

Guidance on Evaluation of Autologous Induced Pluripotent Stem Cells-derived Retinal Pigment Epithelial Cells

1. Introduction

The fundamental technical requirements of the quality and safety of the medical devices or pharmaceuticals processed from autologous human induced pluripotent stem (iPS) cells or the iPS-like cells are stipulated in the Notification Pharmaceutical and Food Safety Bureau (PFSB) 0907 No. 4, “Guideline on ensuring the quality and safety of human autologous induced Pluripotent Stem (-like) cells-derived medical devices or pharmaceuticals” issued by the Director of the PFSB of the Ministry of Health, Labour and Welfare (MHLW) on September 7, 2012.

This guidance shows specific points to consider in the evaluation of quality, efficacy and safety of the autologous human iPS (or iPS-like) cells-derived medical devices that can be specially intended to be used in the treatment of retinal pigment epithelial disorders in addition to the fundamental technical requirements in the Notification mentioned above.

2. Scope

This guidance identifies points to consider in the evaluation of the quality, efficacy, and safety of medical devices processed from autologous human iPS cells, specifically intended to be used in the treatment of retinal pigment epithelial disorders in addition to the fundamental technical requirements.

Further, when it is difficult to determine whether the product being developed could be categorized as a medical device, please consult with the Office of Medical Device Evaluation of the PFSB, the MHLW, if necessary.

3. Role of this guidance

In consideration of the fact that the scope is medical devices processed from autologous human iPS cells that are being developed based on the remarkable technological advances in this area, this guidance is designed to indicate current issues instead of providing a comprehensive list of problems and points to consider. Therefore, it will be revised as technological innovation progresses and knowledge accumulates, and it does not mandate what information must be included in application document for approval.

For the evaluation of products, it is necessary to have a thorough understanding of the characteristics of individual products and to handle the data in a flexible and scientifically rational manner.

It is also recommended that other relevant guidelines from both Japan and overseas should be consulted in addition to this guidance.

4. Terms and definitions

For the purposes of this guidance, the definitions given in the Notification PF5B 0907 No. 4 above and the following apply.

- (1) Retinal pigment epithelial cells: The outermost layer of the 10 retinal layers. These cells form a single layer of epithelial cells and have the ability to phagocytose retinal photoreceptor cells and regenerate visual substances (such as retinal), forming the blood–retinal barrier. These cells are the main sites of lesions in age-related macular degeneration.
- (2) Retinal photoreceptor cells: One of the types of cells in the retina. They are called “light receptors,” converting light energy into electrical energy. They are located at the outermost layer of the neural retina, and their apical regions called “outer segment” is regularly phagocytosed by the retinal pigment epithelia and replaced by new disc membranes that form those outer segments.
- (3) Phagocytic ability: Similar to macrophages, retinal pigment epithelial cells have the ability to incorporate and digest foreign objects, such as bacteria and cell debris. Under normal circumstances, they regularly ingest the apical region of photoreceptor cells.
- (4) Barrier function: Retinal pigment epithelial cells are bound to each other by an adhesive structure that is impervious to substances. This feature is known as the barrier function.
- (5) Cell sheets: This refers to cells that gather to form a sheet-like structure.
- (6) Subretinal transplantation: This refers to a surgical treatment consisting of deliberately creating a space in the subretinal region, which is located between the sensory retina and retinal pigment epithelial cells, in which tissues or devices are inserted.
- (7) Exudative lesions: This refers to a pathological condition in which choroidal neovascularization occurs in association with age-related macular degeneration. Caused by disturbances in the structure of the retina due to the accumulation of exudates and neovascular tissues beneath the retina, the condition involves rapid and severe decrease in visual acuity.
- (8) Examination of the ocular fundus: This refers to a test aimed to identify changes in the retina and choroid by using indirect ophthalmoscope, direct ophthalmoscope, front lenses, etc, projecting light into the ocular fundus from the front of the eye, through the pupil.
- (9) Fundus angiographic test: In this test, a fluorescent substance (fluorescein) is administered intravenously, and the ocular fundus is examined and photographed using a fluorescent camera. This test assists in the evaluation of the barrier function and hemodynamics of the

ocular fundus, as well as in the detection of neovascularization.

- (10) Retinal tomography: This is also known as optical coherence tomography (OCT) and is a test that allows the cross-sectional observation of the layers of the retina in vivo. It is effectively used in the detection of choroidal neovascularization and retinal detachment.
- (11) Retinal sensitivity test: This test examines the area of the subject's visual perception by projecting small lights on the retina and by changing the brightness of each light. It includes tests such as microperimetry and static quantitative perimetry.

5. Points to consider in evaluation

(1) Quality control of products

[1] Characterization items on specification of quality as retinal pigment epithelial cells

a) Cell morphology

Phase contrast microscopy allows visual examination of the specific morphology to retinal pigment epithelial cells (e.g., brown pigments and polygonal, pavement-like shape).

b) Genes specifically expressed in the retinal pigment epithelial cells

Confirm whether retinal pigment epithelium-related genes (such as RPE65, CRALBP, MERTK, and BEST1) are expressed.

c) Cell purity

Cell purity may be confirmed by immunostaining of the combination of more than one antibody such as against RPE65, bestrophin, and PAX6. Alternatively, it may be confirmed through the objective quantification of the number of pigmented cells by microscope-image analysis. Because almost all the pigmented cells in a characteristic morphology from purified cultures of cells with relevant genes are judged as retinal pigment epithelial cells.

d) No undifferentiated cells in the product

Flow cytometry by using immunostains for undifferentiated-cell markers (i.e., Oct3/4, Sox2, and TRA-1-60) and quantification of marker genes (e.g., OCT3/4, Nanog, and Lin28) through quantitative reverse transcription-PCR have been shown in the literature as the methods to detect residual undifferentiated cells in the product.

Among them, the investigation of the Lin28 expression by quantitative RT-PCR is highly specific and sensitive to find the undifferentiated cells¹⁾, and thus, can be a representative method in the evaluation.

In addition, the residual of undifferentiated iPS cells in the product should not necessarily be considered as a sign of tumorigenicity, and items used in non-clinical studies should be referred for tumorigenicity tests.

e) Functional evaluation

The functional properties of retinal pigment epithelial cells compatible for therapeutic use should be checked during the manufacturing process. The following are examples of the commonly performed tests.

- Phagocytic ability: Incorporation of fluorescence-labeled outer segments of the retinal photoreceptor cells or the incorporation of fluorescent beads added to the culture medium into cells is evaluated using flow cytometry.
- Growth factor secreting ability: The secretion amount of vascular endothelial growth factor (VEGF), pigment epithelium-derived factor, and so on are measured using enzyme-linked immunosorbent assay.

[2] Characterization items on specification of quality as retinal pigment epithelial cell sheets

When analyzing the characteristics of retinal pigment epithelial cell sheets, the validity of preparation process of cell sheets in manufacture is clarified by confirmation of the morphology and evaluation of mechanical compatibility and functional properties as shown below.

- a) To confirm sheet morphology, tissue sections of the sheet are examined, or three-dimensional observation of the sheet by using a confocal microscope is conducted to confirm that cells form a sheet.
- b) To check the mechanical compatibility, a graft prepared from the cell sheet detached is verified the absence of breakage or damage in the sheet.
- c) To examine the presence of functional properties (barrier function), the expression of markers associated with barrier function is observed by immunostaining (ZO-1 staining). Or transepithelial electrical resistance and such are measured.

(2) Non-clinical tests

[1] Tumorigenicity tests

To validate the manufacture process, it is useful for the evaluation that tumorigenicity of final products manufactured in the same manner and met the same quality standards is tested at a certain number of local (e.g., under the retina or under the skin) in immunodeficient animals by using the experimental system with a known detection sensitivity. Final products derived from three donors or more, if possible, are tested. Whenever necessary, analyses are conducted in a comprehensive manner by using the soft agar colony formation assay or karyotype analysis, if scientifically relevant. However, the study of tumorigenicity may need to be revised, depending on new data from the clinical applications of the product.

As general principles on non-clinical safety, including tumorigenicity tests, the safety of the final product and the safety of raw materials (iPS cells) are evaluated, in principle, in

distinct from each other. For all non-clinical tests on retinal pigment epithelial cells derived from autologous iPS cells, as functionally matured retinal pigment epithelial cells are used, final products can be supposed to be equivalent to each other in terms of non-clinical safety regardless of their donors or minor changes in the manufacture process, if the points to consider in evaluation shown in this guidance are fully studied and the properties are well characterized.

[2] Potency and performance assessment

As functionally matured cells are transplanted in the retinal pigment epithelial transplantation, in principle, the cells are transplanted subretinally into animal models of retinal pigment epithelial dysfunction, such as the Royal College of Surgeons (RCS) rats and then their protective effect for retina as retinal pigment epithelial cells is confirmed.

[3] Other issues

If special handlings are needed for the insertion of the sheet and such, it is recommended to confirm the items necessary and scientifically appropriate for the clinical application, such as the safety of the handlings and short-term local reactions following transplantation with the handlings, in middle- and/or large-sized animals.

(3) Clinical studies (Clinical trials)

[1] Indications

Diseases involving disorders in the retinal pigment epithelium

Diseases such as age-related macular degeneration, degenerative myopia, Stargardt's disease, traumatic injuries, and retinitis pigmentosa

[2] Items for systemic monitoring

Adequate systemic screening of the presence of malignancies are recommended prior to any surgical procedure to determine whether the tumor could be derived from transplanted cells in case of development of a tumor in tissues other than eye after transplantation. A suitable period is established after transplant surgery, and attention is given in the eventuality that a tumor develops.

[3] Evaluation of transplantation treatment

For the diseases targeted in this guidance, the items for the evaluation of therapeutic effect are mainly categorized into 2 types: a) anatomical evaluation and b) visual performance evaluation. Which should be used as evaluation items and the best timing to do so should be considered according to the targeted diseases and content of treatment. It seems plausible that control groups are selected from the patients who seem adequate for the comparison in consideration of the previous outcome of treatment and their control group, depending on the study design: if patients who did not fully respond to conventional treatment (such as anti-VEGF therapy in age-related macular degeneration) are the enrolled group, or if

patients meeting certain standards are used as controls regardless of the effectiveness of their existing treatment are the ones to be enrolled, a comparison can be conducted with the results of treated patients with conventional treatments in the past, or those of the control groups in these past studies. In cases of hereditary degenerative diseases in which both eyes progress in the consistent time frame, the use of the contralateral eye as a control may be considered appropriate.

The following is a summary of the current trend of the use of evaluation items in the specialized field of ophthalmology. Tests used in the specialized field of ophthalmology have remarkably progressed; suitable evaluation methods should be chosen for each test design at any time.

a) Anatomical evaluation

Fundoscopy examination and diagnostic imaging, such as contrast imaging tests and retinal optical coherence tomography

Tests conducted in ophthalmology have shown remarkable progress in recent years. For example, retinal optical coherence tomography (OCT) allows the observation of tomographic images of the ocular fundus in a non-invasive manner and with high resolution. It facilitates the detection of active exudative lesions such as those found in age-related macular degeneration, as well as the actual quantitative condition of the residual visual cells after treatment, including dry types. Thus, OCT is a highly reliable testing method that may be used for objective evaluation of the time-course evaluation of its protective effect on the retina as well. Therefore, use of diagnostic imaging methods such as OCT is currently the most appropriate evaluation method to determine the survival of transplanted cells and their effects. In addition, making assessments based on contrast imaging tests and OCT is the most appropriate for the evaluation of safety (including rejection and tumor formation), because of their high sensitivity.

b) Visual function test

Visual function tests include assessment of visual acuity, retinal sensitivity, visual field test, and electrophysiological testing

In terms of exudates during choroidal neovascularization, such as that found in pigment epithelial failure in the macular region, as well as that observed in exudative age-related macular degeneration, the pathogenesis of exudation includes progressive degeneration of overlying photoreceptor cells in the macular region. Visual performance depends on the condition of the retinal photoreceptor cells, and the main purpose of the transplantation therapy is to prevent further progression of this macular visual performance disorder (decrease in visual acuity) by supplying healthy pigment epithelia under the residual retinal photoreceptor cells to protect the function of these

photoreceptors. Recovery of lost photoreceptor cells is currently impossible and cannot be considered as a purpose for the pigment epithelial transplantation.

Central vision, or visual acuity, is generally used as an indicator of visual function, although influenced by the position of residual healthy retinal photoreceptor cells in the central region. Visual acuity, thus, is generally better when the residual retinal photoreceptor cells are located closer to the central region. However, in age-related macular degeneration, all retinal photoreceptor cells are not lost uniformly in a concentric manner, and instead, they disappear in a random and disorderly manner. Hence, the range of photoreceptor cells remaining in the macula area is not necessarily correlated with visual acuity. Further, individual differences are also present in the subjective perception of visual performance, such as the identification of important points. Subjectively, the following types of dissociations may actually occur: “During visual acuity tests, numbers are visible, but the subject does not have the impression of being able to see” or “The visual acuity score is low, but surprisingly, there is no disability.”

When the disease is treated early, a high number of photoreceptor cells are protected at the central area, and generally, good visual acuity is maintained. On the other hand, when treatment is conducted at an advanced stage of the disease, the photoreceptor cells in the central area are already lost, and therefore, the chances for any improvement in visual acuity may be significantly reduced. However, if the photoreceptor cells in the surrounding areas can be protected, improvements may be achieved, such as a decrease in the central scotoma (the central blind area).

Therefore, depending on the timing and progression of the disease, when assessment based on visual acuity alone is considered inappropriate for the evaluation of visual function, it is preferable to conduct a comprehensive assessment that includes visual acuity, retinal sensitivity or central area of vision, as well as indexes for macular and local reactivity and the range.

Depending on the disease, if local analyses can be performed, then electrophysiological tests may be conducted to assess the visual performance objectively. In addition, in patients with bilateral impairment, if treatment is conducted on the eye with predominant visual acuity, then NEI VFQ-25 can also be used as an indicator in the evaluation of visual performance to examine the patient’s quality of life²⁾.

6. References

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2. Orr P, Rentz AM, Marfolis MK, Revicki DA, Dolan CM, Colman S, Fine JT, Bressler NM. Validation of the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25) in age related macular degeneration *Invest. Ophthalmol. Vis. Sci*. 2011;52:3354-3359.