(Appendix 2)

Guidance on evaluation of articular cartilage regeneration using human (allogeneic) iPS-like cell-based product

## 1. Introduction

Basic technical requirements for ensuring quality and safety of products obtained by processing human-derived allogeneic induced pluripotent stem cells (iPS cells) or induced pluripotent stem-like cells (iPS-like cells) (hereinafter referred to as "Human (allogeneic) iPS (-like) cell processed products") are defined in the "Guidelines on Ensuring Quality and Safety of Products Derived from Processed Cell and Tissue (Allogeneic iPS (-like) cells)" (PFSB Notification No. 0907-5, dated September 7, 2012, of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare [MHLW]).

The guidance on evaluation provides, in addition to the above basic technical requirements, considerations specific to a particular class of human (allogeneic) iPS (-like) cell processed products that are used as regenerative medical products applied for treatment of articular cartilage damage. The term "Regenerative medical products" is defined in Article 2, Paragraph 9 of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Act No. 145 of 1960) (hereinafter, the same definition is applied).

## 2. Scope of the guidance on evaluation

The guidance on evaluation provides, in addition to the basic technical requirements, points to consider for evaluation of quality, efficacy, and safety of a particular class of human (allogeneic) iPS (-like) cell processed products that are used as regenerative medical products applied for treatment of articular cartilage damage.

## 3. Positioning of the guidance on evaluation

The guidance on evaluation, which applies to human (allogeneic) iPS (-like) cell processed products currently undergoing remarkable development of technologies, provides only points to consider at the present time, but does not intend to cover considerations comprehensively. It is supposed to be revised in response to further technological innovation and accumulation of knowledge and thus not binding on application data.

Product evaluation requires scientifically rational flexibility with full understanding of characteristics of individual products.

In addition to the guidance on evaluation, other related guidelines in and outside Japan should be referred to.

## 4. Definitions of terms

Terms used in the guidance on evaluation are as defined in the "Guidelines on Ensuring Quality and Safety of Products Derived from Processed Cell and Tissue (Allogeneic iPS (-like) cells)" (PFSB Notification No. 0907-5, dated September 7, 2012, of the Pharmaceutical and Food Safety Bureau, MHLW) or defined as follows.

(1) Cartilage tissue: Cartilage has a structure in which chondrocytes are sporadically present in the extracellular matrix. Chondrocytes produce and maintain the extracellular matrix. Cartilage is tissue; cartilage and cartilage tissue are synonymous. In the guidance on evaluation, the term cartilage tissue is used to distinguish cartilage from chondrocytes. In the guidance on evaluation, iPS (-like) cell-processed tissues which are manufactured *in vitro* and equivalent to cartilage tissues in the body are also referred to as cartilage tissues.

(2) Chondrocyte extracellular matrix: The matrix is composed of cartilage-specific collagens (e.g., type II, IX, XI) and proteoglycans and provides resilience and compressibility to the tissue. The matrix also contributes to a mechanical function of cartilage tissue and maintains chondrocytes. The extracellular matrix can be removed from cartilage tissue by enzymatic digestion. When cultured without the matrix, chondrocytes lose their original attributes and are transformed into fibroblast-like cells.

(3) Perichondrium: The term refers to a membranous tissue that surrounds the cartilage tissue at the development stage. The tissue produces factors that regulate chondrocyte differentiation.

(4) Chondrocytes: The term generally refers to cells that are sporadically present in cartilage tissues in the body and secrete collagen (e.g., type II, IX, XI), proteoglycan (mainly aggrecan), and other substances of the extracellular matrix. In the guidance on evaluation, however, the term also refers to iPS (-like) cell-processed cells which are manufactured *in vitro* and equivalent to chondrocytes in the body and their progenitor cells.

(5) Source materials: The term refers to original materials of raw materials or materials used in manufacture of regenerative medical products. (as defined in the Standards for Biological Raw Materials [Public Notice of the Ministry of Health, Labour and Welfare No. 210 of 2003])

(6) Cell bank: The term refers to a collection of a substantial number of aliquots with uniform composition filled in containers stored under a certain storage condition. That is, each container contains an aliquot of a single pool of cells. (as defined in the ICH Q5D "Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products" [PMSB/ELD Notification No. 873, dated July 14, 2000, of the Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare or MHW])

(7) Cross-contamination: The term refers to unintentional transfer of substance from one sample to another sample. It is also referred to as contamination between samples. It means contamination between raw materials used for manufacturing and between intermediates. Potential cases are, for example, where cells derived from a cell bank are unintentionally transferred into a cell population from another cell bank; and where a pre-virus-inactivation raw material is unintentionally transferred into a batch of the post-virus-inactivation raw material.

(8) Cell sheet: The term refers to a sheet formed by cells, which are directly or indirectly bound to each other.

## 5. Points to consider for evaluation

For the time being, the guidance on evaluation is intended to apply to evaluation of cartilage tissue or chondrocytes as human (allogeneic) iPS (-like) cell processed products. The cells to be evaluated are manufactured at the manufacturing site where human (allogeneic) iPS (-like) cells (cell line) established as a cell line and already used as a source material of regenerative medical products are accepted, cultured for generation of a cell bank system, and processed. To establish human (allogeneic) iPS (-like) cells from somatic cells and manufacture regenerative medical products using the established cells as a source material within the same manufacturing site, not only the guidance on evaluation but also the "Guidelines on Ensuring Quality and Safety of Products Derived from Processed Cell and Tissue (Allogeneic iPS (-like) cells)" (PFSB Notification No. 0907-5, dated September 7, 2012, of the Pharmaceutical and Food Safety Bureau, MHLW) should be referred to.

(1) Raw materials

Used as a raw material, iPS (-like) cells should be from a cell line of human (allogeneic) iPS (-like) cells used as a source material for regenerative medical products and controlled in a cell bank system, which needs to be confirmed or reasonably expected to differentiate into chondrocytes, which can form cartilage tissue later, through a certain manufacturing process. If possible, genome sequencing should be performed to rule out mutations in genes related to functions of cartilage tissue or chondrocytes. Genes potentially affecting functions of cartilage tissue or chondrocytes include ones encoding cartilage extracellular matrix proteins (*COL2A1*, *COL9A1*, *COL9A2*, *COL9A3*, *COL11A1*, *COL11A2*, *ACAN*, *HAPLN1*, *COMP*, and *MATN3*), ones correlated with osteoarthritis (Literature 1 in Section 6. Reference data) (*GDF5*), ones responsible for chondrodysplasia (ones responsible for the diseases that are listed in Literature 2 in Section 6. Reference data and also cause abnormalities in cartilage tissues).

For human iPS (-like) cells established through genetic reprogramming by transfection with reprogramming genes in human somatic cells, presence of residual transgenes should be ruled out if possible. If the presence could not be ruled out, the transgenes should be demonstrated to have no adverse effects on quality or safety of the final product, the cartilage tissue or chondrocytes.

(2) Matters warranting special attention in the manufacturing process

For manufacture of cartilage tissue or chondrocytes (final product), the manufacturing method should be clarified and validated for the following items to the extent possible to ensure certain quality.

[1] Presence or absence of lot configuration and specification of lot

Whether the final product and intermediate product are manufactured on a batch basis or not should be clearly stated. If it is manufactured on a batch basis, definition of a batch should be provided.

[2] Manufacturing method

The manufacturing method up to release of the final product should be outlined, including a history from acceptance of iPS (-like) cell line to be used as a source material at the manufacturing site through generation of a cell bank system from human iPS (-like) cells used as a starting material as well as generation of adequately differentiated cells from the starting material. In addition, the treatment, necessary process control, and quality control should be specified in detail.

a) Acceptance inspection

For the human iPS (-like) cell line to be used as a source material, test and inspection items for acceptance at the manufacturing site (e.g., visual inspection, microscopic examination,

viability, characterization of cells, and tests to deny bacteria, fungi, viruses, etc. contamination) should be specified with the criteria for each item. Where necessary and possible, tests for bacteria, fungi, viruses, etc. should be performed to the extent that would not affect the phenotype, genetic traits, characteristics such as specific functions, cell viability, or quality. If the result is positive, stock of the human iPS (-like) cell line and the transportation should be checked for contamination, and a human iPS (-like) cell line should be obtained again.

If testing with a partially processed material is appropriate because of a technical reason, the tests should be performed at an appropriate timepoint after acceptance. For example, the tests may be additionally performed before expansion culture of human (allogeneic) iPS (-like) cell line, which has been accepted in a frozen state based on test and inspection results at the time of manufacture of the rsource material (certificate of analysis) and then thawed. At a stage prior to the start of a clinical trial, values measured with test samples obtained to date should be presented, and based on them, provisional values should be indicated.

b) Cell banking

Methods of generation of cell banks from the iPS (-like) cell line accepted at the manufacturing site, characterization, storage, maintenance, control, and renewal of the cell banks as well as procedures related to other operation processes and tests should be clearly described in detail and justified. ICH Q5D, etc. should be referred to. However, a part of investigation matters may be omitted if justified by evaluation completed in the upstream process.

c) Preparation of cells to be used as a component of the final product

A method of preparing cells to be used as a component of the final product from the cell bank (starting material) (including a differentiation induction method, methods of isolation and culture of intended cells, medium at each stage of culture, culture conditions, culture period, and yield) should be specified and justified to the extent possible.

d) Measures to prevent mix-up and cross contamination during the manufacturing process

In the manufacture of cartilage tissue or chondrocytes derived from iPS (-like) cells (final product), prevention of mix-up and cross contamination during the manufacturing process is of importance, and the preventive measures in the process control should be specified.

(3) Quality control of products

If quality specification values are established at a stage prior to the start of a clinical trial, values measured with test samples obtained to date should be presented, and based on them, provisional values should be indicated.

If technical difficulties preclude tests with the released product itself or a part of it, specification tests with products manufactured in parallel should be performed after being justified.

The transplantation method of cartilage tissue or chondrocytes (final product) manufactured from iPS (-like) cells should be clearly described. Transplantation may be performed by the following procedure: a defect in the articular cartilage is filled directly with the necessary amount of the cartilage tissue followed by fixation with fibrin glue, etc. or undergoes transplantation of a product obtained by further processing the manufactured cartilage tissue (e.g., fusing multiple cartilage tissues or hardening multiple cartilage tissues with a gel into a plate).

Or chondrocytes isolated after digestive enzyme treatment of cartilage tissue produced from iPS (-like) cells themselves or their further processed product may be transplanted. However, it should be noted that chondrocytes obtained by removing the extracellular matrix transform and lose their original attributes.

In this section, [1] explains quality specifications for cartilage tissue produced from iPS (-like) cells, and [2] explains those for cell sheets produced by processing chondrocytes.

[1] Characterization Items for establishment of quality specifications for cartilage tissue

a) Check for appearance

Tissues mostly have a milky white to white surface. In a culture medium containing dye, the tissues are stained with the dye. Cartilage tissues manufactured from iPS (-like) cells may fuse each other during culture. If possible, specifications for size and shape should be established.

b) Cell count and viability

For cell count and viability in the final product, criteria should be also specified. If a method to collect chondrocytes from cartilage tissue efficiently is not established, making it technically difficult to measure cell count and viability and establish specifications for these indicators, surrogate indicators reflecting count and viability of cells in the cartilage tissue may be used. Of note, the surrogate indicators should be justified.

c) Characteristics specific to cartilage tissue

Relative expression levels of chondrocyte marker genes (e.g., *COL2A1*, *COL9A1*, *COL9A2*, *COL9A3*, *COL11A1*, *COL11A2*, *ACAN*, *HAPLN1*) should be determined by mRNA expression analysis. Quantification of protein expression is difficult because there is no established method to extract soluble chondrocyte extracellular matrix proteins from cartilage tissue.

Glycosaminoglycans can be quantified and used as an indicator characteristic of cartilage tissue.

In addition, confirmation should be made that the chondrocyte extracellular matrix in a tissue section is well stained by safranin O staining and type II collagen immunostaining. SOX9 immunostaining in a tissue section allows identification of chondrocytes, and the count indicates efficiency of differentiation into chondrocytes.

Perichondrium-like tissue covering the surface of cartilage tissue expresses type I collagen.

d) Absence of undifferentiated cells

For presence or absence of undifferentiated cells, quantification of marker genes (assessment of expression levels of genes such as *OCT3/4* and N*ANOG*) by quantitative PCR is reported in the literature (Literature 3 in Section 6. Reference data).

It should be noted that presence of undifferentiated iPS (-like) cells does not necessarily lead to tumorigenicity. For tumorigenicity studies, the nonclinical study section should be referred to.

e) Functions

To confirm that the produced cartilage tissue has functional properties consistent with the intended treatment use, the in-process product or the final product should be analyzed. For example, if the final product, cartilage tissue, is expected to have a composition similar to that of the cartilage tissue in the body, measurement of expression level of genes encoding chondrocyte extracellular matrix proteins or histological analysis (safranin O staining or type II collagen immunostaining) may allow prediction or evaluation of the effectiveness of the product in the body before transplantation. In addition, for example, if the final product, cartilage tissue, is expected to have mechanical properties similar to those of the cartilage tissue in the body, measurement of mechanical properties such as viscoelasticity may allow prediction or evaluation of the effectiveness of the product in the body before transplantation. However, it should be noted that mechanical properties key to mechanical functions of cartilage tissue remain to be elucidated.

[2] Characterization Items for establishment of quality specifications for chondrocyte sheet

Before characterization of a chondrocyte sheet, the shape and functional properties should be evaluated as described below, and the manufacturing process of the sheet should be justified as well.

a) Check for appearance

For shape, for example, preparation of tissue sections of the sheet and 3-dimensional observation under a confocal microscope should be performed to confirm that the cells form a sheet.

b) Cell count and viability

Cell count may be determined using a cell suspension prepared from an enzyme-treated portion of the final product by a method using hemocytometer or cell counter. Cell viability may be determined by a trypan blue dye exclusion method, which allows calculation of the numbers of living cells and dead cells. If the product is obtained from a 3-dimensional culture in which cells were spread over scaffolding materials, etc., a cell suspension may be prepared by digesting the scaffolding materials, etc. with proteolytic enzymes for measurement of cell count and viability.

c) Absence of undifferentiated cells

Presence of undifferentiated cells may be assessed based on expression levels of marker genes (e.g., *OCT3/4*, *NANOG*) determined by quantitative RT-PCR or by flow cytometry of cells isolated from the cell sheet in combination with immunostaining of undifferentiated cell markers (OCT3/4, Sox2, TRA-1-60).

It should be noted that presence of undifferentiated iPS (-like) cells does not necessarily lead to tumorigenicity. For tumorigenicity studies, the nonclinical study section should be referred to.

d) Functions

For mechanism of action, the cell sheet is considered to exert the action by producing growth factors that enhance regeneration of damaged cartilage tissue in the recipient body. Measurement of protein and mRNA expression levels of potentially effective growth factors may allow prediction or evaluation of the effectiveness of the product in the body before transplantation. Of note, if growth factors, etc. are used as indicators, whether their levels correlate to effectiveness in the patient should be elucidated in advance.

(4) Stability testing of products

The final product or its critical intermediate products should be subjected to appropriate stability studies under actual storage conditions using the cell viability and surrogate primary efficacy parameters as indicators, in full consideration of the storage and distribution periods and storage form. Storage conditions and shelf-life should be established and justified. Especially if cryopreservation and thawing are involved, impacts of freezing and thawing operations on a cultivable period and quality of the thawed product should be checked. As necessary, the limit of stability should be identified to the extent possible by investigating the long-term stability for periods beyond the respective standard periods of manufacture and storage. However, this does not apply if the product is used immediately after end of manufacture.

If a starting material, intermediate product, or the final product is transported, the respective conditions and procedures (including containers, transportation fluid, and temperature control) should be specified and justified. If the cells are transported in a frozen state, the medium used at the time of freezing or cryopreservation fluid, cryoprotectants, etc. should be appropriately selected as done for the materials used in the manufacturing process. The same applies to the transportation fluid, etc. for transportation in a non-frozen state. The appropriate storage form, temperature conditions, and transportation fluid to maintain the product stability may differ depending on the product form or cell type. For each product, an appropriate combination should be investigated to warrant stability.

(5) Biocompatibility of non-cellular materials and final product

Non-cellular materials related to products include not only materials that come in contact with cells during the manufacturing process but also sub-ingredients constituting a part of the final product with cells and products concomitantly used as sub-components during application (e.g., membranes for topical encapsulation, fibrin glue). For these non-cellular materials, knowledge on quality and safety of the materials themselves as well as on interactions with patients and cells in the product such as biocompatibility should be clearly described. The final product overall should also be evaluated for interaction with the patient's cellular tissue, especially the tissue surrounding the application site. In addition, appropriate information on non-cellular materials turned into sub-ingredients of the final product should be collected in terms of characteristics of their degradation during the manufacturing process (in a medium) and in the body and reabsorption in the body as well as safety of the degradation products. In particular, if bioabsorbable materials are used, necessary tests should be performed for the degradation products. For biocompatibility of non-cellular materials, ISO10993-1, JIS T 0993-1 or ASTM F748-04, and Basic Principles of Biological Safety Evaluation Required for Application for Marketing Approval of Medical Devices (PFSB/ELD/OMDE Notification No. 0301-20, dated March 1, 2012), etc. should be referred to.

(6) Nonclinical studies

[1] Quality control of the final product or tumorigenicity studies for nonclinical safety evaluation

When tumorigenicity of regenerative medical products manufactured by processing iPS (-like) cells is evaluated, it should be noted that “correlation and causal relationship between tumorigenicity of iPS (-like) cells used as a raw material and that of the final product remain to be

elucidated." That is, it must always be noted that for clinical application, in tumorigenicity evaluation, the greatest importance is attached to the final product, an iPS (-like) cell processed product but not iPS (-like) cells used as a raw material. Tumorigenicity studies should be conducted with the final product. Tumorigenicity evaluation using a study system in immunodeficient animals with the known detection limit is useful.

There are two major types of tumorigenicity evaluation of the final product according to the purpose. Tumorigenicity studies are conducted for the purpose of "quality control" (to check an amount of tumorigenic cells such as undifferentiated cells and ones other than the intended cells, possibly supposed to mainly form teratocarcinoma) or for the other purpose of "nonclinical safety evaluation" (to check whether cells in the final product exhibit tumorigenicity in a microenvironment corresponding to the transplantation site in humans). Tumorigenicity evaluation must be performed with either of the two purposes specified. For the former purpose, for example, subcutaneous dose studies in immunodeficient animals (e.g., SCID mice, NOD/SCID/$\gamma$Cnull (NOG) mice, NOD/SCID/IL2r$\gamma$KO (NSG) mice, Rag2-$\gamma$C double-knockout (DKO) mice), which allow simple observation and are highly sensitive, may be conducted (Literature 3 in Section 6. Reference data). For the latter purpose, for example, intraarticular dose studies in immunodeficient animals (e.g., *rnu/rnu* (Nude) rats) may be conducted (Literature 3 in Section 6. Reference data). For either purpose, if cell banks of iPS (-like) cells are generated, the final product manufactured from the above cell bank should be used in tumorigenicity studies in principle. If the final product manufactured from a material other than the above cell banks is used in tumorigenicity studies, such use should be justified. Quality evaluation for tumorigenicity of the final product can be performed by methods other than subcutaneous dose studies in immunodeficient animals. A useful method may be *in vitro* determination of an amount of residual undifferentiated cells in the final product. The *in vitro* determination may be performed by quantitative RT-PCR using undifferentiated cell marker molecule genes (e.g., *OCT3/4*) as indicators (Literature 3 in Section 6. Reference data). Regardless of the method adopted, the detection limit of the study system must be identified before interpretation of the results.

Because intraarticular (clinical route of administration) transplantation involves a surgical procedure, which is highly invasive especially in small animals, it should be noted that the surgical procedure could complicate assessment of the results. The number of cells to be transplanted should be calculated by multiplying the expected clinical dose by the safety factors of the species and interindividual differences if possible. However, adequate consideration should be given to the possibility that the cells transplanted into animals may greatly affect the microenvironment at the administration site owing to the total volume itself, causing artifact changes. That is, the number of cells to be administered should be determined with the importance attached to the objective of tumorigenicity studies with intraarticular transplantation, which is to investigate tumorigenicity of the final product (cells) in the microenvironment corresponding to the transplantation site in humans.

If cartilage tissue or chondrocytes (final product) are manufactured from multiple cell banks of iPS (-like) cells through the same process, and these cell banks have been established by the same method after HLA typing and confirmed to have quality attributes comparable to those of the raw

material of the final product, tumorigenicity evaluation in a microenvironment corresponding to that at the transplantation site in humans should be performed for the final product manufactured from each cell bank in principle. Intraarticular transplantation in immunodeficient animals is a representative approach for tumorigenicity studies of the final product.

[2] Primary efficacy or performance studies of the final product

Expression of the function, durability of the action, and feasibility of clinical effects (Proof-of-Concept or POC) expected for the human (allogeneic) iPS (-like) cell processed product should be presented using animal models appropriate for the target disease to the extent technically possible and scientifically reasonable. Animal models include rats, rabbits, and minipigs with defects made in the articular cartilage. If use of an animal model with endochondral defects is desirable to preclude bone marrow mesenchymal cells from repairing and avoid the influence of rejections of bone marrow cells to heterologous grafts, bred juvenile pigs with thick cartilage may be used. In principle, however, animal models should use sexually mature animals. Furthermore, it should be noted that bred juvenile pigs are more likely to have cartilage damage owing to rapid weight gain. It should be noted that human iPS (-like) cell-derived cartilage tissue is a heterologous graft in a rabbit or minipig animal model when transplanted, requiring use of immunosuppressants, and their effect would be lost in a short period of time, limiting the observation to a short period of time. ICRS score, O'Driscoll score, Wakitani score, etc. may be used to evaluate the response to treatment, but justification should be considered. If cartilage tissue or chondrocytes (final product) are manufactured from multiple cell banks of iPS (-like) cells, and these cell banks have been established by the same method after HLA typing and confirmed to have quality attributes comparable to those of the raw material of the final product, POC may be demonstrated using the final product manufactured from a representative bank.

[3] Others

For items deemed necessary and scientifically valid for clinical application, such as safety of a procedure for transplantation and acute local reactions after transplantation using the procedure, medium- or large-sized animals should be used according to the purpose if possible.

(7) Clinical studies (clinical trials)

Transplantation of cartilage tissue or chondrocytes of a human (allogeneic) iPS (-like) cell processed product, discussed in this guidance, must be clearly described in terms of HLA matching status (mismatching or matching MHC) and use status of immunosuppressants (with or without). The clinical data package and the protocol should be appropriately designed in view of nonclinical data, etc. according to the target disease, intended indication, expected clinical positioning of the treatment method, etc.

Clinical studies should be designed to minimize the risks associated with the study and to maximize the benefits of treatment. Particularly, recommendation is given that a study be conducted in the design with the endpoints appropriately established in view of the origin of the intended cells or tissues, target disease, application method, etc.

For endpoints, the primary and secondary endpoints should be specified according to the final objective. Efficacy endpoints may use subjective clinical assessment scores, activity assessment

scores, and visual analogue scale (VAS) for pain. Structural improvement with repaired tissues may be assessed based on information obtained by MRI, arthroscopy, and biopsy.

[1] Target disease

The product is expected to be indicated for articular cartilage damage. To determine the patient's eligibility, the following items should be considered: age, BMI, joint function, pain, osteoarthritis (severity, definition), time of injury, site, size, depth, number of lesions, prior treatments, coexisting intraarticular lesions (e.g., meniscus injury, anterior cruciate ligament injury), and extra-articular lesions (e.g., deformity, alignment abnormality).

[2] Clinical efficacy evaluation

In clinical evaluation, recommended assessment methods cover joint conditions, pain, and function. In addition, the secondary endpoints should include structural improvement with repaired tissues.

The Knee injury and Osteoarthritis Outcome Score (KOOS) can be used as a clinical assessment method. This scoring system delivers comprehensive assessment of joint conditions, pain, function, and QOL, integrates the whole Western Ontario and McMaster Universities Index (WOMAC), which is a clinical assessment scoring system highly evaluated across the globe, and thus is the most common internationally accepted method to assess chondrocyte-based cell therapies. The International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form-2000 is also used internationally to assess treatment of knee articular cartilage clinically. For both KOOS and IKDC, Japanese versions are available.

[3] Structural assessment

a) Diagnostic imaging assessment

(conventional radiography)

Although conventional radiography does not provide direct assessment of regenerating cartilage, it is simple and useful in assessing bone tissues around the regenerating cartilage. It should be used to assess changes over time if possible.

(MRI)

MRI is the most currently useful clinical diagnostic method for imaging regenerating cartilage. MRI data can be analyzed for comprehensive MRI assessment which focuses on structures of regenerating cartilage and the surrounding tissue or for qualitative MRI assessment which focuses on repaired cartilage.

If possible, comprehensive MRI assessment should be performed using objective criteria such as MOCART (magnetic resonance observation of cartilage repair tissue) to standardize the assessment across multiple study sites. Basic modalities for imaging are proton density-weighted fast spin-echo, fat-suppressed proton density-weighted, and 3-dimensional isotropic voxel sequences. Section images corresponding to the regenerating cartilage site should be assessed.

Qualitative MRI assessment on regenerating cartilage may be performed using techniques such as delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) useful in determining proteoglycan concentrations, T2 mapping useful in determining water content and collagen arrangement, and T1 $\rho$ mapping useful in determining proteoglycan concentrations and water content. However, whether these techniques are useful for qualitative MRI assessment on regenerating cartilage is still under discussion with no consensus reached. The results should be interpreted with caution.

If MRI data are analyzed, the comprehensive MRI assessment should be the first choice, and the qualitative MRI assessment should be used as the secondary analysis.

b) Arthroscopy

Arthroscopy provides assessment of mechanical properties such as hardness in addition to macroscopic assessment and thus is one of methods useful in assessing regenerating cartilage.

For arthroscopy, the International Cartilage Repair Society (ICRS) cartilage repair assessment is widely used. In addition to the ICRS cartilage repair assessment, the Oswestry macroscopic cartilage evaluation score which assesses stiffness by probing is also internationally used, although macroscopic assessment is commonly used.

c) Biopsy

To assess articular cartilage regeneration, biopsy may be performed at the product transplantation site at a certain post-transplantation time point. Such assessment contributes to efficacy evaluation. A bone biopsy needle used for biopsy can reach an adequate depth, allowing subchondral assessment.

Biopsy is generally performed using a bone biopsy needle while checking the repaired and regenerated cartilage part under arthroscopy. The bone biopsy needle should be selected in terms of its size to ensure adequate assessment for repair and regeneration as well as to minimize invasiveness. During the procedure, the site should be monitored under arthroscopy to minimize sampling bias. In addition, clinically documented OsScore, ICRS histological assessment I (Histological assessment of cartilage repair: a report by the histology endpoint committee of ICRS) and II (ICRS II histology score for the assessment of the quality of human carriage repair), etc. (Literature 4 in Section 6. Reference data) should be considered for the assessment. Quantification of the assessment results with full understanding of characteristics of these assessment systems will facilitate comparison of cartilage tissue conditions. For histological staining of samples, staining with safranin O and toluidine blue must be performed to evaluate chondrocyte extracellular matrix, and immunohistological staining of type I collagen and type II collagen must be performed to identify hyaline and fibrous cartilage. Histological evaluation provides information on structural repair and regeneration of the chondrocyte extracellular matrix.

[4] Systemic monitoring items

Subjects should undergo systemic screening for malignant tumor before transplantation, including appropriate medical history taking wherever possible, because if a tumor is found outside the joint after transplantation, whether it is derived from the transplanted cells must be determined. Attention should be paid to tumor development, etc. for an appropriately pre-determined period of time after transplantation procedure.

[5] Items that should be examined in patients without immunosuppressants

The transplantation site should be observed by the following examinations as needed.

a) For anatomical assessment, visual inspection and palpation for inflammatory reactions in the joint as well as diagnostic imaging (e.g., ultrasonography, plain X-ray, CT, and MRI) should be performed to capture changes over a period of time. Attention should be paid not only to the transplantation site but also to the entire joint for inflammation, etc.

b) Laboratory tests, muscle strength tests, etc. should be performed to examine joint functions. If the test results indicate worsening despite previously showing an improving trend after transplantation, joint dysfunction may be caused by a graft failure. In view of such possibility, special attention should be paid to the test results.

c) Blood samples should be collected at regular intervals to monitor the recipient for inflammatory reactions.

[6] Items that should be examined in patients on immunosuppressants

If immunosuppressants are systemically administered, monitoring for systemic complications and periodic blood sampling should be performed in addition to [5].

## 6. Reference data


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