NOTE: The following is a tentative English translation of Annex of Notification 0613-3 issued by the Director of Research and Development Division, Health Policy Bureau, MHLW on June 13, 2016. The translation of the original Japanese version into the English language shall be for convenience of reference only and shall have no legal effect. The Japanese language text shall in any event prevail.

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Points for certified special committees for regenerative medicine to consider when evaluating tumorigenicity assessment in provision plans of regenerative medicine using human pluripotent stem cells

The requirements for non-clinical study necessary for assessing the risk of specified processed cells that are derived from pluripotent stem cells have not been determined yet. Our research group has conducted discussions based on leading-edge knowledge, but a final conclusion with the agreement of all parties was not reached. This report is the opinion that received the approval of the majority after vigorous discussions. The content of this report should be constantly validated and modified to reflect the results of future basic research and careful observation of clinical administration to patients and the knowledge that is built from analysis of these samples.

This report takes maximum consideration in providing a chance of novel therapy to patients who currently suffer from disease with no appropriate therapeutic option, and is prepared with the aim to accumulate scientific data that would contribute to future development, which would enable therapies using pluripotent stem cell-derived, specified cell products to be delivered to patients as safely and quickly as possible.

1. Points to consider on safety required in pluripotent stem cells as raw material

(1) With regard to surplus embryo and cells as raw materials, confirm the following points.

- Informed consent was obtained from the donor.
- Donor screening was appropriately conducted.
- Compliance to other related domestic guidelines and standards was assessed[note1)].

* Clinical use is not allowed unless all of the above 3 items are satisfied.

Note1) Refer to Standards for Biological Raw Materials and No. 4 to 6, MHLW Notification 0907, issued September 7, 2012.

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コメント [1]: "Specified processed cells" is a technical term in Japanese legislations. In the RM Safety Act, cell-based therapeutic products that are manufactured by substantial/more-than-minimal manipulations of somatic/stem cells are called "processed cells". "Processed cells" that are not "cell-processed product" as defined in the PMD Act are called "specified processed cells" in the RM Safety Act. Namely, "cell-processed product" is processed cells that are intended for marketing.

YS 2016/10/19 16:12
コメント [2]: Public Notice of the Ministry of Health, Labour and Welfare No. 375, issued September 26, 2014. Alternatively translated as "Standards for Biological Ingredients"

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コメント [3]: Five guideline documents on ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of human stem cells

(2) **Confirm the following genomic indicators that cannot rule out tumorigenicity in pluripotent stem cells to be used as raw material.**

- Chromosomal abnormalities (Conventional or G-band)
- Structural abnormalities including SNV/Indel of tumor-related genes (Cosmic census & Shibata's list) and copy number variants (CNV)
- Significant residual external factors that may promote tumors

* If any abnormalities related to the above 3 items are found, a strict risk-benefit assessment should be conducted to determine the appropriateness of clinical use [Note 2)]. Pluripotent cells that satisfy these items may be allowed for clinical use under the Act on the Safety of Regenerative Medicine. The explanation document upon consent to target patients should be confirmed to obtain a clear explanation about genomic analysis of pluripotent stem cells to be used as raw material, including the fact that there are still many unknown factors.

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コメント [4]: http://cancer.sanger.ac.uk/census

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コメント [5]: Table 1 of https://www.pmda.go.jp/files/000152599.pdf

Note 2) To minimize the risk to target patients, pluripotent stem cells that have been determined as having no abnormalities related to the above items are recommended for use as much as possible. However, even in cases where abnormalities are found in the genomic analysis of pluripotent stem cells, if there is a possibility that health benefits to target patients exceed the risk, use of these pluripotent stem cells may be allowed. In these cases, during FIH study, until there is a sense of benefit judged from the first several cases, pluripotent stem cells that have been determined as having no abnormalities based on the above items will be used to proceed with caution.

The risk-benefit assessment must be comprehensively judged, with special consideration to evidence, such as availability of alternative therapy and seriousness of the disease.

Use may be allowed depending upon type and number of transplanted cells, site of transplantation, whether there are any alternative therapies, and content of risk management plan. Judgment will be based on whether transplanted cells are terminally differentiated cells, the number of transplanted cells is fairly low, the transplantation site is an environment that is fairly resistant to tumors growth, and whether cell observation after transplantation is easy.

2. Points of review for tumorigenicity assessment of pluripotent stem cell-derived specified processed cells

(1) **Confirm that the previous items are satisfied as raw material for clinical use.**

- If the items are not satisfied, it cannot be used for clinical use.

(2) Confirm the following points, regarding *in vitro* study of the final product.

- Chromosomal abnormalities (Conventional or G-band), structural abnormalities including SNV/Indel of tumor-related genes (Cosmic census + Shibata's list) and copy number variants (CNV), increase in cell sub-population confirmed by somatic cell abnormalities during large-scale culture or those that newly occurred during differentiation of pluripotent stem cells as the raw material

- Residual undifferentiated pluripotent stem cells
- Transformation into cells other than the target, and abnormal growth of cells other than the target cells when cultured longer than the culture period.

* If any abnormalities related to the above 3 items are found, use is not recommended in principle, but in some cases, use may be judged as appropriate after a strict risk-benefit assessment to validate the target disease/administration method, *etc.*[Note 3)]. The explanation document upon consent to target patients should be confirmed to obtain a clear explanation about the risks and benefits.

Note 3) To minimize the risk to target patients, pluripotent stem cell-derived specified processed cells that have been determined as having no abnormalities related to the above items are recommended for use as much as possible. However, even in cases where abnormalities are found in the genomic analysis of pluripotent stem cell-derived specified processed cells, if there is a possibility that health benefits to target patients exceed the risk, use of pluripotent stem cell-derived specified processed cells may be allowed. In these cases, during FIH study, until there is a sense of benefit judged from the first several cases, pluripotent stem cells that have been determined as having no abnormalities based on the above items will be used to proceed with caution.

The risk-benefit assessment must be comprehensively judged, with special consideration to evidence, such as availability of alternative therapy and seriousness of the disease.

Use may be allowed depending upon type and number of transplanted cells, site of transplantation, whether there are any alternative therapies, and content of risk management plan. Judgment will be based on whether transplanted cells are terminally differentiated cells, the number of transplanted cells is fairly low, the transplantation site is an environment that is fairly resistant to tumors growth, and

whether cell observation after transplantation is easy.

(3) With regard to *in vivo* tumorigenicity test of the final product, comprehensively consider the appropriateness of the following 1) to 11).

1) Objective and limits to extrapolation to humans
2) Animal type and immunosuppressive/immunodeficiency state
3) Procedures for administration scheduled in the provision plan of the regenerative medicine
4) Administration site of processed product during the test
5) Administration/transplantation form during the test
6) Scheduled number of cells to be clinically administered and number of cells administered in the *in vivo* tumorigenicity test
7) Observation period of the test and appropriateness of interim analysis, if scheduled
8) Observational endpoints
9) Assessment of observed pathological findings
10) Observation plan after transplantation
11) Storage plan for a portion of the cell-processed product

In vivo tumorigenicity tests do not directly assess the risk of tumorigenicity, but evaluate the absence/presence of hazards and the amount, and the rate of occurrence of factors within immonodeficient animals. In addition, the explanation document upon consent to target patients should be confirmed to obtain a clear explanation that the variety of tumor cells is diverse and that it should be considered that there are cancer cell types that have a low detection rate in *in vivo* tumorigenicity tests.

(4) Confirm the appropriateness of the risk management plan.

- Follow-up plan
- Management plan for occurrence of tumors (surgical removal, drug administration, *etc.*)

(5) Confirm the appropriateness of the provision plan from the viewpoint of potential benefit.

- Alternative therapeutic options and if there are any, compare with the existing therapeutic options
- Prognosis with administration
- Prognosis without administration, *etc.*

3. Reference information

It is known that culture of human cells may cause genetic mutations, such as karyotype changes. Even human diploid fibroblasts that are considered to have stable karyotypes have indicated slight mutations when analyzed by single nucleotide polymorphism (SNP) arrays. Non-diploid karyotypes in apparently normal tissue have also been occasionally observed to have such mutations.

There is no world-wide consensus on the safety of cells with karyotypic abnormalities and cells that have other genetic mutations observed *in vitro*. Genetic information, which is the baseline of genetic stability, differs depending upon cell type and culture methods. There are no cells that indicate an absolute stability in genetic replication when sub-cultured. Therefore, to minimize genetic instability, which is a potential hazard, culture period and number of passage should be restricted and risk assessment for culture conditions and for effect of change should be conducted.

Detection sensitivity to genetic change (mutation type and allele frequency) and the possibility of obtaining appropriate control should be investigated as future issues for genomic information and epigenomic information obtained from cutting-edge technology, such as next-generation sequencers. At the same time, scientific validation of the relationship with tumorigenicity should be advanced and appropriateness for use as a testing method should be assessed. If any mutations could be scientifically apparent as having a relationship with safety, such as tumorigenicity of specified processed cells, tests such as the following would improve the safety of the specified processed cells.

1) Test to detect known tumor-related SNV/Indel and CNV after long-term culture
2) Test to detect known tumor-related epigenome changed after long-term culture
3) Test to detect genetic mutations with known correlation with functional abnormalities of differentiated cells in specified processed cells or with known relationship with the target disease

However, in particular with specified processed cells that are derived from pluripotent stem cells, it is still extremely novel and risk prediction is difficult. Therefore, it is recommended to confirm genetic mutations that are known to be related to any tumor occurrences and to other adverse events, as reference information (supplementary information for reassurance) for discussions on ensuring safety. In other words, it is necessary to clarify the functionality of testing methods, such as the analytical limit of detection of low-allele frequency genetic mutation, and confirm the above points 1) to 3). The judgment on clinical administration of pluripotent stem cell-derivedspecified cell products that have been detected to have the mutations in

points 1) to 3) should be determined, considering the seriousness of disease of the patient and urgency for treatment.

2015 MHLW Grants-in-aid (MHLW Science Special Research Project)
Research on Safety Assessment of Transplanted Cells for Implementing Clinical Research Using iPS Cells

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