(Appendix 1)

# Guidance on Evaluation for the Treatment of Decompensated Cirrhosis using Autologous Bone Marrow-Derived Mesenchymal Stem Cells-Processed Products

## 1. Introduction

Basic technical requirements for ensuring quality and safety of human (autologous) somatic stem cell-processed products, a class of regenerative medical products ("regenerative medical products" 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]. The same shall apply hereinafter.) are defined in the "Guidelines on Ensuring Quality and Safety of Products Derived from Processed Cell and Tissue (Autologous somatic stem cells)" (PFSB Notification No. 0907-2, dated September 7, 2012, of the Pharmaceutical and Food Safety Bureau of the Ministry of Health, Labour and Welfare [MHLW]. Hereinafter referred to as "Guideline for Human (autologous) Somatic Stem Cell-Processed Products"). The guidance on evaluation provides, in addition to the above basic technical requirements, points to consider for a particular class of human (autologous) bone marrow-derived mesenchymal stem cell-processed products that are used as regenerative medical products applied for treatment of decompensated cirrhosis.

## 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 human (autologous) bone marrow-derived mesenchymal stem cell-processed products when applied for treatment of decompensated cirrhosis.

## 3. Positioning of the guidance on evaluation

The guidance on evaluation, which applies to human (autologous) bone marrow-derived mesenchymal stem 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.

For evaluation required for individual products, consultation with the Pharmaceuticals and Medical Devices Agency (PMDA) is strongly recommended. In addition to the guidance on evaluation, other related guidelines in and outside Japan should be referred to.

## 4. Definitions of terms

The terms in the guidance on evaluation are as defined in the "Guideline for Human (autologous) Somatic Stem Cell-Processed Products" or defined as follows.

(1) Hepatic cirrhosis (or cirrhosis): The term refers to a pathological condition representative of end stage of chronic liver disease or a condition of decreased liver function associated with fibrous tissue hyperplasia resulting from a process of regeneration of hepatocytes in response to their necrosis, which is a consequence of repeated liver injury caused by

chronic inflammation, such as hepatitis B, hepatitis C, alcohol, NASH (nonalcoholic steatohepatitis), autoimmune disease, metabolic abnormality, and cholestasis.

(2) Decompensated cirrhosis: The term refers to cirrhosis with symptoms attributable to hepatic failure, such as hepatic encephalopathy, jaundice, ascites, edema, or bleeding tendency. Compensated cirrhosis, on the other hand, has hardly any clinical symptoms and leaves the liver function relatively maintained. In general, cirrhosis with a Child-Pugh score of 7 points or higher given according to (3) is commonly deemed decompensated cirrhosis. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

(3) Child-Pugh score: The term refers to a method to assess functional severity of cirrhosis by rating the following 5 items on a scale of 1 to 3: [1] hepatic encephalopathy (none = 1, mild = 2, coma = 3); [2] ascites (none = 1, mild = 2, moderate or higher = 3); [3] serum bilirubin (mg/dL) (<2.0 = 1, 2.0-3.0 = 2, >3.0 = 3); [4] serum albumin (>3.5 = 1, 2.8-3.5 = 2, <2.8 = 3); and [5] prothrombin time (activity value by thrombo test, %) (>70 = 1, 40-70 = 2, <40 = 3). According to the score, the disease is classified into Class A (5-6 points), Class B (7-9 points), or Class C (10-15 points), and the disease with 7 points or higher is defined as decompensated cirrhosis as done above. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

(4) Mesenchymal stem cells: The term refers to cells that are classified as a type of somatic stem cells derived from mesodermal tissue (mesenchyme) and meet the following 3 conditions: [1] they adhere to a plastic culture container; [2] they are positive for CD105, CD73, and CD90 as well as negative for CD45, CD34, CD14, CD11b, CD79a, CD19, and HLA-Class II (DR); and [3] they have a potential to differentiate into mesenchymal cells (bone, fat, and cartilage). The cells can be isolated from fat tissue, bone marrow, umbilical cord, and pulp. They lack MHC Class-II, modulate immune response by secreting cytokines and growth factors, and are characterized by their nature to promote tissue regeneration and repair.

(5) Model for End-Stage Liver Disease (MELD) score: The term refers to a calculation formula used to predict the short-term prognosis and to determine the indication for liver transplantation in patients with decompensated cirrhosis aged ≥12 years. The score is calculated using the following 3 parameters: [1] serum bilirubin; [2] prothrombin time-international normalized ratio (PT-INR); and [3] serum creatinine.
MELD score = 9.57 ln (serum creatinine, mg/dl) + 3.78 ln (serum bilirubin, mg/dl) + 11.20 ln (PT-INR [blood coagulation ability]) + 6.43

(6) 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])

(7) Raw materials, etc.: The term refers to raw materials or materials or their source materials. (as defined in the Standards for Biological Raw Materials [Public Notice of the Ministry of Health, Labour and Welfare No. 210 of 2003])

(8) 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.

## 5. Points to consider for evaluation

The guidance on evaluation is intended to address processed autologous cells to be applied to the liver for treatment of decompensated cirrhosis. A raw material of the cells is a human (autologous) bone marrow tissue (fluid) containing mesenchymal stem cells, which is accepted and processed at the manufacturing site to manufacture the cells.

(1) Raw materials, etc.

In principle, control items for raw materials (human autologous bone marrow tissue [fluid]) and materials (e.g., bovine serum, culture media) as well as raw materials used for manufacture of the preceding materials should be specified to ensure that the final product has quality required. Especially, the control items for the source materials, etc. must be specified in consideration of their quality (e.g., sterility, impurities) to ensure that their use will not raise safety concerns with the final product. To control the risk of contamination with adventitious agents such as viruses, control items should be specified with reference to necessary information obtained based on the "Standards for Biological Raw Materials." For the scope of raw materials, etc. regulated under the "Standards for Biological Raw Materials," the "Application of Standards for Biological Raw Materials" (Joint Notification of PFSB/ELD Notification No. 10021-1 and PFSB/ELD/OMDE/CMS Notification No. 1002-5, dated October 2, 2014, of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, and Counsellor to the Minister's Secretariat [in charge of review management of medical devices and regenerative medical products], MHLW) should be referred to.

[1] Donor inclusion criteria, eligibility

If human (autologous) bone marrow-derived mesenchymal stem cells are used, the donor and recipient are the same individual, requiring no donor screening, but to prevent cross-contamination and protect safety of manufacturers, conduct of tests for the following viruses (e.g., by a serological method, nucleic acid amplification method) should be considered: hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human T-cell leukemia virus (HTLV), and parvovirus B19.

In addition, medical conditions listed below should be inspected through medical history and interview, and whether the individual is eligible for donation should be determined.

- Infections with bacteria such as *Treponema pallidum*, *Chlamydia*, *Neisseria gonorrhoeae*, and *Mycobacterium tuberculosis*
- Sepsis and suspected sepsis
- Malignancy
- Serious metabolic and endocrine disorders
- Collagen disease and blood disorders
- Transmissible spongiform encephalopathy and suspected transmissible spongiform encephalopathy as well as other dementia
- Pathological condition that precludes collection of cells/tissues to be used as a raw material (e.g., hemorrhagic condition)

[2] Collection of human (autologous) bone marrow tissue (fluid) containing bone marrow-derived mesenchymal stem cells

A reason for selecting collection sites of a human (autologous) bone marrow tissue (fluid) containing bone marrow-derived mesenchymal stem cells and the collection method should be described. The selected site and the method should be justified scientifically and ethically. For the collection method, apparatuses to be used and preventive measures against microbial contamination, mix-up, and cross-contamination should be described specifically.

(2) Matters warranting special attention in the manufacturing process

For manufacture of human (autologous) bone marrow-derived mesenchymal stem cells (final product), the manufacturing method should be clarified and validated for the following items to the extent possible to ensure certain quality.

Manufacturing method

The manufacturing method up to release of the final product should be outlined, including acceptance of a human (autologous) bone marrow tissue (fluid) containing bone marrow-derived mesenchymal stem cells at the manufacturing site and a culture process of human (autologous) bone marrow-derived mesenchymal stem cells, the starting material. In addition, the treatment, necessary process control, and quality control should be specified in detail.

a) Acceptance inspection

For the collected human (autologous) bone marrow tissue (fluid) containing bone marrow-derived mesenchymal stem cells, 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. In addition, the following items should be checked: (A) transportation status of the tissue (e.g., whether the tissue is sealed in an insulated container, how long it has taken from shipping); and (B) appearance of the human (autologous) bone marrow tissue (fluid) containing bone marrow-derived mesenchymal stem cells (e.g., whether the tube for transportation is damaged or leaking, whether the human (autologous) bone marrow tissue (fluid) containing bone marrow-derived mesenchymal stem cells has been immersed in a fluid for transportation, whether the fluid for transportation is free from contamination).

b) Inactivation and removal of bacteria, fungi, viruses, etc.

Where necessary and possible, the collected human (autologous) bone marrow tissue (fluid) containing bone marrow-derived mesenchymal stem cells should be subjected to treatment for inactivation or removal of bacteria, fungi, viruses, etc. to the extent that would not affect the phenotype, genetic traits, specific functions, cell viability, or quality. Measures and evaluation methods for the concerned treatment should be specified.

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

In manufacture of human (autologous) bone marrow-derived mesenchymal stem cells (final products), 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 specifications 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.

[1] Cell morphology

For cell morphology, for example, microscopy (inverted or confocal) may be performed to confirm that human (autologous) bone marrow-derived mesenchymal stem cells are spindle-shaped.

[2] Cell count and viability

Cell count may be determined using a cell suspension of the final product (or using an enzyme-treated portion of the final product if it is cell mass) 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.

[3] Identification

Identity of the intended cells should be confirmed based on appropriately selected items, such as morphological characteristics, biochemical markers, immunological markers, characteristic produced substances, and other appropriate markers of phenotype.

[4] Performance test and characterization

To investigate changes associated with processing, the processed human (autologous) bone marrow-derived mesenchymal stem cells should be analyzed for items, for example, morphological characteristics, growth characteristics, biochemical markers, immunological markers, characteristic produced substances, karyotypes, and other appropriate markers of phenotype (e.g., expression of surface markers CD73, CD90, and CD105). In addition, a functional analysis should be performed as necessary (e.g., confirmation of differentiation into adipocytes, osteocytes, and chondrocytes) (Literature 1). Importance should be attached to analyses and evaluation of quality attributes in view of the mechanism of action of the product under development.

[5] Cell purity

A test method to calculate the proportion of mesenchymal stem cells, the intended cells, and the acceptance criteria should be established. For example, appropriate phenotype markers (e.g., expression of surface markers, CD73, CD90, and CD105) may be used for the calculation. If a human (autologous) bone marrow tissue (fluid) containing bone marrow-derived mesenchymal stem cells is used to culture human (autologous) bone marrow-derived mesenchymal stem cells, unintended cells potentially present in the culture may include hemocytes, fibroblasts, and adipocytes.

[6] Sterility test and mycoplasma test

For sterility of the final product, adequate evaluation using test samples should be performed in advance to ensure sterility throughout the manufacturing process. The final product should be demonstrated to be sterile (absence of common bacteria and fungi) by test prior to application to the patient. Appropriate mycoplasma test should be performed as well. For mycoplasma test, validated nucleic acid amplification methods may be used. If results from the sterility test with the final product are obtained only after administration to the patient, measures to be taken when sterility is denied after that should be specified in advance. In this case, the intermediate product should be demonstrated to be sterile by test, and sterility of the subsequent process up to release of the final product should be strictly controlled. If the test provides results only after administration to the patient, applicability

should be determined based on the latest data, but even in this case, sterility test with the final product must be performed without fail. Use of antibiotics in cell culture systems should be avoided wherever possible, but if they are used, treatment should be performed to ensure that the sterility test is not affected.

[7] Bacterial endotoxins

The test should be performed with the impact of contaminants in the sample taken into account. The acceptance limit does not have to be always specified based on measured values but may be specified in view of the safety margin on the basis of a single dose of the final product, as defined in the Japanese Pharmacopoeia. The test may be included as in-process control test. In this case, the criteria should be specified in view of the process validation results and justified.

[8] Process-related impurities

If bovine serum is used, the specification and test method for residual bovine serum albumin should be established, and the acceptance limit should be specified based on measured values. For animal-derived ingredients, antibiotics, etc., individuals with a history of allergies to these substances should be appropriately assessed for donor eligibility.

(4) Stability testing of products

The final product 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 or the final product is transported, the respective conditions and procedures (including containers, transportation fluid, and temperature control) should be specified and justified. For transportation by air, impacts of air pressure fluctuations and X rays used in the inspection should be taken into account. 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) Nonclinical studies

Because human cell processed products may induce heterologous immune response in animal studies and are not applicable to exposure evaluation as done with low-molecular-weight drugs, etc., quantitative risk assessment is difficult, and nonclinical safety studies are considered to only provide limited safety information. Such limitations must be understood when nonclinical safety studies of human cell processed products are considered. The “Technical Guidance for Quality, Nonclinical Safety Studies and Clinical Studies of

Regenerative Medical Products (Human Cell-Processed Products)" (Notification No. 0614043 of PMDA, dated June 14, 2016, of the Pharmaceuticals and Medical Devices Agency) should be referred to.

[1] General toxicity studies

To avoid heterologous immune response to human cells, immunodeficient animals may be used. Evaluation may be performed in at least 2 groups, using control and test article. For the highest dose, the largest possible number of cells should be specified to evaluate the hazard (toxicity) in consideration of the maximum tolerated dose, maximum feasible dose, and animal welfare. The dosing frequency should reflect the intended dosage regimen in clinical settings, and the route of application should also be the same as that intended in clinical settings if possible. However, repeated-dose studies may not have to be always conducted if repeated administration of a human cell processed product is unlikely to cause product accumulation in the body and thus worsening of toxicity findings. Although the observation period may be up to 14 days after the last dose, which is the shortest period acceptable for evaluation of systemic toxicity, an appropriate study period should be specified with reference to Proof-of-Concept (POC) studies, etc.

[2] Tumorigenicity studies

The human (autologous) bone marrow-derived mesenchymal stem cell-processed products that have been manufactured through culture and processing under the process control in compliance with the Ministerial Ordinance on Good Gene, Cellular and Tissue-based Products Manufacturing Practice (GCTP) for Regenerative Medical Products (MHLW Ordinance No. 93 of 2014) and qualified by cell growth characterization using cells cultured beyond the specified culture period should be subjected to *in vitro* tumorigenicity evaluation but may not have to be subjected to *in vivo* studies in immunodeficient animals.

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

Expression of the function, durability of the action, and feasibility of clinical effects (POC) expected for the human (autologous) bone marrow-derived mesenchymal stem cell-processed product should be presented using animal models appropriate for the target disease to the extent technically possible and scientifically reasonable. Appropriate animal models may be mice and rats (dogs) with carbon tetrachloride-induced cirrhosis, but they should be validated for efficacy or performance evaluation of the final product (desirable effects in the cirrhosis animal model are (a) anti-inflammatory effects such as decreases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT); (b) liver function improvements such as an increase in albumin and a decrease in total bilirubin; and (c) anti-fibrogenesis effects such as improvement in fibrotic area stained by Sirius Red and improvement of hydroxyproline assay results). The mechanism of action to exert the efficacy or performance of the final product should be investigated if possible.

(6) Clinical studies (clinical trials)

The clinical data package and the protocol should be designed in view of nonclinical data, etc. according to the target disease, intended indication or performance, expected clinical positioning of the treatment method, etc. In studies at an early stage of development such as a first-in-human study, safety must be carefully evaluated. In general, to collect efficacy and safety information scientifically in clinical studies regardless of blinding status, comparative

study designs including a concurrent control group such as a placebo group are desirable. However, any clinical study should be appropriately designed in view of objective of the study, study population to be selected, clinical positioning of the regenerative medical product under development, attributes of the product, and characteristics of endpoints. In addition, the endpoints should be specified for each product. For planning a clinical trial in detail, regulatory science strategy consultations (R&D) or clinical trial consultations at the Pharmaceuticals and Medical Devices Agency (PMDA) should be used if possible.

[1] Basic principles on evaluation technology in clinical studies

Clinical trials should be designed to minimize risks related to the protection of the rights, safety and welfare of human subjects as well as the quality of data and to maximize evaluation capability of the efficacy of the test product. 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. The primary efficacy endpoint specified for a confirmatory study should evidently represent clinical significance of the product. Hard endpoints in the treatment of decompensated cirrhosis may include liver-related death and onset of hepatic failure. If possible, endpoints should cover all or a part of parameters appropriate for assessment of anti-inflammatory and liver-damage mitigation, antifibrotic, and liver function improvement effects for a certain period of time after administration of the cells.

[2] Target disease

Decompensated cirrhosis:

Cirrhosis represents the final stage of liver disease. The lesion has regenerative nodules formed across the liver as a consequence of chronic liver disorders, such as hepatitis B, hepatitis C, nonalcoholic steatohepatitis, alcoholic hepatitis, and autoimmune hepatitis, surrounded by fibrous septa. Cirrhosis can be divided into compensated cirrhosis and decompensated cirrhosis: the former has hardly any clinical symptoms and leaves the liver function relatively maintained, while the latter has symptoms attributable to hepatic failure, such as hepatic encephalopathy, jaundice, ascites, edema, or bleeding tendency. In general, Child-Pugh B or severer cirrhosis (with a Child-Pugh score of 7 points or higher or a past history and prior treatment of decompensated cirrhosis) is deemed decompensated cirrhosis.

The liver is known to have a very high regenerative capacity, but in cirrhosis, the regenerative capacity decreases as fibrosis develops. In compensated cirrhosis, removal of underlying causes such as hepatitis B and C viruses, alcohol, and hepatic steatosis is known to mitigate fibrosis and induce regeneration, while in decompensated cirrhosis, the liver is known to be losing capabilities of regenerating and recovering from fibrosis and unlikely to restore the function. At the present time, decompensated cirrhosis is no longer considered irreversible because of emergence of new drug therapies in addition to the existing ones, and these therapies can be expected to improve the liver function (hepatic reserve). If possible, the study population should include patients who have received these basic therapeutic interventions for a certain period of time. Still, development of new therapies such as cell therapy that promotes regeneration and mitigates fibrosis is awaited for the patients who continue to have a Child-Pugh score of 7 points or higher (at 2 consecutive tests at least 90 days apart [within 180 days], as required in the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare) for a certain

period of time despite such therapeutic intervention. Patients with decompensated cirrhosis which persists at Child-Pugh Class B or severer should be included in the study. In addition, especially for patients with advanced cirrhosis, liver transplantation is known as a radical treatment, but a chance of brain-dead liver transplantation occurs infrequently, and liver transplantation cases mostly depend on living donors in Japan. From either donor, chances of liver transplantation are limited. Cell therapy is expected to offer a valuable treatment chance to patients with such a background.

Because a part of decompensated cirrhosis cases can be at Child-Pugh C (severe liver dysfunction), patients with severe liver dysfunction, if enrolled in a study, would be more likely to experience serious adverse events, requiring attention.

In addition, although the following findings are generally applicable to any type of cirrhosis, it is the origin of hepatocellular carcinoma and known to be complicated by gastroesophageal varices. Therefore, screening with preliminary examination and post-treatment follow-up should be carefully performed.

For Child-Pugh score calculation, which is also explained in the clinical efficacy evaluation section below, certain criteria need to be developed, because 2 different forms (activity value, %; international normalized ratio, INR) are used to express prothrombin time (PT), and assessment of encephalopathy and ascites may be affected by subjective judgment. If possible, criteria should be developed as follows: PT should be obtained as an activity value (%); severity of ascites should be assessed with reference to the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare, which specify that the volume of ascites should be estimated based on the ultrasonography findings, body weight change, and volume of drainage by puncture in principle, and presence of approximately ≥1 and ≥3 L of ascites should be assessed as mild and moderate or severe (Child-Pugh score of 2 and 3 points), respectively; and severity of encephalopathy should be assessed according to the coma classification system for hepatic encephalopathy established at the Inuyama Symposium (none, 1 point; mild I to II, 2 points; coma grade III or higher, 3 points), and Covert (subclinical) encephalopathy defined in the International Society for Hepatic Encephalopathy and Metabolism (ISHEN) criteria should be given 1 point.

[3] Clinical efficacy evaluation

A. Clinical information

For clinical information, necessary tests and examinations should be selected according to the purpose based on findings from consultations, hematology tests, imaging, and liver biopsy. Before enrollment in a clinical study, patients should be confirmed to have not had responded to the existing therapeutic interventions with inflammation, fibrosis, or hepatic function remaining unchanged for a certain period of time (e.g., for 3 months or at least 90 days and within 180 days, as required in the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare). Furthermore, efficacy evaluation requires considerations that the patient's response may be modified by additional new drugs (including albumin preparation and fresh frozen plasma), dose changes of drugs, and procedures such as puncture and drainage of ascites.

B. Clinical efficacy evaluation

The primary efficacy evaluation based on occurrence of events (e.g., death, hepatic failure, liver transplantation) would be useful in discussion of the clinical significance. Rating scales, if used, should be ones that have been assessed for reliability and validity and internationally accepted. For the evaluation, a change in rating scale score from baseline and proportion of patients with improvement at the evaluation timepoint should be used. For the change in rating scale score, whether the clinical significance can be justified is of importance, and durability of the effect should weigh as well. Secondary efficacy evaluation is useful not only to validate the results on the primary endpoint but also to discuss the results obtained for the clinical significance (Literature 2).

Cell therapies are expected to alter liver function and fibrosis. To evaluate efficacy, appropriate endpoints should be established. In addition, because a certain period of time is supposed to be taken until improvements on these endpoints, timing of evaluation should be considered in view of the mechanism of action of transplanted cells and feasibility of the study with reference to results from studies at prior phases. If possible, evaluation should be performed not only at the final timepoint (e.g., at 6 months or 1 year) but also at an appropriate frequency to obtain data on changes over time. Parameters of potential interest in evaluation on each aspect are provided below.

a) Improvement of hepatic function (hepatic reserve)

Because liver function can be evaluated by many measures, understanding their characteristics is of importance for the evaluation. Child-Pugh Classification (score) is the representative measure. This is a system used to assess the liver function by scoring values or assessment results on albumin, total bilirubin, PT, ascites, and hepatic encephalopathy The other liver function measures include albumin-bilirubin (ALBI) score or grade, which is calculated from liver function parameter values as done for Child-Pugh score. In addition, because improvement of liver function may be accompanied by alleviation of malaise, itching, edema, etc., evaluation of patient-reported outcomes and physical findings can be useful. In addition, MELD score is also one of the other measures, calculated from values on total bilirubin, PT, and serum creatinine, and reported to be useful especially in predicting prognosis of cirrhosis awaiting liver transplantation. (Literature 3)

b) Antifibrotic effect

The evaluation can be performed by many approaches such as blood marker search (e.g., hyaluronic acid, 7S domain of type IV collagen, P-III-P, M2BPGi, autotaxin), mathematical assessment using blood test results, measurement of liver stiffness using fibroscan, ultrasonography, MRI, etc., and tissue assessment via liver biopsy. If possible, multiple approaches should be used in combination for the evaluation, because there are some cases where data obtained by one approach were not enough to detect a certain trend. Of note, the evaluation of decompensated cirrhosis, which has bleeding tendency, can dispense with liver biopsy if adequately performed by non-invasive approaches.

Blood tests on AST, ALT, alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), PT, platelet count, etc. will provide useful information. Quality-of-life questionnaire scores as well as measurement of liver and spleen volumes can be used as exploratory indirect indicators of hepatic reserve, improvement in liver fibrosis, and regeneration.

For an quantitative indicator of ascites, the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare specify as follows: "the volume of ascites should be estimated based on the ultrasonography findings, body weight change, and volume of drainage by puncture in principle, and presence of approximately ≥1 and ≥3 L of ascites should be assessed as mild and moderate or severe (Child-Pugh score of 2 and 3 points), respectively." In view of the unstable nature of ascites, of which volume is likely to fluctuate, a certain criterion should be in place for evaluation of a therapeutic intervention, for example, resolution of ascites in patients receiving diuretics is given 1 point. The European Association for the Study of Liver (EASL) guidelines presented in the Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology may be used as references. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

For hepatic encephalopathy, multiple classification systems are available, including hepatic encephalopathy coma grades I to V established at the Inuyama Symposium in Japan and internationally the West Heaven Criteria (WHC) and ISHEN criteria. If possible, certain criteria should be established for the evaluation. For example, if possible, according to the hepatic encephalopathy coma grading system established at the Inuyama Symposium, the severity of hepatic encephalopathy should be assessed based on the coma condition (none, 1 point; mild I to II, 2 points; coma grade III or higher, 3 points), and according to the ISHEN criteria, Covert (subclinical) encephalopathy should be given 1 point. The hepatic encephalopathy coma grades established at the Inuyama Symposium and the Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

To express PT, 2 different forms of activity value (%) and INR are used. If possible, both activity value (%) and INR should be determined. For the evaluation, it should be noted that these values can easily vary with use of oral anticoagulants such as warfarin.

C. Safety evaluation

To evaluate safety of human (autologous) bone marrow-derived mesenchymal stem cell-processed products, recipients should be monitored for systemic and local findings as well as subjective symptoms from application of the product to the end of the follow-up period. That is, recipients should be monitored for adverse events, infections, and allergic reactions. After administration of the cells, adequate attention should be paid to findings indicative of embolism and thrombosis. If the cells are administered into a peripheral vein,

measurements to detect potential signs of pulmonary embolism are recommended, covering percutaneous arterial oxygen saturation during administration or for a certain period of time after that as well as D-dimer and fibrinogen degradation products (FDPs) after that. If the cells are administered via the hepatic artery, attention should be paid to not only safety of the catheterization procedure but also post-dose increases in hepatobiliary enzymes. Furthermore, if possible, incidences of decompensated liver disease-related events (esophageal and gastric varices hemorrhage, ascites, hepatorenal syndrome, hepatic encephalopathy), development of hepatocellular carcinoma, and deaths should be followed up concurrently.

## 6. LITERATURE REFERENCES


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## 7. Reference data


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- The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology
- “Disability Accreditation Criteria,” an appendix to the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare (SWWVRB/DHWPD Notification No. 0110001, dated January 10, 2003, of the Department of Health and Welfare for Persons with Disabilities, Social Welfare and War Victims' Relief Bureau, MHLW), the “Partial Amendment of the “Guidance for the Grade Table of Disability Severity (Disability Accreditation Criteria)”” (SWWVRB/DHWPD Notification No. 0204-1, dated February 4, 2016, of the Department of Health and Welfare for Persons with Disabilities, Social Welfare and War Victims' Relief Bureau, MHLW

(Appendix 2)

# Guidance on Evaluation for the Treatment of Decompensated Cirrhosis using Human Allogeneic Adipose Tissue-Derived Mesenchymal Stem Cells-Processed Products

## 1. Introduction

Basic technical requirements for ensuring quality and safety of human (allogeneic) cell-processed products, a class of regenerative medical products ("regenerative medical products" 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]. The same shall apply hereinafter.) are defined in the "Guidelines on Ensuring Quality and Safety of Products Derived from Processed Cell and Tissue (Allogeneic somatic stem cells)" (PFSB Notification No. 0907-3, dated September 7, 2012, of the Pharmaceutical and Food Safety Bureau of the Ministry of Health, Labour and Welfare [MHLW]. Hereinafter referred to as "Guideline for Human (allogeneic) Somatic Stem Cell-Processed Products"). The guidance on evaluation provides, in addition to the above basic technical requirements, points to consider for a particular class of human (allogeneic) adipose tissue-derived mesenchymal stem cell-processed products that are used as regenerative medical products applied for treatment of decompensated cirrhosis.

## 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 human (allogeneic) adipose tissue-derived mesenchymal stem cell-processed products when applied for treatment of decompensated cirrhosis.

## 3. Positioning of the guidance on evaluation

The guidance on evaluation, which applies to human (allogeneic) adipose tissue-derived mesenchymal stem 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.

For evaluation required for individual products, consultation with the Pharmaceuticals and Medical Devices Agency (PMDA) is strongly recommended. In addition to the guidance on evaluation, other related guidelines in and outside Japan should be referred to.

## 4. Definitions of terms

The terms in the guidance on evaluation are as defined in the "Guideline for Human (allogeneic) Somatic Stem Cell-Processed Products" or defined as follows.

(1) Hepatic cirrhosis (or cirrhosis): The term refers to a pathological condition representative of end stage of chronic liver disease or a condition of decreased liver function associated with fibrous tissue hyperplasia resulting from a process of regeneration of hepatocytes in response to their necrosis, which is a consequence of repeated liver injury caused by

chronic inflammation, such as hepatitis B, hepatitis C, alcohol, NASH (nonalcoholic steatohepatitis), autoimmune disease, metabolic abnormality, and cholestasis.

(2) Decompensated cirrhosis: The term refers to cirrhosis with symptoms attributable to hepatic failure, such as hepatic encephalopathy, jaundice, ascites, edema, or bleeding tendency. Compensated cirrhosis, on the other hand, has hardly any clinical symptoms and leaves the liver function relatively maintained. In general, cirrhosis with a Child-Pugh score of 7 points or higher given according to (3) is commonly deemed decompensated cirrhosis. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

(3) Child-Pugh score: The term refers to a method to assess functional severity of cirrhosis by rating the following 5 items on a scale of 1 to 3: [1] hepatic encephalopathy (none = 1, mild = 2, coma = 3); [2] ascites (none = 1, mild = 2, moderate or higher = 3); [3] serum bilirubin (mg/dL) (<2.0 = 1, 2.0-3.0 = 2, >3.0 = 3); [4] serum albumin (>3.5 = 1, 2.8-3.5 = 2, <2.8 = 3); and [5] prothrombin time (activity value by thrombo test, %) (>70 = 1, 40-70 = 2, <40 = 3). According to the score, the disease is classified into Class A (5-6 points), Class B (7-9 points), or Class C (10-15 points), and the disease with 7 points or higher is defined as decompensated cirrhosis as done above. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

(4) Mesenchymal stem cells: The term refers to cells that are classified as a type of somatic stem cells derived from mesodermal tissue (mesenchyme) and meet the following 3 conditions: [1] they adhere to a plastic culture container; [2] they are positive for CD105, CD73, and CD90 as well as negative for CD45, CD34, CD14, CD11b, CD79a, CD19, and HLA-Class II (DR); and [3] they have a potential to differentiate into mesenchymal cells (bone, fat, and cartilage). The cells can be isolated from fat tissue, bone marrow, umbilical cord, and pulp. They lack MHC Class-II, modulate immune response by secreting cytokines and growth factors, and are characterized by their nature to promote tissue regeneration and repair.

(5) Model for End-Stage Liver Disease (MELD) score: The term refers to a calculation formula used to predict the short-term prognosis and to determine the indication for liver transplantation in patients with decompensated cirrhosis aged ≥12 years. The score is calculated using the following 3 parameters: [1] serum bilirubin; [2] prothrombin time-international normalized ratio (PT-INR); and [3] serum creatinine.

MELD score = 9.57 ln (serum creatinine, mg/dl) + 3.78 ln (serum bilirubin, mg/dl) + 11.20 ln (PT-INR [blood coagulation ability]) + 6.43

(6) 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])

(7) Raw materials, etc.: The term refers to raw materials or materials or their source materials. (as defined in the Standards for Biological Raw Materials [Public Notice of the Ministry of Health, Labour and Welfare No. 210 of 2003])

(8) 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 “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])

(9) 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.

## 5. Points to consider for evaluation

The guidance on evaluation is intended to address processed allogeneic cells to be applied to the liver for treatment of decompensated cirrhosis. A raw material of the cells is a human (allogeneic) adipose tissue containing mesenchymal stem cells, which is accepted, cultured for generation of a cell bank system, and processed at the manufacturing site to manufacture the cells.

(1) Raw materials, etc.

In principle, control items for raw materials (human adipose tissue) and materials (e.g., bovine serum, culture media) as well as source materials used for manufacture of the preceding materials should be specified to ensure that the final product has quality required. Especially, the control items for the raw materials, etc. must be specified in consideration of their quality (e.g., sterility, impurities) to ensure that their use will not raise safety concerns with the final product. To control the risk of contamination with adventitious agents such as viruses, control items should be specified with reference to necessary information obtained based on the "Standards for Biological Raw Materials." For the scope of raw materials, etc. regulated under the "Standards for Biological Raw Materials," the "Application of Standards for Biological Raw Materials" (Joint Notification of PFSB/ELD Notification No. 10021-1 and PFSB/ELD/OMDE/CMS Notification No. 1002-5, dated October 2, 2014, of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, and Counsellor to the Minister's Secretariat [in charge of review management of medical devices and regenerative medical products], MHLW) should be referred to.

[1] Donor inclusion criteria, eligibility

Verification should be presented that the donor has been selected in an ethically appropriate manner. Inclusion criteria and eligibility criteria should be defined in consideration of age, gender, ethnic characteristics, medical history, health status, test items for various infections that may be transmitted via collected cells or tissues, immunocompatibility, etc. and justified.

Particularly, hepatitis B virus (HBV) (if possible, not only indicators suggestive of ongoing infection such as HBs antigen and HBV-DNA but also ones suggestive of past infection such as HBs and HBc antibodies should be determined), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human T-cell leukemia virus (HTLV), and parvovirus B19 infections should be ruled out by tests (e.g., serological test, nucleic acid amplification method). Cytomegalovirus (CMV), Epstein-Barr virus (EBV), and West Nile virus (WNV) infections should be ruled out by tests as necessary.

In addition, medical conditions listed below should be inspected through medical history and interview. Based on the obtained information and presence or absence of prior blood transfusion and transplantation therapy, whether the individual is eligible for donation should be determined.

- Infections with bacteria such as *Treponema pallidum*, *Chlamydia*, *Neisseria gonorrhoeae*, and *Mycobacterium tuberculosis*
- Sepsis and suspected sepsis
- Malignancy
- Serious metabolic and endocrine disorders
- Collagen disease and blood disorders
- Liver disease
- Transmissible spongiform encephalopathy and suspected transmissible spongiform encephalopathy as well as other dementia
- Specific genetic diseases and family history
- Pathological condition that precludes collection of cells/tissues to be used as a raw material (e.g., hemorrhagic condition)

[2] Donor records

For cells or tissues used as a raw material, donor records should be organized and retained to ensure that information necessary for ensuring safety is accessible. The measures should be presented in detail.

[3] Collection of human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells

A reason for selecting collection sites of a human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells and the collection method should be described. The selected site and the method should be justified scientifically and ethically. For the collection method, apparatuses to be used and preventive measures against microbial contamination, mix-up, and cross-contamination should be described specifically.

(2) Matters warranting special attention in the manufacturing process

For manufacture of human (allogeneic) adipose tissue-derived mesenchymal stem cells (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 acceptance of a human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells at the manufacturing site, a history of generation of a cell bank system of human (allogeneic) adipose tissue-derived mesenchymal stem cells, the starting material, and a culture process of human (allogeneic) adipose tissue-derived mesenchymal stem cells. In addition, the treatment, necessary process control, and quality control should be specified in detail.

a) Acceptance inspection

For the collected human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells, 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. In addition, the following items should be checked: (A) transportation status of the tissue (e.g., whether the tissue is sealed in an insulated container, how long it has taken from shipping); and (B) appearance of the human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells (e.g., whether the tube for transportation is damaged or leaking, whether the human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells has been immersed in a fluid for transportation, whether the fluid for transportation is free from contamination).

b) Inactivation and removal of bacteria, fungi, viruses, etc.

Where necessary and possible, the collected human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells should be subjected to treatment for inactivation or removal of bacteria, fungi, viruses, etc. to the extent that would not affect the phenotype, genetic traits, specific functions, cell viability, or quality. Measures and evaluation methods for the concerned treatment should be specified.

c) Cell banking

Methods of generation of cell banks of human (allogeneic) adipose tissue-derived mesenchymal stem cells from the human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells 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. To address the risks not attributable to donors during the manufacturing process, especially the risk of viral contamination, necessary tests for viruses should be performed with the master cell bank (MCB) and cells cultured beyond the specified culture period.

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

In manufacture of human (allogeneic) adipose tissue-derived mesenchymal stem cells (final products), 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 specifications 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.

[1] Cell morphology

For cell morphology, for example, microscopy (inverted or confocal) may be performed to confirm that human (allogeneic) adipose tissue-derived mesenchymal stem cells are spindle-shaped.

[2] Cell count and viability

Cell count may be determined using a cell suspension of the final product (or using an enzyme-treated portion of the final product if it is cell mass) 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.

[3] Identification

Identity of the intended cells should be confirmed based on appropriately selected items, such as morphological characteristics, biochemical markers, immunological markers, characteristic produced substances, and other appropriate markers of phenotype.

[4] Performance test and characterization

To investigate changes associated with processing, the processed human (allogeneic) adipose tissue-derived mesenchymal stem cells should be analyzed for items, for example, morphological characteristics, growth characteristics, biochemical markers, immunological markers, characteristic produced substances, karyotypes, and other appropriate markers of phenotype (e.g., expression of surface markers CD73, CD90, and CD105). In addition, a functional analysis should be performed as necessary (e.g., confirmation of differentiation into adipocytes, osteocytes, and chondrocytes) (Literature 1). Importance should be attached to analyses and evaluation of quality attributes in view of the mechanism of action of the product under development.

[5] Cell purity

A test method to calculate the proportion of human (allogeneic) adipose tissue-derived mesenchymal stem cells, the intended cells, and the acceptance criteria should be established. For example, appropriate phenotype markers (e.g., expression of surface markers, CD73, CD90, and CD105) may be used for the calculation. If a human (allogeneic) adipose tissue containing adipose tissue-derived mesenchymal stem cells is used to culture human (allogeneic) adipose tissue-derived mesenchymal stem cells, unintended cells potentially present in the culture may include hemocytes, fibroblasts, and adipocytes.

[6] Sterility test and mycoplasma test

For sterility of the final product, adequate evaluation using test samples should be performed in advance to ensure sterility throughout the manufacturing process. The final product should be demonstrated to be sterile (absence of common bacteria and fungi) by test prior to application to the patient. Appropriate mycoplasma test should be performed as well. For mycoplasma test, validated nucleic acid amplification methods may be used. If results from the sterility test with the final product are obtained only after administration to the patient, measures to be taken when sterility is denied after that should be specified in advance. In this case, the intermediate product should be demonstrated to be sterile by test, and sterility of the subsequent process up to release of the final product should be strictly controlled. If products manufactured through the same process at the same facility have been applied to the other patients, all of them should be demonstrated to be sterile by test. If the product is manufactured on a batch basis and warranted to be hermetically packaged, representative samples may be tested. Use of antibiotics in cell culture systems should be avoided wherever possible, but if they are used, treatment should be performed to ensure that the sterility test is not affected.

[7] Bacterial endotoxins

The test should be performed with the impact of contaminants in the sample taken into account. The acceptance limit does not have to be always specified based on measured

values but may be specified in view of the safety margin on the basis of a single dose of the final product, as defined in the Japanese Pharmacopoeia. The test may be included as in-process control test. In this case, the criteria should be specified in view of the process validation results and justified.

[8] Process-related impurities

If bovine serum is used, the specification and test method for residual bovine serum albumin should be established, and the acceptance limit should be specified based on measured values. For animal-derived ingredients, antibiotics, etc., the package insert should include a statement to the effect that this product should not be used in patients with a history of allergies to these substances.

(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. For transportation by air, impacts of air pressure fluctuations and X rays used in the inspection should be taken into account. 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) Nonclinical studies

Because human cell processed products may induce heterologous immune response in animal studies and are not applicable to exposure evaluation as done with low-molecular-weight drugs, etc., quantitative risk assessment is difficult, and nonclinical safety studies are considered to only provide limited safety information. Such limitations must be understood when nonclinical safety studies of human cell processed products are considered. The “Technical Guidance for Quality, Nonclinical Safety Studies and Clinical Studies of Regenerative Medical Products (Human Cell-Processed Products)” (Notification No. 0614043 of PMDA, dated June 14, 2016, of the Pharmaceuticals and Medical Devices Agency) should be referred to.

[1] General toxicity studies

To avoid heterologous immune response to human cells, immunodeficient animals may be used. Evaluation may be performed in at least 2 groups, using control and test article. For the highest dose, the largest possible number of cells should be specified to evaluate the hazard (toxicity) in consideration of the maximum tolerated dose, maximum feasible dose, and animal welfare. The dosing frequency should reflect the intended dosage regimen in clinical settings, and the route of application should also be the same as that intended in clinical settings if possible. However, repeated-dose studies may not have to be always conducted if repeated administration is unlikely to cause product accumulation in the body and thus worsening of toxicity findings. Although the observation period may be up to 14 days after the last dose, which is the shortest period acceptable for evaluation of systemic toxicity, an appropriate study period should be specified with reference to Proof-of-Concept (POC) studies, etc.

[2] Tumorigenicity studies

The human (allogeneic) adipose tissue-derived mesenchymal stem cell-processed products that have been manufactured through culture and processing under the process control in compliance with the Ministerial Ordinance on Good Gene, Cellular and Tissue-based Products Manufacturing Practice (GCTP) for Regenerative Medical Products (MHLW Ordinance No. 93 of 2014) and qualified by cell growth characterization using cells cultured beyond the specified culture period should be subjected to *in vitro* tumorigenicity evaluation but may not have to be subjected to *in vivo* studies in immunodeficient animals.

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

Expression of the function, durability of the action, and feasibility of clinical effects (POC) expected for the human (allogeneic) adipose tissue-derived mesenchymal stem cell-processed product should be presented using animal models appropriate for the target disease to the extent technically possible and scientifically reasonable. Appropriate animal models may be mice and rats (dogs) with carbon tetrachloride-induced cirrhosis, but they should be validated for efficacy or performance evaluation of the final product (desirable effects in the cirrhosis animal model are (a) anti-inflammatory effects such as decreases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT); (b) liver function improvements such as an increase in albumin and a decrease in total bilirubin; and (c) anti-fibrogenesis effects such as improvement in fibrotic area stained by Sirius Red and improvement of hydroxyproline assay results). The mechanism of action to exert the efficacy or performance of the final product should be investigated if possible.

(6) Clinical studies (clinical trials)

The clinical data package and the protocol should be designed in view of nonclinical data, etc. according to the target disease, intended indication or performance, expected clinical positioning of the treatment method, etc. In studies at an early stage of development such as a first-in-human study, safety must be carefully evaluated. In general, to collect efficacy and safety information scientifically in clinical studies regardless of blinding status, comparative study designs including a concurrent control group such as a placebo group are desirable. However, any clinical study should be appropriately designed based on the potential for development in view of objective of the study, study population to be selected, clinical

positioning of the regenerative medical product under development, attributes of the product, and characteristics of endpoints. In addition, the endpoints should be specified for each product. For planning a clinical trial in detail, regulatory science strategy consultations (R&D) or clinical trial consultations at the Pharmaceuticals and Medical Devices Agency (PMDA) should be used if possible.

[1] Basic principles on evaluation technology in clinical studies

Clinical trials should be designed to minimize risks related to the protection of the rights, safety and welfare of human subjects as well as the quality of data and to maximize evaluation capability of the efficacy of the test product. 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. The primary efficacy endpoint specified for a confirmatory study should evidently represent clinical significance of the product. Hard endpoints in the treatment of decompensated cirrhosis may include liver-related death and onset of hepatic failure. If possible, endpoints should cover all or a part of parameters appropriate for assessment of liver function (hepatic reserve) improvement and antifibrotic effects for a certain period of time after administration of the cells.

[2] Target disease

Decompensated cirrhosis:

Cirrhosis represents the final stage of liver disease. The lesion has regenerative nodules formed across the liver as a consequence of chronic liver disorders, such as hepatitis B, hepatitis C, nonalcoholic steatohepatitis, alcoholic hepatitis, and autoimmune hepatitis, surrounded by fibrous septa. Cirrhosis can be divided into compensated cirrhosis and decompensated cirrhosis: the former has hardly any clinical symptoms and leaves the liver function relatively maintained, while the latter has symptoms attributable to hepatic failure, such as hepatic encephalopathy, jaundice, ascites, edema, or bleeding tendency. In general, Child-Pugh B or severer cirrhosis (with a Child-Pugh score of 7 points or higher or a past history and prior treatment of decompensated cirrhosis) is deemed decompensated cirrhosis.

The liver is known to have a very high regenerative capacity, but in cirrhosis, the regenerative capacity decreases as fibrosis develops. In compensated cirrhosis, removal of underlying causes such as hepatitis B and C viruses, alcohol, and hepatic steatosis is known to mitigate fibrosis and induce regeneration, while in decompensated cirrhosis, the liver is known to be losing capabilities of regenerating and recovering from fibrosis and unlikely to restore the function. At the present time, decompensated cirrhosis is no longer considered irreversible because of emergence of new drug therapies in addition to the existing ones, and these therapies can be expected to improve the liver function (hepatic reserve). If possible, the study population should include patients who have received these basic therapeutic interventions for a certain period of time. Still, development of new therapies such as cell therapy that promotes regeneration and mitigates fibrosis is awaited for the patients who continue to have a Child-Pugh score of 7 points or higher (at 2 consecutive tests at least 90 days apart [within 180 days], as required in the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare) for a certain period of time despite such therapeutic intervention. Patients with decompensated cirrhosis

which persists at Child-Pugh Class B or severer should be included in the study. In addition, especially for patients with advanced cirrhosis, liver transplantation is known as a radical treatment, but a chance of brain-dead liver transplantation occurs infrequently, and liver transplantation cases mostly depend on living donors in Japan. From either donor, chances of liver transplantation are limited. Cell therapy is expected to offer a valuable treatment chance to patients with such a background.

Because a part of decompensated cirrhosis cases can be at Child-Pugh C (severe liver dysfunction), patients with severe liver dysfunction, if enrolled in a study, would be more likely to experience serious adverse events, requiring attention.

In addition, although the following findings are generally applicable to any type of cirrhosis, it is the origin of hepatocellular carcinoma and known to be complicated by gastroesophageal varices. Therefore, screening with preliminary examination and post-treatment follow-up should be carefully performed.

For Child-Pugh score calculation, which is also explained in the clinical efficacy evaluation section below, certain criteria need to be developed, because 2 different forms (activity value, %; international normalized ratio, INR) are used to express prothrombin time (PT), and assessment of encephalopathy and ascites may be affected by subjective judgment. If possible, criteria should be developed as follows: PT should be obtained as an activity value (%); severity of ascites should be assessed with reference to the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare, which specify that the volume of ascites should be estimated based on the ultrasonography findings, body weight change, and volume of drainage by puncture in principle, and presence of approximately ≥1 and ≥3 L of ascites should be assessed as mild and moderate or severe (Child-Pugh score of 2 and 3 points), respectively; and severity of encephalopathy should be assessed according to the coma classification system for hepatic encephalopathy established at the Inuyama Symposium (none, 1 point; mild I to II, 2 points; coma grade III or higher, 3 points), and Covert (subclinical) encephalopathy defined in the International Society for Hepatic Encephalopathy and Metabolism (ISHEN) criteria should be given 1 point.

[3] Clinical efficacy evaluation

A. Clinical information

For clinical information, necessary tests and examinations should be selected according to the purpose based on findings from consultations, hematology tests, imaging, and liver biopsy. Before enrollment in a clinical study, patients should be confirmed to have not had responded to the existing therapeutic interventions with inflammation, fibrosis, or hepatic function remaining unchanged for a certain period of time (e.g., for 3 months or at least 90 days and within 180 days, as required in the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare). Furthermore, efficacy evaluation requires considerations that the patient's response may be modified by additional new drugs (including albumin preparation and fresh frozen plasma), dose changes of drugs, and procedures such as puncture and drainage of ascites.

B. Efficacy evaluation

The primary efficacy evaluation based on occurrence of events (e.g., death, hepatic failure, liver transplantation) would be useful in discussion of the clinical significance.

Rating scales, if used, should be ones that have been assessed for reliability and validity and internationally accepted. For the evaluation, a change in rating scale score from baseline and proportion of patients with improvement at the evaluation timepoint should be used. For the change in rating scale score, whether the clinical significance can be justified is of importance, and durability of the effect should weigh as well. Secondary efficacy evaluation is useful not only to validate the results on the primary endpoint but also to discuss the results obtained for the clinical significance (Literature 2).

Cell therapies are expected to alter liver function and fibrosis. To evaluate efficacy, appropriate endpoints should be established. In addition, because a certain period of time is supposed to be taken until improvements on these endpoints, timing of evaluation should be considered in view of the mechanism of action of transplanted cells and feasibility of the study with reference to results from studies at prior phases. If possible, evaluation should be performed not only at the final timepoint (e.g., at 6 months or 1 year) but also at an appropriate frequency to obtain data on changes over time. Parameters of potential interest in evaluation on each aspect are provided below.

a) Improvement of hepatic function (hepatic reserve)

Because liver function can be evaluated by many measures, understanding their characteristics is of importance for the evaluation. Child-Pugh Classification (score) is the representative measure. This is a system used to assess the liver function by scoring values or assessment results on albumin, total bilirubin, PT, ascites, and hepatic encephalopathy The other liver function measures include albumin-bilirubin (ALBI) score or grade, which is calculated from liver function parameter values as done for Child-Pugh score. In addition, because improvement of liver function may be accompanied by alleviation of malaise, itching, edema, etc., evaluation of patient-reported outcomes and physical findings can be useful. In addition, MELD score is also one of the other measures, calculated from values on total bilirubin, PT, and serum creatinine, and reported to be useful especially in predicting prognosis of cirrhosis awaiting liver transplantation (Literature 3).

b) Antifibrotic effect

The evaluation can be performed by many approaches such as blood marker search (e.g., hyaluronic acid, 7S domain of type IV collagen, P-III-P, M2BPGi, autotaxin), mathematical assessment using blood test results, measurement of liver stiffness using fibroscan, ultrasonography, MRI, etc., and tissue assessment via liver biopsy. If possible, multiple approaches should be used in combination for the evaluation, because there are some cases where data obtained by one approach were not enough to detect a certain trend. Of note, the evaluation of decompensated cirrhosis, which has bleeding tendency, can dispense with liver biopsy if adequately performed by non-invasive approaches.

Blood tests on AST, ALT, alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), PT, platelet count, etc. will provide useful information.

Assessment of quality of life and measurement of liver and spleen volumes can also be exploratory indicators of indirect liver function (hepatic functional reserve), improvement in liver fibrosis and regeneration.

For an quantitative indicator of ascites, the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare specify as follows: “the volume of ascites should be estimated based on the ultrasonography findings, body weight change, and volume of drainage by puncture in principle, and presence of approximately ≥1 and ≥3 L of ascites should be assessed as mild and moderate or severe (Child-Pugh score of 2 and 3 points), respectively.” In view of the unstable nature of ascites, of which volume is likely to fluctuate, a certain criterion should be in place for evaluation of a therapeutic intervention, for example, resolution of ascites in patients receiving diuretics is given 1 point. The European Association for the Study of Liver (EASL) guidelines presented in the Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology may be used as references. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

For hepatic encephalopathy, multiple classification systems are available, including hepatic encephalopathy coma grades I to V established at the Inuyama Symposium in Japan, as shown below, and internationally the West Heaven Criteria (WHC) and ISHEN criteria. If possible, certain criteria should be established for the evaluation. For example, if possible, according to the hepatic encephalopathy coma grading system established at the Inuyama Symposium, the severity of hepatic encephalopathy should be assessed based on the coma condition (none, 1 point; mild I to II, 2 points; coma grade III or higher, 3 points), and according to the ISHEN criteria, Covert (subclinical) encephalopathy should be given 1 point. The hepatic encephalopathy coma grades established at the Inuyama Symposium and the Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

To express PT, 2 different forms of activity value (%) and INR are used. If possible, both activity value (%) and INR should be determined. For the evaluation, it should be noted that these values can easily vary with use of oral anticoagulants such as warfarin.

C. Safety evaluation

To evaluate safety of human (allogeneic) adipose tissue-derived mesenchymal stem cell-processed products, recipients should be monitored for systemic and local findings as well as subjective symptoms from application of the product to the end of the follow-up period. That is, recipients should be monitored for adverse events, infections, and allergic reactions. In particular, because human (allogeneic) cells are used, recipients should be adequately monitored for potential risks of viral infection, GVHD, etc.

After administration of the cells, adequate attention should be paid to findings indicative of embolism and thrombosis. If the cells are administered into a peripheral vein, measurements to detect potential signs of pulmonary embolism are recommended, covering percutaneous arterial oxygen saturation during administration or for a certain period of time after that as well as D-dimer and fibrinogen degradation products (FDPs) after that. If the cells are administered via the hepatic artery, attention should be paid to not only safety of the catheterization procedure but also post-dose increases in hepatobiliary enzymes. Furthermore, if possible, incidences of decompensated liver disease-related events (esophageal and gastric varices hemorrhage, ascites, hepatorenal syndrome, hepatic encephalopathy), development of hepatocellular carcinoma, and deaths should be followed up concurrently.

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(Appendix 3)

Guidance on Evaluation for the Treatment of Decompensated Cirrhosis using Human Autologous CD34-Positive Peripheral Blood Stem Cells-Processed Products

## 1. Introduction

Basic technical requirements for ensuring quality and safety of human (autologous) somatic stem cell-processed products, a class of regenerative medical products ("regenerative medical products" 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]. The same shall apply hereinafter.) are defined in the "Guidelines on Ensuring Quality and Safety of Products Derived from Processed Cell and Tissue (Autologous somatic stem cells)" (PFSB Notification No. 0907-2, dated September 7, 2012, of the Pharmaceutical and Food Safety Bureau of the Ministry of Health, Labour and Welfare [MHLW]. Hereinafter referred to as "Guideline for Human (autologous) Somatic Stem Cell-Processed Products"). The guidance on evaluation provides, in addition to the above basic technical requirements, points to consider for a particular class of human (autologous) peripheral blood CD34-positive cell-processed products that are used as regenerative medical products applied for treatment of decompensated cirrhosis.

## 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 human (autologous) peripheral blood CD34-positive cell-processed products when applied for treatment of decompensated cirrhosis.

## 3. Positioning of the guidance on evaluation

The guidance on evaluation, which applies to human (autologous) peripheral blood CD34-positive 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.

For evaluation required for individual products, consultation with the Pharmaceuticals and Medical Devices Agency (PMDA) is strongly recommended. In addition to the guidance on evaluation, other related guidelines in and outside Japan should be referred to.

## 4. Definitions of terms

The terms in the guidance on evaluation are as defined in the "Guideline for Human (autologous) Somatic Stem Cell-Processed Products" or defined as follows.

(1) Hepatic cirrhosis (or cirrhosis): The term refers to a pathological condition representative of end stage of chronic liver disease or a condition of decreased liver function associated with fibrous tissue hyperplasia resulting from a process of regeneration of hepatocytes in response to their necrosis, which is a consequence of repeated liver injury caused by chronic inflammation, such as hepatitis B, hepatitis C, alcohol, NASH (nonalcoholic steatohepatitis), autoimmune disease, metabolic abnormality, and cholestasis.

(2) Decompensated cirrhosis: The term refers to cirrhosis with symptoms attributable to hepatic failure, such as hepatic encephalopathy, jaundice, ascites, edema, or bleeding tendency. Compensated cirrhosis, on the other hand, has hardly any clinical symptoms and leaves the liver function relatively maintained. In general, cirrhosis with a Child-Pugh score of 7 points or higher given according to (3) is commonly deemed decompensated cirrhosis. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

(3) Child-Pugh score: The term refers to a method to assess functional severity of cirrhosis by rating the following 5 items on a scale of 1 to 3: [1] hepatic encephalopathy (none = 1, mild = 2, coma = 3); [2] ascites (none = 1, mild = 2, moderate or higher = 3); [3] serum bilirubin (mg/dL) (<2.0 = 1, 2.0-3.0 = 2, >3.0 = 3); [4] serum albumin (>3.5 = 1, 2.8-3.5 = 2, <2.8 = 3); and [5] prothrombin time (activity value by thrombo test, %) (>70 = 1, 40-70 = 2, <40 = 3). According to the score, the disease is classified into Class A (5-6 points), Class B (7-9 points), or Class C (10-15 points), and the disease with 7 points or higher is defined as decompensated cirrhosis as done above. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

(4) Peripheral blood mononuclear cells: The term refers to mononuclear cells including monocytes and lymphocytes isolated from peripheral blood. The cells can be isolated by removing plasma components, red blood cells, platelets, and granulocytes from freshly collected human blood.

(5) CD34: The term refers to a cell surface marker expressed on many types of stem cells, including hematopoietic stem cells and vascular endothelial progenitor cells in bone marrow and peripheral blood, satellite cells in skeletal muscle, and hair follicle stem cells in the epithelium. Cells expressing this marker are referred to as CD34-positive cells.

(6) Model for End-Stage Liver Disease (MELD) score: The term refers to a calculation formula used to predict the short-term prognosis and to determine the indication for liver transplantation in patients with decompensated cirrhosis aged ≥12 years. The score is calculated using the following 3 parameters: [1] serum bilirubin; [2] prothrombin time-international normalized ratio (PT-INR); and [3] serum creatinine.
MELD score = 9.57 ln (serum creatinine, mg/dl) + 3.78 ln (serum bilirubin, mg/dl) + 11.20 ln (PT-INR [blood coagulation ability]) + 6.43

(7) 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])

(8) Raw materials, etc.: The term refers to raw materials or materials or their source materials. (as defined in the Standards for Biological Raw Materials [Public Notice of the Ministry of Health, Labour and Welfare No. 210 of 2003])

(9) 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.

## 5. Points to consider for evaluation

The guidance on evaluation is intended to address processed autologous cells to be applied to the liver for treatment of decompensated cirrhosis. A raw material of the cells is a suspension of human (autologous) peripheral blood mononuclear cells containing CD34-positive cells, which is accepted and processed at the manufacturing site to manufacture the cells.

(1) Raw materials, etc.:

In principle, control items for raw materials (human [autologous] peripheral blood mononuclear cells) and a dedicated magnetic cell isolator (an isolator capable of isolating CD34-positive cells using stem cell specific antibodies immobilized to magnetic beads [referring to antibodies against CD34 antigen in this document]) as well as source materials used for manufacture of the preceding materials and device should be specified to ensure that the final product has quality required. Especially, the control items for the source materials, etc. must be specified in consideration of their quality (e.g., sterility, impurities) to ensure that their use will not raise safety concerns with the final product.

Human (autologous) peripheral blood CD34-positive cells to be transplanted are manufactured by isolating only CD34-positive cells from a suspension of peripheral blood mononuclear cells using the dedicated magnetic cell isolator, and the manufacture does not involve any expansion culture process.

[1] Donor inclusion criteria, eligibility

If human (autologous) peripheral blood CD34-positive cells are used, the donor and recipient are the same individual, requiring no donor screening, but to prevent cross-contamination and protect safety of manufacturers, conduct of tests for the following viruses (e.g., by a serological method, nucleic acid amplification method) should be considered: hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and human T-cell leukemia virus (HTLV).

In addition, medical conditions listed below should be inspected through medical history and interview, and whether the individual is eligible for donation should be determined.

- Infections with bacteria such as *Treponema pallidum*, *Chlamydia*, *Neisseria gonorrhoeae*, and *Mycobacterium tuberculosis*
- Sepsis and suspected sepsis
- Malignancy
- Serious metabolic and endocrine disorders
- Collagen disease and blood disorders
- Transmissible spongiform encephalopathy and suspected transmissible spongiform encephalopathy as well as other dementia
- Pathological condition that precludes collection of cells to be used as a raw material (e.g., hemorrhagic condition)

[2] Collection of suspension of human (autologous) peripheral blood mononuclear cells containing CD34-positive cells

The methods to collect a suspension of human (autologous) peripheral blood mononuclear cells containing CD34-positive cells should be described and justified for the establishment scientifically and ethically. For the collection method, apparatuses to be used and preventive measures against microbial contamination, mix-up, and cross-contamination should be described specifically.

(2) Matters warranting special attention in the manufacturing process

For manufacture (isolation) of peripheral blood CD34-positive cells, the isolation method should be clarified and validated for the following items to the extent possible to ensure certain quality.

[1] Expiration date of kit

The magnetic cell isolation system is composed of the main body of the magnetic cell isolator and its dedicated product (disposable set for isolation). The product dedicated to the magnetic cell isolator has an expiration date. Before its use, the product should be confirmed to be before the expiration.

[2] Manufacturing method

The magnetic cell isolation system is capable of collecting CD34-positive cells selectively from a heterogeneous cell population in peripheral blood in a closed system through the following process: anti-CD34 antibodies immobilized to magnetic beads are firstly applied to collected apheresis harvest, allowing them to capture CD34-positive cells; and only the captured CD34-positive cells are then retained in the cell isolation column by magnetic force and finally eluted by removing the magnet.

The manufacturing method to isolate human (autologous) peripheral blood CD34-positive cells, the starting material should be outlined, including acceptance of a suspension of human (autologous) peripheral blood mononuclear cells at the manufacturing site. In addition, the treatment, necessary process control, and quality control should be specified in detail. For manufacture, a disposable set for isolation, which is the product dedicated to the magnetic cell isolator, and the dedicated isolation program should be used.

a) Acceptance inspection

When a suspension of peripheral blood mononuclear cells collected by apheresis (in an apheresis harvest bag) is accepted at the manufacturing site, pathogen test results of the donor should be checked. In addition, the following items should be checked: (A) transportation status of the cells (e.g., whether the primary container is covered with cushioning materials, whether the primary container has been transported with refrigerants in a cell transportation container); and (B) appearance of the apheresis harvest bag (e.g., whether the bag is soiled, damaged, opened, and/or leaking).

The product dedicated to the magnetic cell isolator should be checked for appearance before opening (e.g., whether the package is soiled, damaged, opened, and/or leaking).

b) Measures to prevent mix-up and contamination of cells, etc.

To prevent contamination, tools and containers that come in direct contact with the cells, etc. should be single-use products. To prevent mix-up and cross contamination during the manufacturing process, the preventive measures in the process control should be specified. For example, handling cell materials from different donors at the same time should be prohibited.

(3) Quality control of products

If quality specifications 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.

Cell quality test

[1] Cell count and viability test

For cell count and viability in the final product, criteria should be specified. Cell count may be determined using a cell suspension prepared from a portion of the final product by a method using hemocytometer or cell counter. Cell viability may be determined by flow cytometry according to the protocol based on the guideline of the International Society for Hematotherapy and Graft Engineering (ISHAGE) (ISHAGE protocol), which allows calculation of the numbers of living cells and dead cells.

Measurement of CD34-positive cells must be performed according to the ISHAGE protocol based on the ISHAGE guideline, recommended by the International Society for Cell & Gene Therapy (Literatures 1 and 2) The flow cytometry system (registered as a medical device by submission of the notification) should be subjected to precision control at each medical institution. The measurement is recommended to be performed by clinical laboratory technicians familiar with the ISHAGE protocol at each medical institution.

[2] Purity

Just isolated CD34-positive cells should be confirmed to be the intended cells by 2-color flow cytometry analysis using anti-CD34 and anti-CD45 antibodies in combination or by 3-4-color flow cytometry analysis additionally using a reagent for dead cell detection and/or internal standard particle reagent for absolute count determination in combination. Premixed antibody reagents and measurement kits covering parameters recommended in the ISHAGE guideline are commercially available, and they may be used for the confirmation.

[3] Sterility test

For sterility of the final product, adequate evaluation using test samples should be performed in advance to ensure sterility throughout the manufacturing process. Because results from the sterility test with the final product are obtained only after administration to the patient, measures to be taken when sterility is denied after that should be specified in advance.

[4] Bacterial endotoxins

The test should be performed with the impact of contaminants in the sample taken into account. The acceptance limit does not have to be always specified based on measured values but may be specified in view of the safety margin on the basis of a single dose of the final product, as defined in the Japanese Pharmacopoeia. The test may be included as in-process control test. In this case, the criteria should be specified in view of the process validation results and justified.

(4) Stability testing of products

For the final product, stability should be evaluated throughout its life up to time of use including transportation, in terms of cell viability and purity in [1] Cell count and viability test and [2] Purity under Cell quality test in (3) Quality control of products, and the storage condition and shelf life should be justified.

(5) Nonclinical studies

Because human cell processed products may induce heterologous immune response in animal studies and are not applicable to exposure evaluation as done with low-molecular-

weight drugs, etc., quantitative risk assessment is difficult, and nonclinical safety studies are considered to only provide limited safety information. Such limitations must be understood when nonclinical safety studies of human cell processed products are considered. The "Technical Guidance for Quality, Nonclinical Safety Studies and Clinical Studies of Regenerative Medical Products (Human Cell-Processed Products)" (Notification No. 0614043 of PMDA, dated June 14, 2016, of the Pharmaceuticals and Medical Devices Agency) should be referred to.

[1] General toxicity studies

To avoid heterologous immune response to human cells, immunodeficient animals may be used. Evaluation may be performed in at least 2 groups, using control and test article. For the highest dose, the largest possible number of cells should be specified to evaluate the hazard (toxicity) in consideration of the maximum tolerated dose, maximum feasible dose, and animal welfare. The dosing frequency should reflect the intended dosage regimen in clinical settings, and the route of application should also be the same as that intended in clinical settings if possible. However, repeated-dose studies may not have to be always conducted if repeated administration of a human cell processed product is unlikely to cause product accumulation in the body and thus worsening of toxicity findings. Although the observation period may be up to 14 days after the last dose, which is the shortest period acceptable for evaluation of systemic toxicity, an appropriate study period should be specified with reference to Proof-of-Concept (POC) studies, etc.

[2] Tumorigenicity studies

The human (autologous) peripheral blood CD34-positive cell-processed products that have been manufactured through processing under the process control in compliance with the Ministerial Ordinance on Good Gene, Cellular and Tissue-based Products Manufacturing Practice (GCTP) for Regenerative Medical Products (MHLW Ordinance No. 93 of 2014) do not have to be subjected to *in vitro* studies or *in vivo* tumorigenicity study in immunodeficient animals in principle, in view of preceding experience with transplantation of autologous peripheral blood CD34-positive cells.

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

Expression of the function, durability of the action, and feasibility of clinical effects (POC) expected for the human (autologous) peripheral blood CD34-positive cell-processed product should be presented using animal models appropriate for the target disease to the extent technically possible and scientifically reasonable Appropriate animal models may be mice and rats (dogs) with carbon tetrachloride-induced cirrhosis, but they should be validated for efficacy or performance evaluation of the final product (desirable effects in the cirrhosis animal model are (a) anti-inflammatory effects such as decreases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT); (b) liver function improvements such as an increase in albumin and a decrease in total bilirubin; and (c) anti-fibrogenesis effects such as improvement in fibrotic area stained by Sirius Red and improvement of hydroxyproline assay results). The mechanism of action to exert the efficacy or performance of the final product should be investigated if possible.

(6) Clinical studies (clinical trials)

The clinical data package and the protocol should be designed in view of nonclinical data, etc. according to the target disease, intended indication or performance, expected clinical positioning of the treatment method, etc. In studies at an early stage of development such as a first-in-human study, safety must be carefully evaluated. In general, to collect efficacy and safety information scientifically in clinical studies regardless of blinding status, comparative study designs including a concurrent internal control group such as a placebo group are desirable. However, any clinical study should be appropriately designed in view of objective of the study, study population to be selected, clinical positioning of the regenerative medical product under development, attributes of the product, and characteristics of endpoints. In addition, the endpoints should be specified for each product. For planning a clinical trial in detail, regulatory science strategy consultations (R&D) or clinical trial consultations at the Pharmaceuticals and Medical Devices Agency (PMDA) should be used if possible.

[1] Basic principles on evaluation technology in clinical studies

Clinical trials should be designed to minimize risks related to the protection of the rights, safety and welfare of human subjects as well as the quality of data and to maximize evaluation capability of the efficacy of the test product. 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. The primary efficacy endpoint specified for a confirmatory study should evidently represent clinical significance of the product. Hard endpoints in the treatment of decompensated cirrhosis may include liver-related death and onset of hepatic failure. If possible, endpoints should cover all or a part of parameters appropriate for assessment of anti-inflammatory and liver-damage mitigation, antifibrotic, and liver function improvement effects for a certain period of time after cell transplantation.

[2] Target disease

Decompensated cirrhosis:

Cirrhosis represents the final stage of liver disease. The lesion has regenerative nodules formed across the liver as a consequence of chronic liver disorders, such as hepatitis B, hepatitis C, nonalcoholic steatohepatitis, alcoholic hepatitis, and autoimmune hepatitis, surrounded by fibrous septa. Cirrhosis can be divided into compensated cirrhosis and decompensated cirrhosis: the former has hardly any clinical symptoms and leaves the liver function relatively maintained, while the latter has symptoms attributable to hepatic failure, such as hepatic encephalopathy, jaundice, ascites, edema, or bleeding tendency. In general, Child-Pugh B or severer cirrhosis (with a Child-Pugh score of 7 points or higher or a past history and prior treatment of decompensated cirrhosis) is deemed decompensated cirrhosis.

The liver is known to have a very high regenerative capacity, but in cirrhosis, the regenerative capacity decreases as fibrosis develops. In compensated cirrhosis, removal of underlying causes such as hepatitis B and C viruses, alcohol, and hepatic steatosis is known to mitigate fibrosis and induce regeneration, while in decompensated cirrhosis, the liver is known to be losing capabilities of regenerating and recovering from fibrosis and unlikely to restore the function. At the present time, decompensated cirrhosis is no longer considered irreversible because of emergence of new drug therapies in addition to the existing ones, and

these therapies can be expected to improve the liver function (hepatic reserve). If possible, the study population should include patients who have received these basic therapeutic interventions for a certain period of time. Still, development of new therapies such as cell therapy that promotes regeneration and mitigates fibrosis is awaited for the patients who continue to have a Child-Pugh score of 7 points or higher (at 2 consecutive tests at least 90 days apart [within 180 days], as required in the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare) for a certain period of time despite such therapeutic intervention. Patients with decompensated cirrhosis which persists at Child-Pugh Class B or severer should be included in the study. In addition, especially for patients with advanced cirrhosis, liver transplantation is known as a radical treatment, but a chance of brain-dead liver transplantation occurs infrequently, and liver transplantation cases mostly depend on living donors in Japan. From either donor, chances of liver transplantation are limited. Cell therapy is expected to offer a valuable treatment chance to patients with such a background.

Because a part of decompensated cirrhosis cases can be at Child-Pugh C (severe liver dysfunction), patients with severe liver dysfunction, if enrolled in a study, would be more likely to experience serious adverse events, requiring attention.

In addition, although the following findings are generally applicable to any type of cirrhosis, it is the origin of hepatocellular carcinoma and known to be complicated by gastroesophageal varices. Therefore, screening with preliminary examination and post-treatment follow-up should be carefully performed.

For Child-Pugh score calculation, which is also explained in the clinical efficacy evaluation section below, certain criteria need to be developed, because 2 different forms (activity value, %; international normalized ratio, INR) are used to express prothrombin time (PT), and assessment of encephalopathy and ascites may be affected by subjective judgment. If possible, criteria should be developed as follows: PT should be obtained as an activity value (%); severity of ascites should be assessed with reference to the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare, which specify that the volume of ascites should be estimated based on the ultrasonography findings, body weight change, and volume of drainage by puncture in principle, and presence of approximately ≥1 and ≥3 L of ascites should be assessed as mild and moderate or severe (Child-Pugh score of 2 and 3 points), respectively; and severity of encephalopathy should be assessed according to the coma classification system for hepatic encephalopathy established at the Inuyama Symposium (none, 1 point; mild I to II, 2 points; coma grade III or higher, 3 points), and Covert (subclinical) encephalopathy defined in the International Society for Hepatic Encephalopathy and Metabolism (ISHEN) criteria should be given 1 point (Literature 3).

In addition, the product-specific considerations include the following:

a) Exclusion of patients at high risk of adverse events related to drugs or equipment used for stem cell harvest (e.g., patients with concurrent interstitial pneumonia, splenomegaly, or blood cell proliferative disorders may have high risk of adverse events related to granulocyte colony-stimulating factor [G-CSF] preparations)
b) Exclusion of patients at high risk of adverse events involving vascular regeneration (e.g., patients with concurrent proliferative diabetic retinopathy or malignant tumor)

[3] Clinical efficacy evaluation

A. Clinical information

For clinical information, necessary tests and examinations should be selected according to the purpose based on findings from consultations, hematology tests, imaging, and liver biopsy. Before enrollment in a clinical study, patients should be confirmed to have not had responded to the existing therapeutic interventions with inflammation, fibrosis, or hepatic function remaining unchanged for a certain period of time (e.g., for 3 months or at least 90 days and within 180 days, as required in the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare). Furthermore, efficacy evaluation requires considerations that the patient's response may be modified by additional new drugs (including albumin preparation and fresh frozen plasma), dose changes of drugs, and procedures such as puncture and drainage of ascites.

B. Efficacy evaluation

The primary efficacy evaluation based on occurrence of events (e.g., death, hepatic failure, liver transplantation) would be useful in discussion of the clinical significance. Rating scales, if used, should be ones that have been assessed for reliability and validity and internationally accepted. For the evaluation, a change in rating scale score from baseline and proportion of patients with improvement at the evaluation timepoint should be used. For the change in rating scale score, whether the clinical significance can be justified is of importance, and durability of the effect should weigh as well. Secondary efficacy evaluation is useful not only to validate the results on the primary endpoint but also to discuss the results obtained for the clinical significance (Literature 3). Cell therapies are expected to alter liver function and fibrosis. To evaluate efficacy, appropriate endpoints should be established. In addition, because a certain period of time is supposed to be taken until improvements on these endpoints, timing of evaluation should be considered in view of the mechanism of action of transplanted cells and feasibility of the study with reference to results from studies at prior phases. If possible, evaluation should be performed not only at the final timepoint (e.g., at 6 months or 1 year) but also at an appropriate frequency to obtain data on changes over time. Parameters of potential interest in evaluation on each aspect are provided below.

a) Improvement of hepatic function (hepatic reserve)

Because liver function can be evaluated by many measures, understanding their characteristics is of importance for the evaluation. Child-Pugh Classification (score) is the representative measure. This is a system used to assess the liver function by scoring values or assessment results on albumin, total bilirubin, PT, ascites, and hepatic encephalopathy. The other liver function measures include albumin-bilirubin (ALBI) score or grade, which is calculated from liver function parameter values as done for Child-Pugh score. In addition, because improvement of liver function may be accompanied by alleviation of malaise, itching, edema, etc., interview surveys for patient-reported outcomes and evaluation of physical findings can be useful. In addition, MELD score is also one of the other measures, calculated from values on total bilirubin, PT, and serum creatinine, and reported to be useful especially in predicting prognosis of cirrhosis awaiting liver transplantation (Literature 4).

b) Antifibrotic effect

The evaluation can be performed by many approaches such as blood marker search (e.g., hyaluronic acid, 7S domain of type IV collagen, P-III-P, M2BPGi, autotaxin), mathematical assessment using blood test results, measurement of liver stiffness using fibroscan, ultrasonography, MRI, etc., and tissue assessment via liver biopsy. If possible, multiple approaches should be used in combination for the evaluation, because there are some cases where data obtained by one approach were not enough to detect a certain trend. Of note, the evaluation of decompensated cirrhosis, which has bleeding tendency, can dispense with liver biopsy if adequately performed by non-invasive approaches.

Blood tests on AST, ALT, alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), PT, platelet count, etc. will provide useful information. Quality-of-life questionnaire scores as well as measurement of liver and spleen volumes can be used as exploratory indirect indicators of hepatic reserve, improvement in liver fibrosis, and regeneration.

For an quantitative indicator of ascites, the Criteria for Disability Accreditation of Severe Hepatic Impairment by the Ministry of Health, Labour and Welfare specify as follows: “the volume of ascites should be estimated based on the ultrasonography findings, body weight change, and volume of drainage by puncture in principle, and presence of approximately ≥1 and ≥3 L of ascites should be assessed as mild and moderate or severe (Child-Pugh score of 2 and 3 points), respectively.” In view of the unstable nature of ascites, of which volume is likely to fluctuate, a certain criterion should be in place for evaluation of a therapeutic intervention, for example, resolution of ascites in patients receiving diuretics is given 1 point. The European Association for the Study of Liver (EASL) guidelines presented in the Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology may be used as references. The Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

For hepatic encephalopathy, multiple classification systems are available, including hepatic encephalopathy coma grades I to V established at the Inuyama Symposium in Japan and internationally the West Heaven Criteria (WHC) and ISHEN criteria. If possible, certain criteria should be established for the evaluation. For example, if possible, according to the hepatic encephalopathy coma grading system established at the Inuyama Symposium, the severity of hepatic encephalopathy should be assessed based on the coma condition (none, 1 point; mild I to II, 2 points; coma grade III or higher, 3 points), and according to the ISHEN criteria, Covert (subclinical) encephalopathy should be given 1 point. The hepatic encephalopathy coma grades established at the Inuyama Symposium and the Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020 (3rd Edition) of the Japanese Society of Gastroenterology and the Japan Society of Hepatology should be referred to.

To express PT, 2 different forms of activity value (%) and INR are used. If possible, both activity value (%) and INR should be determined. For the evaluation, it should be noted that these values can easily vary with use of oral anticoagulants such as warfarin.

C. Safety evaluation

To evaluate safety of h human (autologous) peripheral blood CD34-positive cell-processed products, recipients should be monitored for systemic and local findings as well as subjective symptoms from application of the product to the end of the follow-up period. That is, recipients should be monitored for adverse events, infections, and allergic reactions. If the cells are administered via the hepatic artery, attention should be paid to not only safety of the catheterization procedure but also post-dose increases in hepatobiliary enzymes. Furthermore, if possible, incidences of decompensated liver disease-related events (esophageal and gastric varices hemorrhage, ascites, hepatorenal syndrome, hepatic encephalopathy), development of hepatocellular carcinoma, and deaths should be followed up concurrently.

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