but 2 patients provided the tissue leading to successful manufacture of Nepic, and for the efficacy, 1 patient (Subject number B-2) achieved corneal epithelium reconstruction. For the safety, no particular problems have been observed. Accordingly, even patients with bilateral LSCD are considered potentially eligible for manufacture and transplantation of Nepic as long as they are capable of providing the corneal limbal tissue necessary for preparation of Nepic. Experience with collection of the corneal limbal tissue from patients with bilateral LSCD, however, is considerably limited, and the tissue collection has a risk of extending the conjunctivalization lesion in the donor eye. For application of Nepic to patients with bilateral LSCD, therefore, the following caution statement should be included in the "Precautions Concerning Indication or Performance" section: Eligible patients must be carefully selected by physicians with a full understanding of the information about patient characteristics, such as donor eye conditions of the patients enrolled in clinical studies, presented in the "Clinical Studies" section in the package insert. The applicant proposed the guide for appropriate tissue collection requiring ≥50% of the corneal limbus area to remain normal, but the rationale for setting this threshold is weak at present. Thus, the concerned guide should not be included in the information materials to be distributed to medical institutions, and information about the donor eye condition at screening of the patients with bilateral LSCD enrolled in the clinical studies should be provided to medical institutions using photographs, etc.

On the assumption that inflammation and infections associated with causative etiology of LSCD are well-controlled, the above applicant's explanations 1) to 7) except for 2) are acceptable. Concerning SJS of known cause in 2), a case report showed that SJS at a chronic phase, which seemed stable, was aggregated over years (Japan Cornea Conference 2018). In light of this report, PMDA considered it unacceptable to indicate patients with SJS for Nepic because there is no experience with manufacture of Nepic from a tissue of such patients, and thus the efficacy and safety of Nepic are unknown. In addition, with reference to exclusion of aniridia from the indication in 5), patients with a congenital disease other than aniridia, which leads to dysgenesis of corneal epithelial stem cells, are also considered ineligible for manufacture and transplantation of Nepic because it is difficult to manufacture Nepic. Furthermore, patients with idiopathic LSCD are considered ineligible for manufacture and transplantation of Nepic for the following reasons: 1 patient with idiopathic LSCD (Subject number A-3) was enrolled in the clinical study but did not achieve corneal epithelium reconstruction, and the background leading to LSCD remained unknown; and the concerned pathological condition may affect the ability of the corneal limbal tissue used as the raw material to produce Nepic as well as the efficacy and safety of Nepic including the donor eye.

In addition to the above, "severe" specified in the proposed "Indication or Performance" section is not clearly defined in clinical settings, and PMDA considers that the "Indication or Performance" should be limbal stem cell deficiency with a specific description of the eligible patients to clarify the indication of Nepic.

Accordingly, the "Indication or Performance" and "Precautions Concerning Indication or Performance" sections of Nepic should be established as shown below.

Indication or Performance

Limbal stem cell deficiency. The following patients, however, will be excluded:

- Patients with Stevens-Johnson syndrome
- Patients with ocular pemphigoid
- Patients with aniridia or other congenital corneal epithelial stem cell dysplasia
- Patients with recurrent pterygium
- Patients with idiopathic limbal stem cell deficiency

Precautions Concerning Indication or Performance

- Nepic should be used in the following patients:
  "In the affected eye, <50% of the corneal limbus remains normal, and conjunctivalization extends to an area within 5 mm in diameter including the central cornea in the affected eye" or "removal of conjunctival scar tissue in the affected eye (amniotic membrane transplantation where necessary) is not effective, and conjunctivalization extends to an area within 5 mm in diameter including the central cornea in the affected eye."
- Because Nepic is not intended to treat any cause of limbal stem cell deficiency, Nepic should be used after the causative disease of limbal stem cell deficiency is controlled or the cause is removed.
- Experience with collection of the corneal limbal tissue from patients with bilateral limbal stem cell deficiency is considerably limited, and the tissue collection has a risk of extending the conjunctivalization lesion in the donor eye. For application of Nepic to patients with bilateral limbal

stem cell deficiency, eligible patients must be carefully selected by physicians with a full understanding of the information about patient characteristics, such as donor eye conditions of the patients enrolled in clinical studies, presented in the "Clinical Studies" section.

7.R.6 Dosage and administration or method of use

The proposed "Dosage and Administration or Method of Use" and "Precautions Concerning Dosage and Administration or Method of Use" of Nepic were established based on EYE-01M study as shown below.

Dosage and Administration or Method of Use

Operations performed before manufacture of corneal epithelial cell sheet

1. Corneal limbal tissue is collected from the patient. An area appropriate for the tissue collection is selected in view of the corneal limbus condition. The collected corneal limbal tissue is placed in a tissue transport tube and sent to Japan Tissue Engineering Co., Ltd.
2. Blood is collected in accordance with a conventional procedure. The collected blood is placed in a blood storage tube and sent to Japan Tissue Engineering Co., Ltd. This blood specimen is used as the reserve sample.

Operations in transplantation of corneal epithelial cell sheet

The corneal epithelial cell sheet is rinsed with a pre-treatment fluid filled in a pre-treatment fluid bottle, immersed in this fluid, detached with a ring-shaped culture disk from the corneal epithelium culture dish, and transplanted onto the eye surface including the corneal limbus region.

Precautions Concerning Dosage and Administration or Method of Use

Precautions before manufacture of corneal epithelial cell sheet

1. The transplantation plan including scheduled dates of tissue collection and transplantation should be developed using the specified format sent from Japan Tissue Engineering Co., Ltd.
2. It should be confirmed that the tissue transport tube and blood storage tube are containers dedicated to the patient by checking the labels before tissue collection.
3. Collection of the corneal limbal tissue and its immersion into the tissue transportation fluid should be performed in a clean environment.
4. The corneal limbal tissue piece that is intact and includes the basal lamina should be collected.
5. Alternative treatment should be considered in advance because the cultured corneal epithelium package may not be released or the transplanted corneal epithelial cell sheet may not survive.[13)]

Precautions during corneal epithelial cell sheet transplantation

1. It should be confirmed that the transportation container is sealed at the delivery of the cultured corneal epithelium package. If the seal is broken, the package should not be opened, and Japan Tissue Engineering Co., Ltd. should be contacted.
2. To prevent mix-up, it should be confirmed that the corneal epithelial cell sheet to be transplanted is dedicated to the patient by checking the label on the cultured corneal epithelium package.
3. The cultured corneal epithelium package should be stored in a transportation container or at 20°C to 28°C until just before use.

[13)] The concerned matter was added after the application.

4. To protect from drying, the corneal epithelial cell sheet should be kept immersed in the pre-treatment fluid in the culture dish for cultured corneal epithelium until just before transplantation.
5. If symblepharon may occur, an appropriate procedure should be performed before transplantation.
6. If the conjunctival scar tissue may be observed on the eye surface, it should be removed from the eye surface wherever possible before transplantation because it may interfere with survival of the corneal epithelial cell sheet.
7. Any fluid should be removed from the eye surface before placing the corneal epithelial cell sheet.
8. The corneal epithelial cell sheet should be placed on the eye surface in the right-side-up position, preventing the sheet from being placed upside-down.
9. Onto the corneal epithelial cell sheet placed on the eye surface, an intraocular perfusate should be slowly dropped to protect it from drying.
10. The corneal epithelial cell sheet should be released from a ring-shaped culture disk by applying a knife to the internal circumference of the ring.
11. The rim of the corneal epithelial cell sheet should be sutured where necessary.
12. After the transplantation, the therapeutic soft contact lens should be applied and tarsorrhaphy should be performed where necessary.

PMDA asked the applicant to explain how to determine the collection site of the corneal limbal tissue.

The applicant's response:
After marketing of Nepic, patients at Stage IIB or III, corresponding to the patient population of EYE-01M study, will undergo a procedure for collecting the normal corneal limbal tissue specified in EYE-01M study (approximately 2 × 3 mm piece of the corneal limbal tissue that is determined to be free from abnormalities such as inflammation and infection by the investigator or sub-investigator under slit lamp microscope should be collected from the contralateral eye [donor eye] of the recipient eye in the patient).

Unlike patients at Stage IIB or III, patients at Stage IIA in the severity classification who have undergone removal of conjunctival scar tissue (amniotic membrane transplantation where necessary) but failed corneal epithelium reconstruction, the corneal limbal tissue may be collected from the affected eye, not limited to the contralateral eye, for the following reasons:

- Patients at Stage IIA have the affected eye in which ≥50% of the total corneal limbus remains normal, and collection of the corneal limbal tissue from an area that is confirmed to be free from inflammation and infection even in the affected eye under slit lamp microscope is unlikely to pose a high risk.

Although the tissue collection was performed at the 12 o'clock position in most of the patients in EYE-01M study, such a position is not essential, and thus it is considered unnecessary to specify the direction of the procedure for collecting the corneal limbal tissue in the "Dosage and Administration or Method of Use" section of Nepic.

PMDA's view:
The "Dosage and Administration or Method of Use" may be established based on the conditions in EYE-01M study, which demonstrated clinical usefulness of Nepic.

PMDA considers that the collection of corneal limbal tissue from the affected eye is inappropriate for patients at Stage IIA in the severity classification who have undergone removal of conjunctival scar tissue (amniotic membrane transplantation where necessary) but failed corneal epithelium reconstruction, because Nepic has not been manufactured from the tissue collected from the affected eye, and the efficacy and safety of Nepic remain unknown, including invasion associated with the tissue collection from the eye that has undergone procedures such as removal of conjunctival scar tissue. Therefore, the "Dosage and Administration or Method of Use" section should specify that the tissue be collected from the contralateral eye.

The above applicant's explanation on how to determine the collection site of the corneal limbal tissue is largely understandable. However, the corneal limbal tissue was collected at the 12 o'clock position in all the subjects in EYE-01M study except for 1 subject with a particular reason, and thus it remains unclear whether the corneal limbal tissue collected from the other area than the 12 o'clock position has an ability to produce Nepic successfully. PMDA considers it necessary to include information about the collection site of the corneal limbal tissue in the clinical study in the "Clinical Studies" section and the following statement in the "Precautions Concerning Dosage and Administration or Method of Use" section of the package insert of Nepic: The collection site of the corneal limbal tissue should be determined based on the collection site in the clinical study.

In addition to the above, the following points should be clarified in the "Dosage and Administration or Method of Use" section but not in the "Precautions Concerning Dosage and Administration or Method of Use" section because they are important information for transplantation of Nepic:

- The corneal limbal tissue should be collected from the area of the donor eye that is confirmed to be free from inflammation and infection and to have no sign of conjunctivalization.
- Conjunctival scar tissue should be removed from the eye surface wherever possible and transplantation of Nepic should be performed.
- The rim of the corneal epithelial cell sheet should be sutured where necessary. After the transplantation of Nepic, the therapeutic soft contact lens should be applied. In addition, tarsorrhaphy should be performed where necessary.

PMDA concluded that the "Dosage and Administration or Method of Use" and "Precautions Concerning Dosage and Administration or Method of Use" sections should be specified as shown below.

Dosage and Administration or Method of Use

Operations in manufacture of corneal epithelial cell sheet

1. An area in the patient's donor eye (contralateral eye of the eye planned to receive the product as a graft or the recipient eye) is confirmed to be free from inflammation and infection and approximately 2 × 3 mm piece of the corneal limbal tissue in area that have no sign of conjunctivalization is collected. The collected corneal limbal tissue is placed in a tissue transport tube and sent to the manufacturer.
2. Blood is collected in accordance with a conventional procedure. The collected blood is placed in a blood storage tube and sent to the manufacturer. This blood specimen is used as the reserve sample.

Operations in transplantation of corneal epithelial cell sheet

The corneal epithelial cell sheet is rinsed with a pre-treatment fluid and immersed in this fluid, and detached with a ring-shaped culture disk from the corneal epithelium culture dish. Conjunctival scar tissue is removed from the eye surface of the patient wherever possible and the graft is transplanted onto the eye surface including the corneal limbus. The rim of the corneal epithelial cell sheet is sutured where necessary. After the transplantation, the therapeutic soft contact lens is applied. In addition, tarsorrhaphy is performed where necessary.

Precautions Concerning Dosage and Administration or Method of Use

Precautions during manufacture of corneal epithelial cell sheet

1. The transplantation plan including scheduled dates of tissue collection and transplantation should be developed using the specified format sent from Japan Tissue Engineering Co., Ltd.
2. It should be confirmed that the tissue transport tube and blood storage tube are containers dedicated to the patient by checking the labels before tissue collection.
3. Collection of the corneal limbal tissue and its immersion into the tissue transportation fluid should be performed in a clean environment.
4. The corneal limbal tissue piece that is intact and includes the basal lamina should be collected.
5. The collection site of the corneal limbal tissue should be determined based on the "Clinical Studies" section.
6. Alternative treatment should be considered in advance because the cultured corneal epithelium package may not be released or the transplanted corneal epithelial cell sheet may not survive.

Precautions during corneal epithelial cell sheet transplantation

1. It should be confirmed that the transportation container is sealed at the delivery of the cultured corneal epithelium package. If the seal is broken, the package should not be opened, and Japan Tissue Engineering Co., Ltd. should be contacted.
2. To prevent mix-up, it should be confirmed that the corneal epithelial cell sheet to be transplanted is dedicated to the patient by checking the label on the cultured corneal epithelium package.
3. The cultured corneal epithelium package should be stored in a transportation container or at 20°C to 28°C until just before use.
4. To protect from drying, the corneal epithelial cell sheet should be kept immersed in the pre-treatment fluid in the culture dish for cultured corneal epithelium until just before transplantation.
5. If symblepharon may occur, an appropriate procedure should be performed before transplantation.
6. Any fluid should be removed from the eye surface before placing the corneal epithelial cell sheet.
7. The corneal epithelial cell sheet should be placed on the eye surface in the right-side-up position, preventing the sheet from being placed upside-down.
8. The corneal epithelial cell sheet should be released from a ring-shaped culture disk by applying a knife to the internal circumference of the ring.
9. Onto the corneal epithelial cell sheet placed on the eye surface, an intraocular perfusate should be slowly dropped to protect it from drying.

8. Data Relating to Risk Analysis and Outline of the Review Conducted by PMDA

The applicant's explanation about a post-marketing surveillance plan of Nepic:

Clinical experience with Nepic is very limited, and the safety information about Nepic has not been adequately collected. The applicant therefore plans a post-marketing surveillance to evaluate the safety and efficacy of Nepic in all patients treated with Nepic in post-marketing clinical practice.

The safety specification of this surveillance is to collect all the adverse events associated with use of Nepic.

The sample size for the surveillance is planned to be 40 patients per year in light of the expected number of patients receiving Nepic after marketing.

The follow-up period was specified as a period from the tissue collection for manufacture of Nepic to Week 52 of transplantation in light of a report on transplantation of autologous cultured corneal epithelium in patients with LSCD (*N Engl J Med*. 2010;363:147-155) showing that if the corneal epithelium remains stable for 1 year after transplantation, the effectiveness will be continued thereafter.

PMDA's view:
Because clinical experience with Nepic is very limited, a post-marketing surveillance needs to be conducted in all patients treated with Nepic to collect information about the safety and efficacy of Nepic after marketing in a prompt and unbiased manner. PMDA accepted the above applicant's explanation on the surveillance plan (safety specification, planned sample size for the surveillance, and follow-up period).

The post-marketing surveillance should collect information that includes the causative etiology, the eye surface condition of the donor eye, collection site of the corneal limbal tissue, adverse events occurring in the donor eye, and result of manufacture of Nepic. Information about appropriate tissue collection for manufacture of Nepic, if additionally available, should be provided to healthcare professionals in an appropriate and prompt manner.

9. Results of Compliance Assessment Concerning the New Regenerative Medical Product Application Data and Conclusion Reached by PMDA

9.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The new regenerative medical product application data were subjected to a document-based compliance inspection and a data integrity assessment in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. On the basis of the inspection and assessment, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

9.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The new regenerative medical product application data (7-1, 7-2) were subjected to an on-site GCP inspection,[14)] in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. On the basis of the inspection, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

10. Overall Evaluation during Preparation of the Review Report (1)

On the basis of the data submitted, PMDA has concluded that Nepic has a certain level of efficacy in the treatment of "limbal stem cell deficiency," and that Nepic has acceptable safety in view of its benefits. Nepic is clinically meaningful because it provides a new treatment option for patients with LSCD.

PMDA has concluded that Nepic may be approved if Nepic is not considered to have any particular problems based on comments from the Expert Discussion.

[14)] Although an application of Nepic was submitted under a category of regenerative medical products, to the clinical study conducted before enforcement of the GCP Ordinance for regenerative medical products, from which data are included in 7-1, the GCP Ordinance for medical devices was applied.

Review Report (2)

February 7, 2020

Product Submitted for Approval

Brand Name	Nepic
Non-proprietary Name	Human (autologous) corneal limbus-derived corneal epithelial cell sheet
Applicant	Japan Tissue Engineering Co., Ltd.
Date of Application	March 20, 2019

List of Abbreviations
See Appendix.

1. Content of the Review

Comments made during the Expert Discussion and the subsequent review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized below. The expert advisors present during the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for marketing approval, in accordance with the provisions of the Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

1.1 Efficacy

As a result of the review in Section "7.R.2 Efficacy" of the Review Report (1), PMDA has concluded that Nepic is shown to demonstrate a certain level of efficacy in the treatment of LSCD.

The above conclusion of PMDA was supported by the expert advisors at the Expert Discussion. The following comments were raised from the expert advisors:

- In Subject number B-2, the visual acuity was decreased soon after transplantation of Nepic (Week 2 of transplantation) and not improved until the cataract surgery (Week 79 of transplantation). Even after the surgery, the visual acuity remained at a similar level to that at screening. It is important to discuss reasons why the severity of LSCD was improved to Stage IA, but the visual acuity was not improved as well as the efficacy and safety of Nepic in the concerned subject.
- Because the LSCD severity rating results differed between the investigator and data monitoring committee, it is important to consider measures to standardize the severity rating for the post-marketing surveillance.

PMDA asked the applicant to explain the above comments raised by the expert advisors.

The applicant's responses:

- Changes in visual acuity as well as the efficacy and safety of Nepic in Subject number B-2 are considered as follows:
  - ✓ The decrease in visual acuity from the screening to Week 2 of transplantation was potentially caused by existing roughness of the corneal surface in this subject. The tear fluid distribution on such a rough surface would affect light refraction, leading to the change in visual acuity. In addition, superficial punctate keratopathy, lacrimation, photophobia, and eye pain occurred at Week 2 of transplantation. These multiple factors are considered to have led to the temporal decrease in visual acuity soon after the transplantation.
  - ✓ The visual acuity was further decreased owing to cataract progression at Week 24 of transplantation, and the decrease was continued even thereafter. At Week 79 of transplantation, cataract surgery was performed, but with the effect of the rough corneal surface, the visual acuity remained at a similar level to that at screening.
  - ✓ As described above, the cataract progression and rough corneal surface prevented the visual acuity improvement as intended throughout the period of EYE-01M study and EYE-01M-FU study. However, the efficacy and safety of Nepic was shown in this subject because the LSCD severity was rated as Stage IA at both Weeks 52 and 104 of transplantation, and no problematic adverse events occurred in either EYE-01M study or EYE-01M-FU study.

- To standardize the LSCD severity rating, the following actions are planned:
  - ✓ In the post-marketing surveillance, the data monitoring committee does not conduct the severity rating. The physician using Nepic will rate the LSCD severity by the following procedure: The affected eye should be monitored over time based on the past medical record and observed in detail by applying slit light from various angles to the anterior segment extensively under slit lamp microscope.
  - ✓ To reduce variations in severity rating among physicians, a seminar will be held for physicians using Nepic. The seminar will cover points to be noted in slit lamp microscopy for judging conjunctivalization and training on the procedure for LSCD severity rating to ensure the standardization.

PMDA's view:

Although information about changes in visual acuity in Subject number B-2 obtained in the clinical studies is limited, the applicant's explanation is understandable to some extent. PMDA accepted the applicant's explanation on the efficacy and safety of Nepic in the concerned subject.

In addition to the above, PMDA accepted the applicant's explanation on the following measures for the post-marketing surveillance: Physicians using Nepic will rate the LSCD severity; and the seminar including training of the procedure for LSCD severity rating to ensure its standardization will be held for the user physicians. The standardization of LSCD severity rating is highly critical in determining whether the patient is eligible for manufacture and transplantation of Nepic and evaluating the efficacy. Nepic should be therefore used by physicians with adequate knowledge and experience in treating LSCD who have attended the seminar on the usage of Nepic and LSCD severity rating.

1.2 Safety

As a result of the review in Section "7.R.3 Safety" of the Review Report (1), PMDA has concluded that adverse events requiring special attention during use of Nepic are "eye pain and foreign body sensation in eyes" (events on the donor eye associated with tissue collection) as well as "intraocular pressure increased, ocular hypertension, and glaucoma," "corneal epithelium defect and punctate keratitis," and "corneal neovascularisation."

The above conclusion of PMDA was supported by the expert advisors at the Expert Discussion.

1.3 Clinical positioning and indication or performance

As a result of the review in Sections "7.R.4 Clinical positioning" and "7.R.5 Indication or performance" of the Review Report (1), PMDA has concluded that the "Indication or Performance" and "Precautions Concerning Indication or Performance" should be specified as shown below.

Indication or Performance

Limbal stem cell deficiency. The following patients, however, will be excluded:

- Patients with Stevens-Johnson syndrome
- Patients with ocular pemphigoid
- Patients with aniridia or other congenital corneal epithelial stem cell dysplasia
- Patients with recurrent pterygium
- Patients with idiopathic limbal stem cell deficiency

Precautions Concerning Indication or Performance

- Nepic should be used in the following patients:
  "In the affected eye, <50% of the corneal limbus remains normal, and conjunctivalization extends to an area within 5 mm in diameter including the central cornea in the affected eye" or "removal of conjunctival scar tissue in the affected eye (amniotic membrane transplantation where necessary) is not effective, and conjunctivalization extends to an area within 5 mm in diameter including the central cornea in the affected eye."
- Because Nepic is not intended to treat any cause of limbal stem cell deficiency, Nepic should be used after the causative disease of limbal stem cell deficiency is controlled or the cause is removed.
- Experience with collection of the corneal limbal tissue from patients with bilateral limbal stem cell deficiency is considerably limited, and the tissue collection has a risk of extending the conjunctivalization lesion in the donor eye. For application of Nepic to patients with bilateral limbal stem cell deficiency, eligible patients must be carefully selected by physicians with a full understanding of the information about patient characteristics, such as donor eye conditions of the patients enrolled in clinical studies, presented in the "Clinical Studies" section.

In response to the above, the following comments were raised from the expert advisors. The expert advisors supported conclusion of PMDA on the other matters.

- In light of the following points, SJS may be included in the indication of Nepic:
  - ✓ In patients with SJS, if the corneal limbus is not damaged at an acute phase, LSCD may not develop, or in some of these patients, the corneal limbus in one eye may remain intact without

conjunctivalization (monocular LSCD). If the extent of corneal damage differs between left and right eyes, cells to be used as a raw material of Nepic may be collected from the eye with milder severity.

- ✓ Ophthalmologists specialized in cornea can readily discriminate between the intact eye without conjunctivalization and the damaged one.
- ✓ It is clinically significant to provide a therapeutic option to patients with SJS, of which effective treatment methods are not available.

- In patients with SJS, however, collection of the corneal limbal tissue has a risk of aggravating the donor eye condition owing to the invasive procedure. Use of Nepic is still considered acceptable on the assumption that eligible patients must be carefully selected by the physician and the patients should be adequately informed of this risk.
- The report at the "Japan Cornea Conference 2018" PMDA cited in Section 7.R.5 of the Review Report (1) has not been published as an article, it cannot serve as an appropriate ground for the conclusion that patients with SJS are not eligible for manufacture and transplantation of Nepic.
- Although graft-versus-host disease (GVHD) may induce LSCD, an investigation is required to determine as to whether it may be included in the indication.

PMDA's view:

(a) SJS

PMDA understands the comments that cells to be used as a raw material of Nepic may be collected from the mildly affected eye; and it is clinically significant to provide a therapeutic option to patients with SJS, of which effective treatment methods are not available. In light of the following points, on the other hand, it is difficult to include SJS in the indication of Nepic from a risk-benefit assessment, which indicates that collection of the corneal limbal tissue has a risk of aggravating the donor eye condition and experience with manufacture and use of Nepic in the clinical studies did not involve patients with SJS.

- Although consensus about the mechanism of development of SJS has not been established, SJS is inferred to develop as immunological changes triggered by drugs, mycoplasma infection, viral infection, etc. (*Journal of Japanese Ophthalmological Society.* 2017;121:42-86). Further careful consideration should be given to the risk of aggravating the donor eye condition because in patients with LSCD intrinsically caused by SJS, the invasive procedure for tissue collection has a risk of inducing inflammation, and patients with SJS in whom the cornea damage differs in severity between the left and right eyes live relying on the visual acuity of the eye with milder severity.
- For the natural course of the eyes in patients with SJS, some patients who had initially had the intact cornea free from inflammation and progressive conjunctival scar formation later had the aggravated eye surface at the chronic phase (*Br J Ophthalmol.* 2007;91:1048-53).
- Although the criteria for the donor eye that allows safe tissue collection in patients with SJS were discussed, information is not enough to establish the criteria.

PMDA explained the above to the expert advisers, and the expert advisers supported the following PMDA's conclusion: It is difficult to include SJS in the indication of Nepic from a risk-benefit viewpoint because the criteria for the donor eye that allow safe tissue collection cannot be established, and the efficacy and safety remain unclear owing to a lack of experience with SJS in manufacture of Nepic.

(b) GVHD

For the following reasons, PMDA has concluded that patients with GVHD are not eligible for manufacture and transplantation of Nepic:

- In patients with ocular GVHD, fibrogenesis develops in the corneal limbus region in response to inflammation, adversely affecting corneal epithelial stem cells, and dry eye induces the chronic inflammatory process (*Cornea.* 2019;83:364-75).
- Of patients with ocular GVHD who had undergone cataract surgery, multiple patients experienced corneal perforation, corneal melt, etc. (*Cornea.* 2015;34:506-11, *J Cataract Refract Surg.* 2016;42:833-9)
- Comorbid dry eye, meibomian gland dysfunction, etc. have a risk of adversely affecting survival of the transplanted Nepic.

The above conclusion of PMDA was supported by the expert advisors.

PMDA has concluded that in the "Precautions Concerning Indication or Performance" section, descriptions about severity of patients with LSCD eligible for manufacture and transplantation of Nepic should be modified, as described below, in accordance with definitions of LSCD severity classification used in EYE-01M study and EYE-01M-FU study. The PMDA's conclusion was supported by the expert advisors.

- "In the affected eye, conjunctivalization involves ≥50% of the total corneal limbus area and extends to an area within 5 mm in diameter including the central cornea in the affected eye" or "removal of conjunctival scar tissue in the affected eye (amniotic membrane transplantation where necessary) is not effective, and conjunctivalization extends to an area within 5 mm in diameter including the central cornea in the affected eye."

On the basis of the above, the "Indication or Performance" and "Precautions Concerning Indication or Performance" sections of Nepic should be established as shown below.

Indication or Performance

Limbal stem cell deficiency. The following patients, however, will be excluded:

- Patients with Stevens-Johnson syndrome
- Patients with ocular pemphigoid
- Patients with graft versus host disease
- Patients with aniridia or other congenital corneal epithelial stem cell dysplasia
- Patients with recurrent pterygium
- Patients with idiopathic limbal stem cell deficiency

Precautions Concerning Indication or Performance

- Nepic should be used in the following patients.
  "In the affected eye, conjunctivalization involves ≥50% of the total corneal limbus area and extends to an area within 5 mm in diameter including the central cornea in the affected eye" or "removal of conjunctival scar tissue in the affected eye (amniotic membrane transplantation where necessary) is not effective, and conjunctivalization extends to an area within 5 mm in diameter including the central cornea in the affected eye."

- Because Nepic is not intended to treat any cause of limbal stem cell deficiency, Nepic should be used after the causative disease of limbal stem cell deficiency is controlled or the cause is removed.
- Experience with collection of the corneal limbal tissue from patients with bilateral limbal stem cell deficiency is considerably limited, and the tissue collection has a risk of extending the conjunctivalization lesion in the donor eye. For application of Nepic to patients with bilateral limbal stem cell deficiency, eligible patients must be carefully selected by physicians with a full understanding of the information about patient characteristics, such as donor eye conditions of the patients enrolled in clinical studies, presented in the "Clinical Studies" section.

PMDA asked the applicant to establish the "Indication or Performance" and "Precautions Concerning Indication or Performance" sections as described above. The applicant responded appropriately, and PMDA accepted.

1.4 Dosage and administration or method of use

As a result of the review in Section "7.R.6 Dosage and administration or method of use" of the Review Report (1), PMDA has concluded that the "Dosage and Administration or Method of Use" and "Precautions Concerning Dosage and Administration or Method of Use" sections should be as specified in the corresponding sections of the Review Report (1).

The above conclusion of PMDA was supported by the expert advisors at the Expert Discussion, and PMDA has concluded that the sections should be modified as shown below.

Dosage and Administration or Method of Use

Operations in manufacture of corneal epithelial cell sheet

1. An area in the patient's donor eye (contralateral eye of the eye planned to receive the product as a graft or the recipient eye) is confirmed to be free from inflammation and infection and approximately 2 × 3 mm piece of the corneal limbal tissue in area that have no sign of conjunctivalization is collected. The collected corneal limbal tissue is placed in a tissue transport tube and sent to the manufacturer.
2. Blood is collected in accordance with a conventional procedure. The collected blood is placed in a blood storage tube and sent to the manufacturer. This blood specimen is used as the reserve sample.

Operations in transplantation of corneal epithelial cell sheet

The corneal epithelial cell sheet is rinsed with a pre-treatment fluid and immersed in this fluid, and detached with a ring-shaped culture disk from the corneal epithelium culture dish. Conjunctival scar tissue is removed from the eye surface of the patient wherever possible and the corneal epithelial cell sheet is transplanted onto the eye surface including the corneal limbus. The rim of the corneal epithelial cell sheet is sutured where necessary. After the transplantation, the therapeutic contact lens is applied and tarsorrhaphy is performed where necessary.

Precautions Concerning Dosage and Administration or Method of Use

Precautions during manufacture of corneal epithelial cell sheet

1. The transplantation plan including scheduled dates of tissue collection and transplantation should be developed using the specified format designated by the marketing authorization holder.

2. It should be confirmed that the tissue transport tube and blood storage tube are containers dedicated to the patient by checking the labels before tissue collection.
3. Collection of the corneal limbal tissue and its immersion into the tissue transportation fluid should be performed in a clean environment.
4. The corneal limbal tissue piece that is intact and includes the basal lamina should be collected.
5. The collection site of the corneal limbal tissue should be determined based on the "Clinical Studies" section.
6. Alternative treatment should be considered in advance because the cultured corneal epithelium package may not be released or the transplanted corneal epithelial cell sheet may not survive.

Precautions during corneal epithelial cell sheet transplantation

1. It should be confirmed that the transportation container is sealed at the delivery of the cultured corneal epithelium package. If the seal is broken, the package should not be opened, and the marketing authorization holder should be contacted.
2. To prevent mix-up, it should be confirmed that the corneal epithelial cell sheet to be transplanted is dedicated to the patient by checking the label on the cultured corneal epithelium package.
3. The cultured corneal epithelium package should be stored in a transportation container or at 20°C to 28°C until just before use.
4. To protect from drying, the corneal epithelial cell sheet should be kept immersed in the pre-treatment fluid in the culture dish for cultured corneal epithelium until just before transplantation.
5. If symblepharon may occur, an appropriate procedure should be performed before transplantation.
6. Any fluid should be removed from the eye surface before placing the corneal epithelial cell sheet.
7. The corneal epithelial cell sheet should be placed on the eye surface in the right-side-up position, preventing the sheet from being placed upside-down.
8. Onto the corneal epithelial cell sheet placed on the eye surface, an intraocular perfusate should be slowly dropped to protect it from drying.
9. The corneal epithelial cell sheet should be released from a ring-shaped culture disk by applying a knife to the internal circumference of the ring.

PMDA asked the applicant to revise the "Dosage and Administration or Method of Use" and "Precautions Concerning Dosage and Administration or Method of Use" sections as described above. The applicant responded appropriately, and PMDA accepted.

1.5 Post-marketing surveillance plan (draft)

In the present application, the applicant proposed a plan of post-marketing surveillance covering all patients treated with Nepic to evaluate the safety of Nepic in post-marketing clinical practice. The planned sample size was 40 patients per year, and the observation period was from the tissue collection for manufacture of Nepic to Week 52 of transplantation.

As a result of the review in Section "8. Data Relating to Risk Analysis and Outline of the Review Conducted by PMDA" of the Review Report (1), PMDA has concluded that the post-marketing surveillance plan is acceptable as developed by the applicant.

The above conclusion of PMDA was supported by the expert advisors at the Expert Discussion.

In view of the above discussion and the following correction presented by the applicant, PMDA has concluded that the post-marketing surveillance should be conducted as provided in Table 47.

Major correction

- In light of the number of patients awaiting corneal transplant, the number of patients who might use Nepic was surveyed again. On the basis of the survey result, the planned sample size of the surveillance was revised to approximately 120 patients per year.

Table 47. Outline of post-marketing surveillance (draft)

Objective	Evaluation of the safety and efficacy of Nepic
Survey method	All-case surveillance
Observation period	From the tissue collection for manufacture of Nepic to Week 52 of transplantation.
Population	Patients with LSCD
Planned sample size	Approximately 120 patients per year
Main survey items	Safety specification: All adverse events associated with use of Nepic Efficacy specification: LSCD severity, corrected visual acuity, etc.

1.6 Others

1.6.1 Quality

1.6.1.1 [redacted]

The applicant specified [redacted] as the critical quality attribute and included [redacted] of the final product as the verification item [see Section 1.6.1.2]. In addition, through investigation of the test methods, [redacted] of the corneal epithelial cell sheet was adopted.

PMDA accepted the applicant's measure.

1.6.1.2 Verification

On the basis of the risk evaluation, the applicant included items listed in Table 48 in addition to critical manufacturing process parameters, in-process control tests, and specifications of the final product.

Table 48. Verification items for manufacturing process and final product

[redacted]

* For [redacted], the in-process control test is specified as the verification item.

PMDA accepted the quality control strategy established by the applicant because it covered items deemed critical in ensuring the quality of the product based on the risk evaluation.

1.6.1.3 Control of secondary components

The applicant's explanation about the container integrity of the secondary components:
By specifying [redacted] in the manufacturing process, container integrity of the tissue transport tube and pre-treatment fluid bottle was controlled. In association with the above measure, "[redacted]" in the in-process control test and "[redacted]" for [redacted] in the specifications of the tissue transport set and pre-treatment fluid bottle were to be omitted.

PMDA accepted the applicant's measure.

1.6.2 Designation as designated regenerative medical product

In accordance with the "Concept for designation of biological products and specified biological products as well as designated regenerative medical products" (PFSB/ELD Notifications No. 1105-1 and 2 dated November 5, 2014), PMDA has concluded that Nepic should be designated as a designated regenerative medical product because mouse cells (3T3-J2 cells) are used as feeder cells in the manufacturing process of Nepic, which is a product using the autologous corneal limbal tissue as a raw material; and the manufacturing process does not include inactivation or removal of pathogens.

2. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved after modifying the indication or performance and the dosage and administrations or method of use as shown below, with the following approval conditions. Because the product is designated as an orphan regenerative medical product, the re-examination period is 10 years. The product is designated as a specified regenerative medical product.

Indication or Performance

Limbal stem cell deficiency. The following patients, however, will be excluded:

- Patients with Stevens-Johnson syndrome
- Patients with ocular pemphigoid
- Patients with graft versus host disease
- Patients with aniridia or other congenital corneal epithelial stem cell dysplasia
- Patients with recurrent pterygium
- Patients with idiopathic limbal stem cell deficiency

Dosage and Administration or Method of Use

Operations in manufacture of corneal epithelial cell sheet

1. An area in the patient's donor eye (contralateral eye of the eye planned to receive the product as a graft or the recipient eye) is confirmed to be free from inflammation and infection and approximately 2 × 3 mm piece of the corneal limbal tissue in area that have no sign of conjunctivalization is collected. The collected corneal limbal tissue is placed in a tissue transport tube and sent to the manufacturer.
2. Blood is collected in accordance with a conventional procedure. The collected blood is placed in a blood storage tube and sent to the manufacturer. This blood specimen is used as the reserve sample.

Operations in transplantation of corneal epithelial cell sheet

The corneal epithelial cell sheet is rinsed with a pre-treatment fluid and immersed in this fluid, and detached with a ring-shaped culture disk from the corneal epithelium culture dish. Conjunctival scar tissue is removed from the eye surface of the patient wherever possible and the corneal epithelial cell sheet is transplanted onto the eye surface including the corneal limbus. The rim of the corneal epithelial cell sheet is sutured where necessary. After the transplantation, the therapeutic contact lens is applied and tarsorrhaphy is performed where necessary.

Approval Conditions

1. The applicant is required to take necessary measures such as dissemination of the guideline for proper use prepared in cooperation with relevant academic societies and conducting seminars to ensure that physicians with adequate knowledge and experience in limbal stem cell deficiency acquire full skills of the product usage and knowledge in complications associated with the procedures and that the physicians use the product in compliance with the "Indication or Performance" as well as "Dosage and Administration or Method of Use" at medical institutions with an established system for treatment of limbal stem cell deficiency.
2. Since only a limited number of patients participated in clinical studies of the product, the applicant is required to conduct a use-results survey covering all patients treated with the product in principle until the end of the re-examination period in order to understand the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure proper use of the product.
3. The applicant is required to take necessary measures such as storage of reserve samples of the final product and retention of use records for 30 years to ensure appropriate handling in view of a risk of xenogeneic transplantation related to mouse embryonic 3T3-J2 cells used as feeder cells in the manufacturing process of the product.

Appendix

List of Abbreviations

ADL	Activity of daily living
[redacted]	[redacted]
CI	Confidence interval
CTCAE	Common Terminology Criteria for Adverse Events
ELISA	Enzyme linked immunosorbent assay
ETDRS	Early Treatment Diabetic Retinopathy Study
FAS	Full analysis set
GVHD	Graft versus host disease
HCL	Hard contact lens
HE	Hematoxylin-eosin
ICH Q5A (R1) guideline	"Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin" (PMSB/ELD Notification No. 329 dated February 22, 2000)
ICH Q5D guideline	"Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products" (PMSB/ELD Notification No. 873 dated July 14, 2000)
[redacted]	[redacted]
LogMAR	Logarithmic minimum angle of resolution
LSCD	Limbal Stem Cell Deficiency
MCB	Master cell bank
MedDRA/J	Medical Dictionary for Regulatory Activities Japanese version
[redacted]	[redacted]
MWCB	Master working cell bank
Nepic	Nepic
PAS	Periodic acid-Schiff
PMDA	Pharmaceuticals and Medical Devices Agency
[redacted]	[redacted]
SJS	Stevens-Johnson syndrome
[redacted]	[redacted]
WCB	Working cell bank
WHO	World Health Organization
[redacted]	[redacted]