PSEHB/MDED Notification No. 0226-1
February 26, 2021

Attention to: Commissioner of Prefectural Health Department (Bureau)

Director of the Medical Device Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare
(Official seal omitted)

Release of Evaluation Guidance for Next-Generation Medical Devices and Cellular- and Tissue-Based Products

The Ministry of Health, Labour and Welfare has been working on evaluation guidance for next-generation medical devices and cellular- and tissue-based products that are of high medical needs and feasible to streamline product development and expedite approval review processes by preparing technical evaluation guidance used during the review process and releasing them in advance.

As provided in an appendix to this document, the Guidance on Evaluation for the Treatment of Subacute Spinal Cord Injury using Human Allogeneic iPS (-like) Cell-Processed Products is compiled, specifying data potentially necessary for and points of the evaluation. Please ensure that companies concerned under your jurisdiction are notified that the above guidance should be used as reference with the following noted before submission of application for marketing approval.

Of note, a duplicate copy of this notification will be sent to the following organization heads: the Chief Executive of the Pharmaceuticals and Medical Devices Agency, the Chairperson of the Japan Federation of Medical Devices Associations, the Chairperson of the American Medical Devices and Diagnostics Manufacturers' Association, the Chairperson of the Medical Equipment/IVD Committee of the European Business Council, the President of the Federation of Pharmaceutical Manufacturers' Associations of Japan, the President of the Japan Pharmaceutical Manufacturers Association, the Chairperson of the Japan-Based Executive Committee of the Pharmaceutical Research and Manufacturers of America, the Chair of the European Federation of Pharmaceutical Industries and Associations, Japan, the Chairperson of the Forum for Innovative Regenerative Medicine, the President of the Japanese Society for Regenerative Medicine, and the President of the Japan Society of Gene and Cell Therapy.

1. The guidance on evaluation provides matters of interest in product evaluation (endpoints) to expedite preparation of approval application data and the review. The guidance on evaluation is not positioned as legal standards but provides endpoints potentially applicable to next-generation medical devices and cellular- and tissue-

based products currently undergoing remarkable development of technologies. It should be noted that other endpoints than ones provided in the guidance may be needed or some of the endpoints provided in the guidance may be omitted depending on characteristics of the product.

2. When preparing documents and data necessary for approval application of individual products, the applicant is recommended to investigate the matters provided in the guidance on evaluation in advance and also utilize face-to-face consultations at the Pharmaceuticals and Medical Devices Agency at the earliest possible time.

(Appendix)

Guidance on Evaluation for the Treatment of Subacute Spinal Cord Injury using Human Allogeneic iPS (-like) Cell-Processed Products

1. Introduction

Basic technical requirements for ensuring quality and safety of products obtained by processing human-derived allogeneic induced pluripotent stem cells (iPS cells) or induced pluripotent stem-like cells (iPS-like cells) (hereinafter referred to as "Human (allogeneic) iPS (-like) cell processed products") are defined in the "Guidelines on Ensuring Quality and Safety of Products Derived from Processed Cell and Tissue (Allogeneic iPS (-like) cells)" (PFSB Notification No. 0907-5, dated September 7, 2012, of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare [MHLW]). The guidance on evaluation provides, in addition to the above basic technical requirements, considerations specific to a particular class of human (allogeneic) iPS (-like) cell processed products that are used as regenerative medical products applied for treatment of subacute spinal cord injury (traumatic). The term "Regenerative medical products" is defined in Article 2, Paragraph 9 of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Act No. 145 of 1960) (hereinafter, the same definition is applied).

2. Scope of the guidance on evaluation

The guidance on evaluation provides, in addition to the basic technical requirements, points to consider for evaluation of quality, efficacy, and safety of a particular class of human (allogeneic) iPS (-like) cell processed products that are used as regenerative medical products intraspinally transplanted for treatment of subacute spinal cord injury (traumatic) .

3. Positioning of the guidance on evaluation

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

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

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

4. Definitions of terms

(1) Subacute phase of a spinal cord injury: The acute phase of a spinal cord injury spans from occurrence of the injury to 1 week post-injury, and the chronic phase spans up to 6 months to 1

year post-injury. The subacute phase falls between the acute and chronic phases. In the guidance on evaluation, the "subacute phase" is defined as a period from 2 weeks to 2 months post-injury.

(2) Intraspinal transplantation (transplantation into nerve tissue): Direct injection is one route of administration used in cell transplantation therapy for spinal cord injury. In the guidance on evaluation, injections into the center and peripheral area of the injury are collectively referred to as "Intraspinal transplantation or transplantation into nerve tissue."

(3) Severity of a spinal cord injury: Spinal cord injury is roughly divided into complete paralysis with total loss of movement and sensation below the neurological level of injury and paresis with some function retained. Frequently used general severity classifications are the Frankel Grade and American Spinal Injury Association Impairment Scale (AIS), which classifies injury cases into the following 5 grades: A, Complete; B, Sensory Incomplete (sensory but not motor function is preserved); C, Motor Incomplete; D, Motor Incomplete (functions for standing and walking are preserved); and E, Normal.

(4) Neurological level of a spinal cord injury: Clinical presentation of a spinal cord injury greatly varies depending on the neurological level of injury. Cervical spinal cord injury causes quadriplegia, while thoracic spinal cord injury causes leg paralysis.

(5) Neurons, neural stem cells, and neural progenitor cells: Neural stem cells are a cell population that is self-renewal and multipotent. Cells destined to differentiate into a particular type of cells after several divisions are referred to as "Neural progenitor cells." Cells that have completed the differentiation process are referred to as "Neurons."

(6) Neural cells and post-differentiation neural cells: In the guidance on evaluation, the term "Post-differentiation neural cells" refers to neurons, astrocytes, and oligodendrocytes, three primary cell types in the nervous system, that are derived from iPS (-like) cells through differentiation process *in vitro* or in the recipient body after transplantation.

(7) Cell clusters (neurosphere structure): It is formed in a suspended culture of neural stem cells and progenitor cells with specific growth factors and a mixed cluster of cells at various differentiation stages, including undifferentiated neural stem cells and progenitor cells

(8) MRI (diffusion tensor imaging, DTI): Diffusion-weighted tensor imaging (DTI) is an MRI technique to assess diffusion directions of water molecules. Diffusion sensor tractography is an imaging technique scanning a specific neural tract along the craniocaudal axis with DTI parameters and useful in visualizing white matter damage.

(9) 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 ICH Q5D "Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products" [PMSB/ELD Notification No. 873, dated July 14, 2000, of the Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare or MHW])

5. Points to consider for evaluation

For the time being, the guidance on evaluation is intended to apply to evaluation of neural progenitor cells or other post-differentiation neural cells as human (allogeneic) iPS (-like) cell processed products. The cells to be evaluated are manufactured at the manufacturing site where human (allogeneic) iPS (-

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

(1) Raw materials, etc.

Used as the raw materials, etc., iPS (-like) cells should be from a cell line of human (allogeneic) iPS (-like) cells used as a raw material for regenerative medical products and controlled in a cell bank system, which needs to be confirmed or reasonably expected to differentiate into neural progenitor cells or other post-differentiation neural cells through a certain manufacturing process.

For human iPS cells established through genetic reprogramming by transfection with reprogramming genes in human somatic cells, presence of residual transgenes should be ruled out if possible. If the presence could not be ruled out, the transgenes should be demonstrated to have no adverse effects on quality or safety of the final product, the neural progenitor cells and other post-differentiation neural cells.

(2) Matters warranting special attention in the manufacturing process

For the manufacture of neural progenitor cells and other post-differentiation neural cells (final products), the manufacturing method should be clarified and validated for the following items to the extent possible to ensure certain quality.

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

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

[2] Manufacturing method

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

a) Acceptance inspection

For the human iPS(-like) cell line to be used as a source material, test and inspection items for acceptance at the manufacturing site (e.g., visual inspection, microscopic examination, viability, characterization of cells, and tests to deny bacteria, fungi, viruses, etc. contamination) should be specified with the criteria for each item. Where necessary and possible, tests for bacteria, fungi, viruses, etc. should be performed to the extent that would not affect the phenotype, genetic traits, characteristics such as specific functions, cell viability, or quality. If the result is positive, stock

of the human iPS (-like) cell line and the transportation should be checked for contamination, and a human iPS (-like) cell line should be obtained again.

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

b) Cell banking

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

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

A method of preparing the cells, to be used as a component of the final product, from the iPS (-like) cell line accepted as the raw materials, etc. at the manufacturing site and from its cell bank (including a differentiation induction method, methods of isolation and culture of intended cells, medium at each stage of culture, culture conditions, culture period, and yield) should be specified and justified to the extent possible. If the final product is presented in a frozen state, methods to freeze the cells and prepare a dosing cell suspension for transplantation from the frozen cells (e.g., methods to thaw the cells and prepare the final dosing suspension) should be specified and justified to the extent possible as well.

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

In the manufacture of neural progenitor cells and other post-differentiation neural cells derived from iPS (-like) 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.

e) Monitoring of cell growth

For neural progenitor cells, an increasing number of cell divisions is suggested to contribute to the possibility of chromosomal and genomic instability. Throughout the manufacturing process up to release of the final product, cell growth should be controlled within an appropriate range not affecting cell quality by avoiding excessive passages and cell divisions if possible. The measures for such control should be specified.

(3) Quality control of products

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

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

The transplantation method of neural progenitor cells and other post-differentiation neural cells (final product) manufactured from iPS (-like) cells should be clearly described. Neural progenitor cells and other post-differentiation neural cells (final product) manufactured from iPS (-like) cells may be transplanted in a state either of isolated cells or of cell cluster (neurosphere structure) as a dose containing the necessary number of cells directly into the site or peripheral area of the spinal cord injury.

a) Cell morphology

For cell morphology of the final product, in manufacture using a suspended culture method, translucent to pale white cell clusters (neurospheres structure) with a smooth surface are mostly formed. If possible, morphological characteristics and size should be visually checked, and the records should be retained. Consideration may be given to establishment of criteria for the morphological characteristics and size where necessary. In manufacture by the monolayer culture method using culture flasks, etc., cells should be visually confirmed to have documented morphological characteristics as intended, and the records should be retained if possible.

b) Cell count and viability

For cell count and viability in the final product, criteria should be also specified. Cell count may be determined using a cell suspension prepared from an enzyme-treated portion of the final product by a method using hemocytometer or cell counter. Cell viability may be determined by a trypan blue dye exclusion method, which allows calculation of the numbers of living cells and dead cells. In addition, cells may be stained with fluorescence such as DAPI and acridine orange for measurement.

If technical difficulties are found in cell count and viability assays with cell clusters (neurospheres structure), surrogate indicators supporting the count and viability of cells contained in the cell clusters (neurospheres structure) may be used. Of note, the surrogate indicators should be justified.

c) Cell specificity

If the final product is neural progenitor cells, relative expression levels of neural progenitor cell marker genes (e.g., *SOX1*, *PAX6*, *NES*) should be determined by mRNA expression analysis. For quantification of protein expression, methods such as cell immunostaining and flow cytometry should be used to determine expression levels of SOX1, PSA-NCAM, etc. If possible, multiple different analysis methods selected from the above ones should be applied to evaluate identity and count of neural progenitor cells.

If the final product is other post-differentiation neural cells, methods such as mRNA expression analysis, cell immunostaining, and flow cytometry may be used to evaluate expression levels of marker molecules characteristic of neurons, glial cells, or other progenitor cells. If possible, multiple different analysis methods selected from the above ones should be applied to evaluate identity and count of post-differentiation neural cells.

d) Absence of undifferentiated cells

Presence of undifferentiated cells may be assessed based on expression levels of marker genes (e.g., *POU5F1* [*OCT3/4*], *NANOG*) determined by quantitative RT-PCR or based on expression levels of marker antigens characteristic of undifferentiated cells (e.g., POU5F1 [OCT3/4], TRA1-60 antigen, SSEA-3 antigen) determined by cell immunostaining or flow cytometry using antibodies recognizing these antigens. In addition, a backward culture method in which the final product is returned to the culture condition for iPS (-like) cells used as the raw materials, etc., and incubated for a certain period may be used. If possible, multiple different analysis methods selected from the above ones should be applied to evaluate presence of undifferentiated cells.

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

e) Chromosome and genome structures

The final product is required to be evaluated for the chromosome and genome structures. For chromosome structure, the chromosome karyotype should be analyzed by the Giemsa staining and G-banding method, etc. if possible. For others, a microarray method may be used to evaluate the genome structure at the whole genome level.

f) Functions

To confirm that the produced cells have functional properties consistent with the intended treatment use, the in-process product or the final product should be analyzed. For example, the final product of neural progenitor cells should be confirmed to be capable of differentiating into various types of neural cells by the following method: neural progenitor cells are cultured under conditions enhancing their differentiation *in vitro* for a certain period of time followed by cell immunostaining to visualize expression of markers characteristic of neurons (e.g., TUBB3 [tubulin beta 3 class III]) and glial cells (e.g., GFAP). In addition, to quantify the number of differentiated neurons, an immunostaining method using anti-HuC/D antibody (antigen, ELAVL3/4) and anti-NeuN antibody (antigen, RBFOX3) may be used.

For the final product of other post-differentiation neural cells, methods such as mRNA expression analysis, cell immunostaining, and flow cytometry may be used to evaluate expression levels of marker molecules characteristic of neurons, glial cells, or other progenitor cells assumed to be relevant to the indication. If humoral factors, etc. derived from the cells are assumed to be relevant to the indication of the final product, their amounts secreted can be evaluated.

(4) Stability testing of products

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

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

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

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

(6) Nonclinical studies

[1] Tumorigenicity studies for nonclinical safety evaluation

When tumorigenicity of regenerative medical products manufactured by processing iPS (-like) cells is evaluated, it should be noted that “correlation and causal relationship between tumorigenicity of iPS (-like) cells used as the raw materials, etc. and that of the final product remain to be elucidated.” That is, it must always be noted that for clinical application, in tumorigenicity evaluation, the greatest importance is attached to the final product, an iPS (-like) cell processed product but not iPS (-like) cells used as the raw materials, etc. Tumorigenicity studies should be conducted with the final product. Tumorigenicity evaluation using a study system in immunodeficient animals with the known detection limit is useful. For conduct of tumorigenicity studies, the “Guideline for Tests for Detecting Undifferentiated Pluripotent Stem Cells and Transformed Cells, Tumorigenicity Studies, and Genetic Stability Evaluation of Human Cell-Processed Products” (PSEHB/MDED Notification No. 0627-1, dated June 27, 2019, of the Medical

Device Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, MHLW) should be referred to.

If possible, tumorigenicity studies for nonclinical safety evaluation should be conducted by performing transplantation in immunodeficient animals (e.g., NOG mouse, NSG mouse), because they are highly sensitive. If cell banks of iPS (-like) cells are generated, the final product manufactured from the above cell bank should be used in tumorigenicity studies in principle. If the final product manufactured from a material other than the above cell banks is used in tumorigenicity studies, such use should be justified.

Because intraspinal (clinical route of administration) transplantation involves a surgical procedure, which is highly invasive especially in small animals, it should be noted that the surgical procedure could complicate assessment of the results. The number of cells to be transplanted should be calculated by multiplying the expected clinical dose by the safety factors of the species and interindividual differences if possible. However, adequate consideration should be given to the possibility that the cells transplanted into animals may greatly affect the microenvironment at the administration site owing to the total volume itself, causing artifact changes. That is, the number of cells to be administered should be determined with the importance attached to the objective of tumorigenicity studies with intraspinal transplantation, which is to investigate tumorigenicity of the final product (cells) in the microenvironment corresponding to the transplantation site in humans. To evaluate tumorigenicity of cells transplanted in high volume, an intracranial (intracerebral) transplantation method using immunodeficient animals may be used as an alternative, which allows evaluation in the microenvironment close to that at the intraspinal transplantation site. In this case, the study results should be interpreted with importance attached to differences in microenvironment between the spinal cord and brain tissue.

If neural progenitor cells or other post-differentiation neural cells (final product) are manufactured from multiple cell banks of iPS (-like) cells through the same process, and these cell banks have been established by the same method after HLA typing and confirmed to have quality attributes comparable to those of the raw materials, etc. of the final product, tumorigenicity evaluation in a microenvironment corresponding to that at the transplantation site in humans should be performed for the final product manufactured from each cell bank in principle. Intraspinal or intracerebral transplantation in immunodeficient animals is a representative approach for tumorigenicity studies of the final product.

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

Expression of the function, durability of the action, and feasibility of clinical effects (Proof-of-Concept or POC) expected for the human (allogeneic) iPS (-like) cell processed product should be presented using animal models appropriate for the target disease to the extent technically possible and scientifically reasonable. Animal models include mice, rats, and monkeys with spinal cord injury. It should be noted that human iPS (-like) cell-derived neural progenitor cells and other post-differentiation neural cells (final products) are heterologous grafts in a rat or monkey animal model when transplanted, requiring use of immunosuppressants, and their effect would be lost in a short period of time, limiting the observation to a short period of time. Behavioral observation may be performed to evaluate the response to treatment, but justification should be considered. If neural progenitor cells or other post-differentiation neural cells (final product) are manufactured from

multiple cell banks of iPS (-like) cells, and these cell banks have been established by the same method after HLA typing and confirmed to have quality attributes comparable to those of the raw material of the final product, POC may be demonstrated using the final product manufactured from a representative bank.

[3] Others

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

(7) Clinical studies (clinical trials)

1. Study population

To ensure that a clinical study is conducted in a population suitable for efficacy and safety evaluation, inclusion/exclusion criteria should be established, using widely accepted diagnostic criteria, severity classification, etc. Because clinical presentation of a spinal cord injury greatly varies depending on the neurological level and severity of injury, the inclusion/exclusion criteria to narrow a population suitable for efficacy evaluation may have to be specified by limiting the severity and neurological level of injury to some extent according to attributes of cells to be transplanted and purpose of the study. However, to discuss efficacy and safety of the product used in injuries that would have been excluded from clinical studies based on the severity or neurological level, potential generalization of the results obtained in clinical studies and information gathering through additional clinical studies should be considered. Prior consultations with the Pharmaceuticals and Medical Devices Agency are recommended.

1.1. Inclusion criteria for enrollment

1.1.1. Timing of investigational therapeutic intervention

For a clinical study in patients with spinal cord injury, timing of a therapeutic intervention should be specified as a post-injury timepoint. Sooner after injury, neurological symptoms are more unstable owing to influence of spinal shock,[1)] potentially precluding appropriate assessment of paralysis severity and neurological symptoms in an acute phase of injury and consequently making the efficacy evaluation difficult.[1,2)] Several days after injury, neurological symptoms in subjects can be assessed in detail, because they are reported to be slightly stabilized at this timepoint.[1)] In a chronic phase when several months have passed since injury, cavity and scar formations are reported to weaken the effect of regenerative therapy.[3)] Appropriate timing of intervention should be individually considered according to attributes of cells to be transplanted and purpose of the study.

1.1.2. Neurological level of injury in target patients

To ensure appropriate efficacy evaluation, limiting the neurological level of injury in target patients should be considered, because an extent of recovery from spinal cord injury differs depending on the neurological level of injury.[4)]

As a difference in extent of injury, complete paralysis is found in a greater proportion of patients with thoracic spinal cord injury than that of patients with cervical spinal cord injury.[5)] In patients with thoracic spinal cord injury, motor paralysis remains unchanged even if the neurological level of injury is lowered by recovery from paralysis (meaning that a decrease in neurological level of injury does not correlate to a change in neurological assessment result such as American Spinal Injury Association [ASIA] motor score). It should be therefore noted that the ASIA motor score does not necessarily reflect recovery from paralysis. On the other hand, in patients with cervical spinal cord injury, a slight lowering in neurological level of injury can be sharply detected as an improvement in motor function. As described above, if both patients with cervical spinal cord injury and those with thoracic spinal cord injury are evaluated in the same clinical study, the primary endpoint should be carefully specified before start of the clinical study. If use of the common primary endpoint is difficult, clinical studies should be separately conducted in each patient population.

Because cervical spinal cord injury at a high neurological level may cause serious respiratory paralysis, whether patients with respiratory paralysis will be enrolled in the study population should be considered in advance.

1.1.3. Severity of injury in target patients

In patients with spinal cord injury, an extent of recovery may differ depending on severity at the time of injury, widely accepted severity classification must be specified in the inclusion criteria to ensure selection of appropriate patients for the clinical study. Cell transplantation therapy is expected to be used in patients with relatively severe paralysis such as those with complete paralysis or severe incomplete paralysis. Establishment of the inclusion criteria based on severity of injury in target patients should be considered according to mechanism of action of cells to be transplanted and purpose of the study.

1.2. Exclusion criteria

Exclusion criteria should be specified with importance attached to factors potentially affecting prognosis of spinal cord injury such as surgery, steroid therapy, and other drugs. For the effect of surgery on outcome of paralysis caused by spinal cord injury, consensus has not been reached. A report shows that surgery at an early phase improved outcome of paralysis,[6)] while the other reports show that presence or absence of surgery was not related to outcome of paralysis.[7,8)] However, for dislocation, reposition at an early phase is recommended.[9)] Because the effect of surgery on efficacy and safety evaluation remains unclear at the present time, handling of presence or absence of surgery, for example, specifying it in the exclusion criteria or using it as an allocation factor, should be considered at the planning stage of each clinical study. In addition, enrollment of patients treated with steroids or drugs potentially affecting behavior of transplanted cells should be individually considered at the planning stage as a factor that has a potential effect on efficacy and safety evaluation.

1.3. Elderly and young people

Because spinal cord injury commonly occurs in the elderly (aged 65 years or older), efficacy and safety evaluation in view of the “Guideline for Clinical Evaluation of Drugs Used in the Elderly”

(PAB/NDD Notification No. 104, dated December 2, 1993, of the New Drug Division, Pharmaceutical Affairs Bureau, MHW) and "Questions and Answers for 'Guidelines for Clinical Evaluation of Drugs Used in the Elderly'" (Administrative Notice, dated September 17, 2010, of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW). Because recovery from spinal cord injury is reported to be significantly slower in the elderly than in non-elderly,[10,11,12,13] consideration should be given to ensure elderly and non-elderly can be separately evaluated by defining elderly or non-elderly status as an allocation factor or planning a sub-group analysis before start of a clinical study.

Enrollment of the elderly and children should also be considered.

2. Sample size determination

The sample size should be determined based on statistical consideration and according to the study objectives, hypotheses to be tested, and study design.

3. Efficacy evaluation

In general, the primary efficacy evaluation should be performed using rating scales 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. 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.[14]

Measures such as training of raters should be fully considered to ensure uniform assessment among raters and to minimize variations among them. Especially for a global study, consideration should be given to ensure that the evaluation method is standardized across regions where the study is conducted. In addition, raters should be qualified before start of a clinical study.

3.1. Primary endpoint

The true purpose of treatment of spinal cord injury is to improve not only neurological functions but also quality of life (QOL) including functional outcome and activity of daily living (ADL).[15] However, objective assessment of QOL improvement by treatment of spinal cord injury is difficult at the present time. In addition, up to now, there are no neurological assessment methods for spinal cord injury of which results have been securely demonstrated to be related to QOL.

In the clinical studies conducted to date, neurological assessment methods such as ASIA scoring system (motor score and sensory score, especially in patients with thoracic spinal cord injury, the scores are important in determining the neurological level of injury), ASIA Impairment Scale (AIS), or Frankel Grade were commonly specified as methods to measure the efficacy endpoint.[9,10] The ASIA scoring system allows assessment of neurological symptoms in detail in a relatively reproducible manner[16] but does not directly cover functions for activities of daily living On the other hand, AIS and Frankel Grade systems, which allow simple assessment of paralysis overall, are useful and thus frequently used in clinical settings but reported to poorly reflect actual ADL.[17] Thus, a reverse phenomenon where the ASIA score is worsen despite of improved AIS may occur.[18]

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 but also at an appropriate frequency to obtain data on changes over time.

In addition, even if a neurological assessment method is specified as a method to measure the primary endpoint, results may have different meanings between cervical spinal cord injury and thoracic spinal cord injury. In patients with cervical spinal cord injury, ASIA motor score can reflect both improvement and worsening in detail, and the primary endpoint may be conventionally specified as improvement of ASIA motor score. On the other hand, in patients with thoracic spinal cord injury, even if the neurological level of injury is lowered by recovery from paralysis, the motor function of lower extremities would remain unchanged, and thus improvement of motor function cannot be captured by the ASIA motor score. Therefore, capturing a lowering in neurological level of injury by assessment of the ASIA sensory score should be considered. As described above, if both patients with cervical spinal cord injury and those with thoracic spinal cord injury are enrolled in one clinical study, the primary endpoint should be carefully specified. If use of the common primary endpoint is difficult, clinical studies should be separately conducted in each patient population.

3.2. Secondary endpoints

Secondary endpoints should be specified as efficacy endpoints to supplement the primary endpoint. In addition, in a study where a neurological assessment method such as ASIA scoring system is specified as a method to measure the primary endpoint, assessment on functional outcome should be specified.

Secondary endpoints should include responder rates such as a proportion of subjects with AIS Grade improved by ≥1 grade and may use neurological assessment methods for the Neurological Level of Injury (NLI), etc. and ADL/QOL assessment methods for ADL (e.g., Spinal Cord Independence Measure [SCIM], ADL assessment method specific to spinal cord injury), comprehensive QOL (e.g., Euro-QoL 5-dimension [EQ5D], MOS 36-Item Short-Form Health Survey [SF-36]), etc.

If a neurological assessment method such as ASIA scoring system is specified as a method to measure the primary endpoint, whether assessment on functional outcome and ADL/QOL is used in the secondary endpoints should be considered.[14)] Recently, SCIM has been recommended for ADL assessment in patients with spinal cord injury.[16)] In patients with cervical spinal cord injury, a lowering in NLI is reported to correlate with a change in SCIM self-care sub-scale score.[19)] However, a part of SCIM items are specific to cervical spinal cord injury and thus not applicable to thoracic spinal cord injury. Consideration should be given to ensure that the secondary endpoint can be properly assessed in each patient.

Because spinal cord injury may cause vesicorectal disorder, of which symptoms can remarkably impair ADL/QOL of the subjects, consideration should be given to ensure that vesicorectal disorder is properly assessed using appropriate measures such as SCIM sub-scale items. Spinal cord injury pain is also a pathological condition that may remarkably impair ADL/QOL of the patients and thus should be considered as an item to be potentially added to the endpoints.

Imaging and electrophysiological examinations are used to assess treatment of spinal cord injury objectively. Simple spine MRI is recommended for imaging the spine after treatment of spinal cord injury to check survival of transplanted cells, presence or absence of neoplastic transformation, and spinal canal stenosis associated with neoplastic transformation. Although whether contrast-

enhanced MRI is useful in assessing responses to treatment of spinal cord injury remains unclear, the imaging technique may be considered to assess the spinal condition after cell transplantation in detail, including tumorigenesis. In addition, diffusion tensor imaging (DTI), on which reports have been published in recent years, should be considered as a measure to evaluate spinal cord regeneration if possible.

Electrophysiological examinations (e.g., evoked electromyography with central magnetic stimulation) is also recommended for functional assessment after treatment of spinal cord injury. Wherever possible, objective measures should be used.

4. Safety evaluation

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product (including regenerative medical products; the same applies in this section) and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product. If an adverse event is observed, name of the event, severity, outcome, time of onset and outcome confirmed, use status of investigational drugs as well as presence or absence of treatment and its details should be recorded in a case report form, and whether it corresponds to a serious adverse event and whether its causal relationship to the investigational drug can be ruled out should be assessed.

In addition, the Council for International Organizations of Medical Science (CIOMS) VI Working Group has proposed that the name of an adverse event entered in a case report form should be the diagnosis instead of individual symptoms and signs wherever possible.[20] The Point to Consider guideline of the Medical Dictionary for Regulatory Activities Terminology (MedDRA) instructs the reporting method of adverse events, stating that entry of the diagnosis only, if comprised of symptoms and signs, would not constitute a loss of information.[21] However, it should be noted that collection and evaluation of individual symptoms and signs other than the diagnosis reported as an adverse event may be of importance in some cases that involve specific symptoms and signs of interest. For details, the “Guideline of Structure and Content of Clinical Study Reports” (PAB/ELD Notification No. 335, dated May 1, 1996, of the Evaluation and Licensing Division, Pharmaceutical Affairs Bureau, MHW) and “Questions and Answers: Guideline of Structure and Content of Clinical Study Reports” (Administrative Notice, dated October 18, 2012, of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW) should be referred to.

In clinical studies, adverse events of special interest including ones characteristic of cell transplantation and ones related to pathological conditions of spinal cord injury should be collected. The adverse events of special interest are as follows:

Significant adverse events

[1] Neoplastic transformation
[2] Infection
[3] Rejection
[4] Adverse events associated with procedure for transplantation (e.g., bleeding, cavity formation)
[5] Syringomyelia
[6] Adverse events associated with use of immunosuppressants

[7] Worsening of paralysis
[8] Pneumonia
[9] Respiratory failure
[10] Deep vein thrombosis/pulmonary infarction
[11] Drug-induced hypersensitivity syndrome
[12] Urinary tract infection
[13] Pressure ulcer
[14] Joint contractures
[15] Spinal cord injury pain
[16] Gastrointestinal ulceration
[17] Cerebral infarction

5. Handling of prohibited concomitant drugs, prohibited concomitant therapies, and rehabilitation

5.1. Prohibited concomitant drugs

For drugs potentially affecting efficacy evaluation, their concomitant use should be prohibited wherever possible if justified from ethical and clinical viewpoints in advance. For drugs excluded from the prohibited concomitant drugs because of ethical or clinical reasons, the protocol should specify that their dosage regimens (frequency for as-needed use) should not be changed during the study.

5.2 Handling of rehabilitation

Rehabilitation is a factor that affects functional recovery from spinal cord injury. In a clinical study, consideration should be given to differences in rehabilitation administered among subjects, which can affect efficacy evaluation. At the same time, however, rehabilitation after therapeutic intervention should be flexibly considered, not limited to standard practice. In particular, aggressive rehabilitation in patients with body parts affected by complete motor paralysis and with focus on limb and trunk functions, which are supposed to be hardly restored to practical level, is not administered as standard practice at the present time. If regenerative medicine is provided to improve the functions that have been supposed to be hardly restored based on the previous reports, rehabilitation suitable for the purpose should be selected. If possible, use of devices based on advanced rehabilitation technologies (e.g., robots, functional electrical stimulation) and stimulation therapies (e.g., magnetic stimulation, electrical stimulation) should be considered. If the endpoint to evaluate the therapeutic effect in a clinical study involves symptoms that can be improved by such rehabilitation alone (e.g., paresis area), an appropriate control group should be included. Historical controls may be used as the control group. In view of difficulty in standardizing rehabilitation programs among patients, details and time of rehabilitation actually administered to individual patients should be documented to allow retrospective comparisons.