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The Manufacturing, Engineering, and Regulation of Pluripotent Stem Cell-Derived Therapies
PART 1: Characterization of the Starting Material

Japanese Regulatory Perspectives on the Significance of Genomic Mutation Analysis in the Tumorigenicity Evaluation of Pluripotent Stem Cell-Derived Therapeutic Products

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The human body is a mosaic of different genomes

Survey finds that 'normal' human tissues are riddled with mutations.

Nature (NEWS on 06 June 2019)

https://www.nature.com/articles/d41586-019-01780-9

RESEARCH ARTICLE

RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues

Keren Yizhak[1], François Aguet[1], Jaegil Kim[1], Julian M. Hess[1], Kirsten Kübler[1,2,3], Jonna Grimsby[1], Ruslana Frazer[1], Hailei Zhang[1], Nicholas J. Haradhvala[1,2], Daniel Rosebrock[1], Dimitri Livitz[1], Xiao Li[1], Eila Arich-Landkof[1,2], Noam Shoresh[1], Chip Stewart[1], Ayellet V. Segrè[1,3,4], Philip A. Branton[5], Paz Polak[6], Kristin G. Ardlie[1], Gad Getz[1,2,3,7,*]

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Science 07 Jun 2019:
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Somatic mosaicism in normal tissues

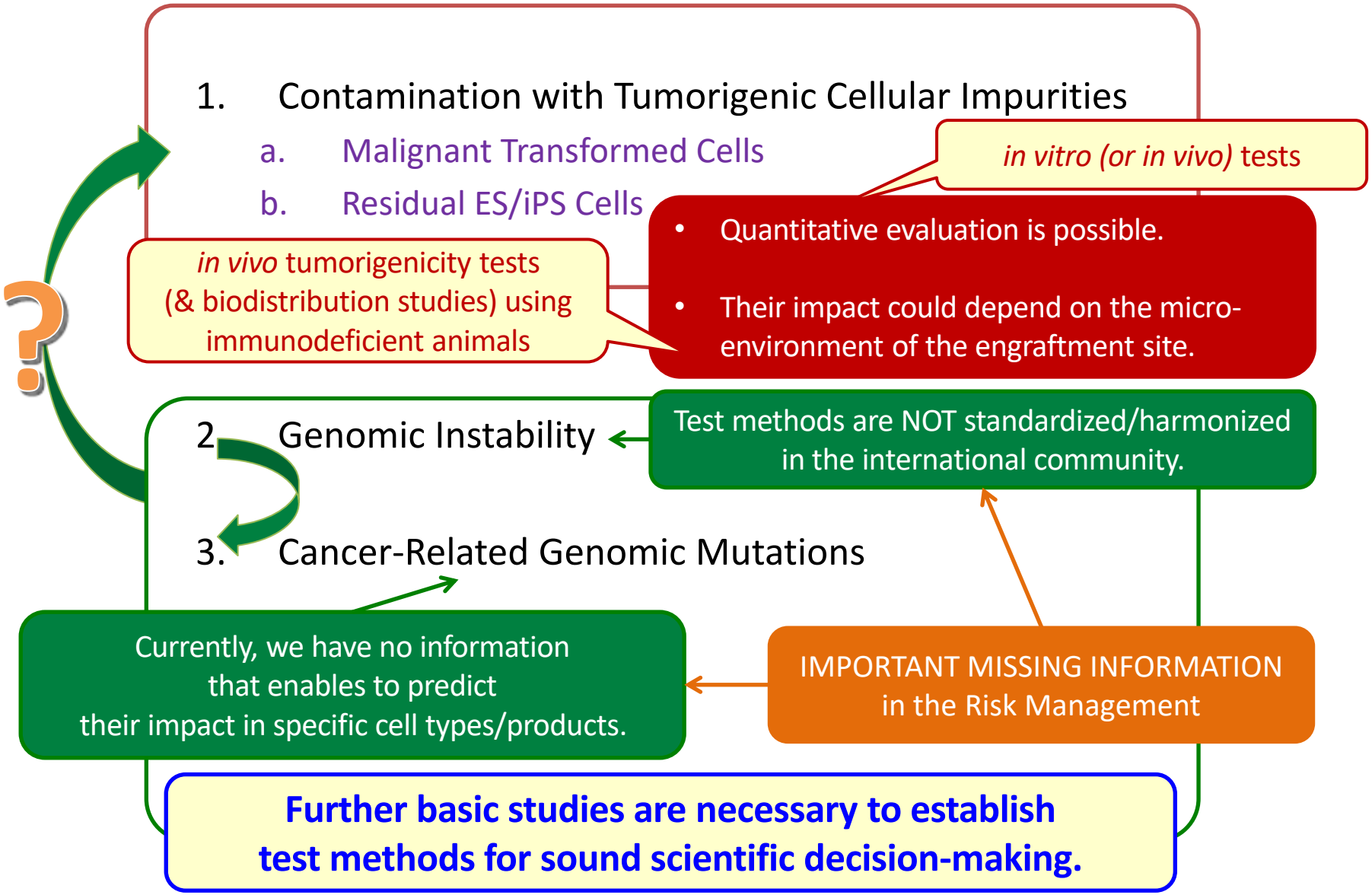
Somatic cells can accumulate mutations over the course of an individual's lifetime. This generates cells that differ genetically at specific loci within the genome. To explore how this genetic diversity in individuals contributes to disease, Yizhak *et al.* developed a method to detect mutations from RNA sequencing data (see the Perspective by Tomasetti). Applying this method to Cancer Genome Atlas samples and normal samples from the Genotype-Tissue Expression (GTEx) project generated a tissue-specific study of mutation accumulation. Somatic mutations were detected in nearly all individuals and across many normal human tissues in genomic regions called cancer hotspots and in genes that play a role in cancer. Interestingly, the skin, lung, and esophagus exhibited the most mutations, suggesting that the environment generates many human mutations.

…means "we currently have no way"

"Researchers now need to find ways to sort out which of those cells will become tumours and which are 'normal' "

Cristian Tomasetti, Johns Hopkins Medicine

Potential Hazards for the Tumorigenicity Risk of Pluripotent Stem Cell-Derived Therapeutic Products



Annex of Notification 0309-1 issued March 9, 2021

by the Director of **Research and Development Division, Health Policy Bureau**, MHLW

A Revised Version of the Annex of Notification 0613-3 issued June 13, 2016

Title:

"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"

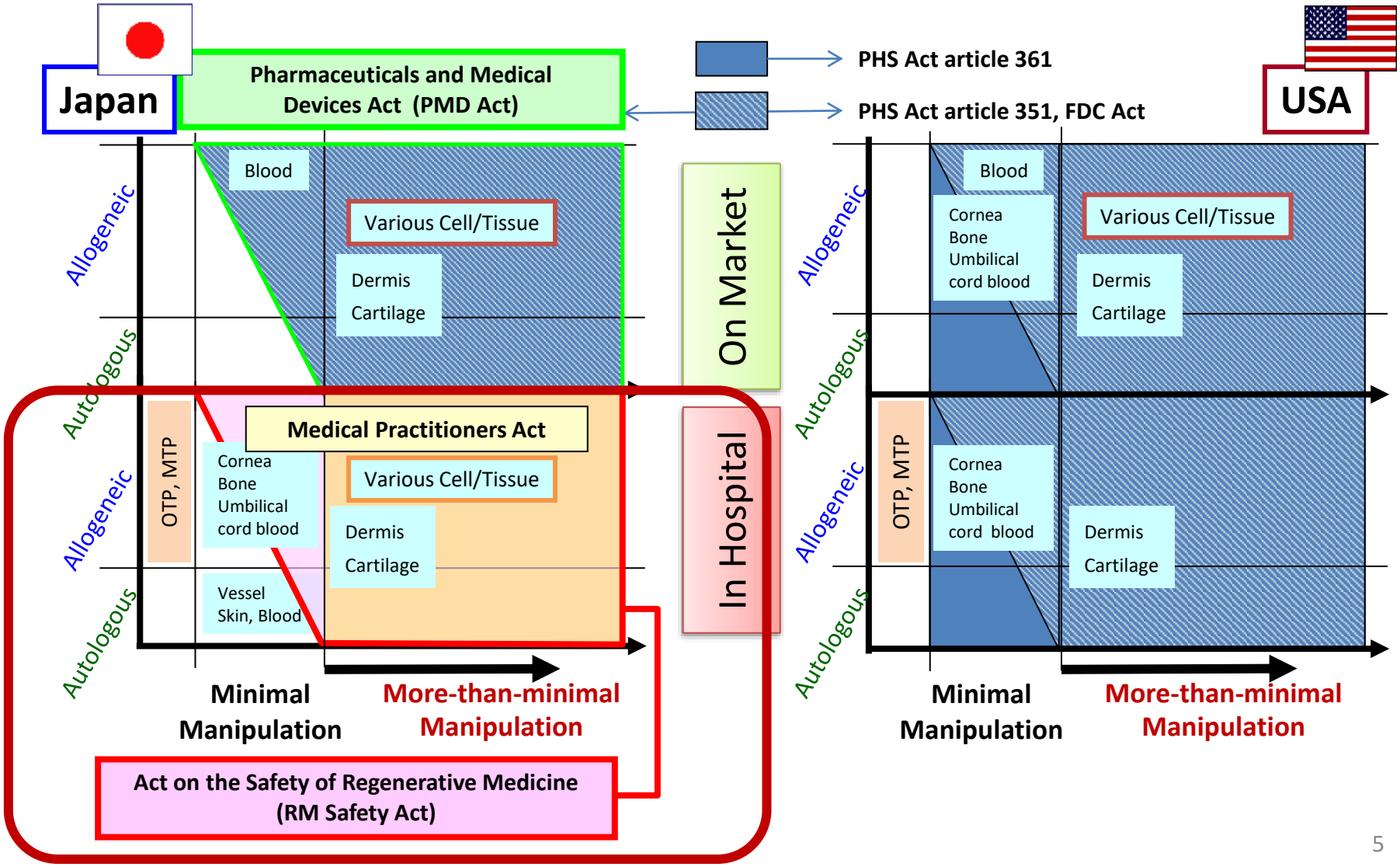
Scope:

Regenerative medicine using hPSCs under the Act on the Safety of Regenerative Medicine (RM Safety Act)

Contents:

Discussions of a scientific research group of MHLW on safety assessment of transplanted cells for implementing clinical research using iPS/ES cells

Regulation for regenerative medicine (RM)/cell therapy (CT)



Development Pathways for RM products in Japan

☐ Conventional development pathway

Non-commercial Clinical Research → Clinical Trial (confirmation of efficacy and safety) → Approval → Marketing

→ Similar to IND/IMP and MAA in US and EU/UK

☐ Special development pathway that accommodates early practical application of RM products

Non-commercial Clinical Research → Clinical Trial (likely to predict efficacy, and confirming safety) → Conditional/ Term-limited Approval → Marketing Further confirmation of efficacy and safety → Approval (or Revocation) → Marketing Continues

Post-marketing safety measures must be taken, including prior informed consent of risk to patients

RM Safety Act (including GCTP)

PMD Act + GCTP (GMP for RM products) + the other GXPs

- If data from the clinical trial are likely predict efficacy and confirming safety, conditional/term-limited marketing authorization for RM products might be granted to timely provide the products to patients.
- The PMD Act requires further confirmation of safety and efficacy during the post-marketing phase.

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Reviewed by PMDA (incorporated administrative agency)

Reviewed by Certified Special Committees for Regenerative Medicine (institutional committees)

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the Scope of the Annex of Notification 0309-1 (2021)

PTC for evaluation of tumorigenicity assessment in provision plans of RM using human PSCs

0. Introduction

1. Points to consider on safety required in pluripotent stem cells as raw material
   - (1) Surplus embryos and cells as raw materials
   - (2) Genomic indicators that cannot rule out tumorigenicity in pluripotent stem cells to be used as raw material

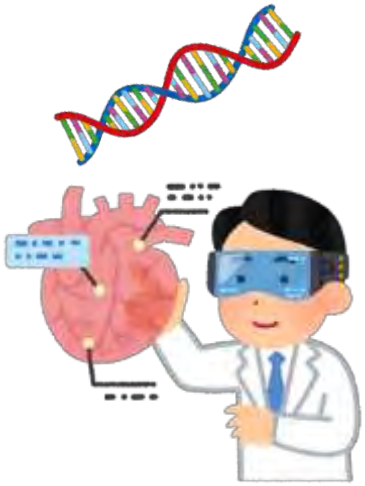
2. Points of review for tumorigenicity assessment of pluripotent stem cell-derived products
   - (1) Quality of raw materials
   - (2) In vitro study of the final product
   - (3) In vivo tumorigenicity test of the final product
   - (4) Risk management plan
   - (5) Appropriateness of the provision plan from the viewpoint of potential benefit

3. Reference information

Introduction

"Among non-clinical studies for the risk assessment of products derived from pluripotent stem cells (PSCs), the requirements for the evaluation of their tumorigenicity have not yet been established."

"The purpose of this report is to accumulate scientific data that will contribute to the future development of therapies using PSC-derived products in order to bring them to patients safely and as quickly as possible."



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Reference Information

"Genomic mutations, such as karyotype changes, are known to be detected in cultures of human cells. **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 genomic mutations observed *in vitro*. Genomic information, which is the baseline of genomic stability, differs depending upon cell type and culture methods. There are no cells that indicate an absolute stability in genomic replication when sub-cultured. Therefore, **to minimize genomic 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 genomic 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."**

Reference Information (cont'd)

"**If any mutations could be scientifically apparent as having a relationship with safety, such as tumorigenicity in cell products**, tests such as the following would improve the safety of cell products:

(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 genomic mutations with known correlation with functional abnormalities in differentiated cells of cell products or with known relationship with the target disease

However, in particular with pluripotent stem cell-derived products, it is still extremely novel and risk prediction is difficult. Therefore, it is **recommended** to confirm genomic 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.

It's just a recommendation for reassurance, not a strict regulatory requirement.

In other words, it is necessary to clarify the functionality of testing methods, such as the analytical limit of detection of low-allele frequency genomic mutation, and confirm the above points (1) to (3). The decision on clinical administration of pluripotent stem cell-derived products that have been detected to have the mutations in points (1) to (3) should be made, considering the seriousness of disease of the patient and urgency for treatment."

"Validation of analytical procedures" is critical.

PTC for evaluation of tumorigenicity assessment in provision plans of RM using human PSCs

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Annex of Notification 0309-1 issued March 9, 2021

Genomic indicators that cannot rule out tumorigenicity in pluripotent stem cells to be used **as raw material**

"Confirm:

- Chromosomal abnormalities (conventional karyotyping or G-band)
- SNVs/Indels of tumor-related genes (COSMIC Cancer Gene Census Tier1 http://cancer.sanger.ac.uk/census , "Shibata's list" https://www.pmda.go.jp/files/000152599.pdf)
  & Structural abnormalities including copy number variants (CNVs)
- Significant residual external factors that may promote tumors

approx. 600 genes in total

If an abnormality related to any of the above 3 items is found, a strict assessment of risks and potential benefits should be conducted to determine the appropriateness of clinical use. PSCs 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 the subject(s) 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."

PTC for evaluation of tumorigenicity assessment in provision plans of RM using human PSCs

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Annex of Notification 0309-1 issued March 9, 2021

In vitro study of the **final product**

"Confirm:

- A) **Chromosomal abnormalities (conventional karyotyping or G-band)**
  B) **SNVs/Indels of tumor-related genes**
  (COSMIC Cancer Gene Census Tier1 http://cancer.sanger.ac.uk/census
  and "Shibata's list" https://www.pmda.go.jp/files/000152599.pdf)
  & Structural abnormalities including copy number variants (CNVs)
- **Mutations added by updating the list of cancer-related genes** [If they are found in the final product already administered in a clinical trial, the risk should be assessed, and the information should be provided to the subject(s)].
- **Residual undifferentiated PSCs**
- **Unexpected cell transformation & abnormal growth of cells other than the desired cells** when cultured longer than the culture period.

If an abnormality related to any of the above 4 items is found, use is not recommended in principle, but in some cases, use may be justified after a strict assessment of risks and potential benefits to validate the target disease, administration method, etc. The explanation document upon consent to the subject(s) should be confirmed to obtain a clear explanation about the risks and benefits."

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… requires quality/non-clinical data for the assessment of the safety and efficacy of a specific regenerative medical product, **not for contributing to the future development of therapies using PSC-derived products.**

the Scope of the Annex of Notification 0309-1 (2021)

Non-Commercial Clinical Studies under the RM Safety Act and Clinical Trials under the PMD ACT for PSC-Derived Products in Japan

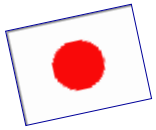
Final Product	Starting Cells	Target Disease	Institution(s)	Type of Clinical Trial	Year of IMP Approval	Year of FIH Trial
Retinal pigment epithelial cells	Autologous iPSCs	Exudative age-related macular degeneration	FBRI, RIKEN	Non-commercial clinical research under the RM Safety Act	2013	2014
Retinal pigment epithelial cells	Allogeneic iPSCs	Exudative age-related macular degeneration	Kobe City Medical Center, Osaka Univ., Kyoto Univ., RIKEN	Non-commercial clinical research under the RM Safety Act	2017	2017
Dopaminergic neural progenitor cells	Allogeneic iPSCs	Parkinson's disease	Kyoto Univ.	Clinical trial under the PMD Act	2018	2018
Platelets	Autologous iPSCs	Aplastic anemia	Kyoto Univ.	Non-commercial clinical research under the RM Safety Act	2018	2019
Corneal epithelial cells	Allogeneic iPSCs	Corneal epithelial stem cell exhaustion	Osaka Univ.	Non-commercial clinical research under the RM Safety Act	2019	2019
Hepatocytes	ESCs (Allogeneic)	Congenital urea cycle disorder	NCCHD	Clinical trial under the PMD Act	2019	2019
Cardiomyocytes	Allogeneic iPSCs	Ischemic cardiomyopathy	Osaka Univ.	Clinical trial under the PMD Act	2019	2020
Retinal photoreceptor cells	Allogeneic iPSCs	Retinitis pigmentosa	Kobe City Eye Hospital	Non-commercial clinical research under the RM Safety Act	2020	2020
NKT cells	Allogeneic iPSCs	Recurrent or advanced head and neck cancer	Chiba Univ., RIKEN	Clinical trial under the PMD Act	2020	2020

Summary

1. Genomic abnormality is a potential hazard for the tumorigenicity risk of cell therapy products (CTPs), including PSC-derived products.

2. However, significance and methodology of the genomic analyses are still open questions in the risk assessment of CTPs, as well as cells used as their raw materials.

3. In Japan, the Annex of Notification 0309-1 (2021) tentatively recommends extensive analyses of genomic abnormalities including SNVs/Indels and CNVs in PSC-derived products and PSCs as their raw materials, prior to the First-in-Human non-commercial clinical research under the RM Safety Act, in order to accumulate scientific data that will contribute to the future development of therapies using PSC-derived products.



Also, note that **the cost for WGS** conducted prior to a non-commercial clinical research on a PSC-derived product **under the RM Safety Act is usually covered by a part of public research grants,** not as in commercial clinical trials under the PMD Act.

Thank you for your attention!

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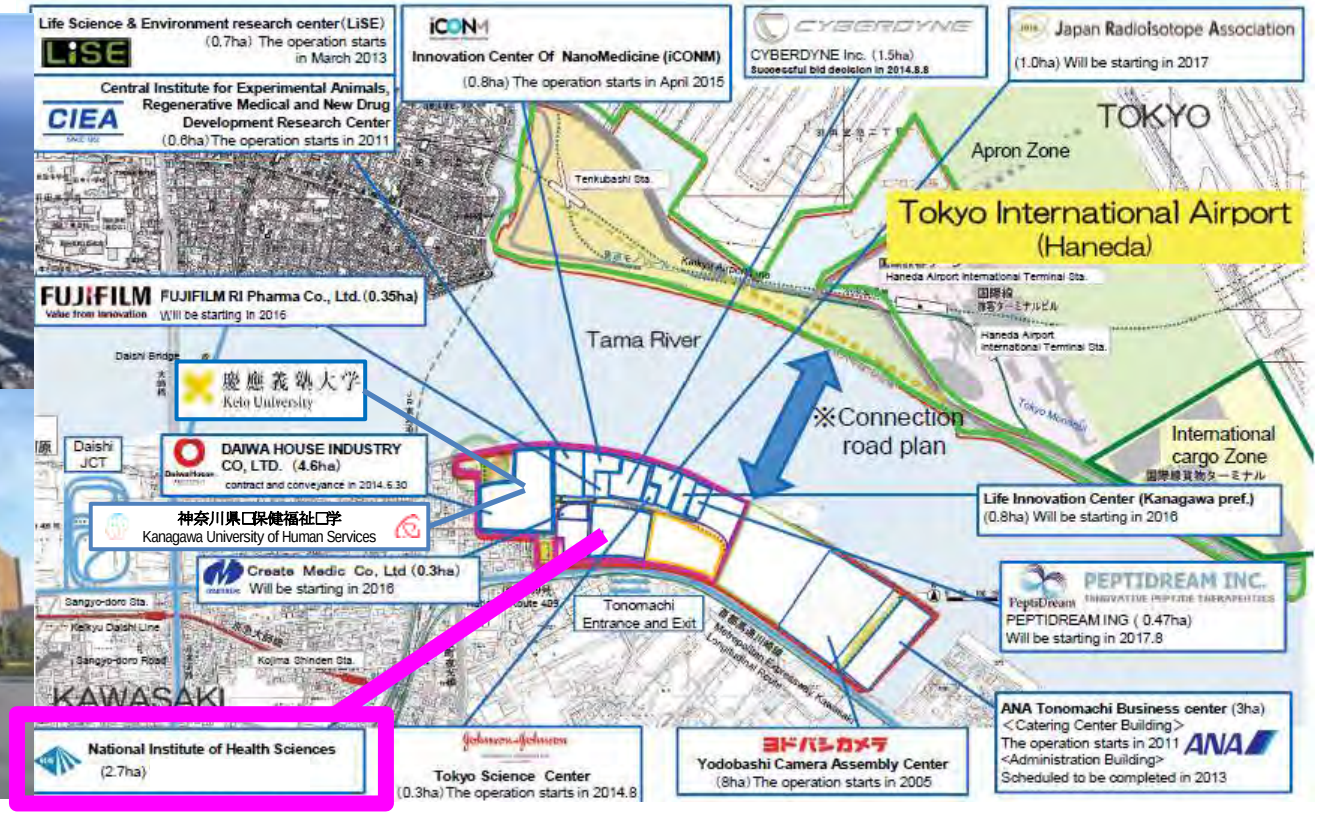

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* https://www.wikiwand.com/ja/%E3%83%AB%E3%83%95%E3%83%88%E3%83%8F%E3%83%B3%E3%82%B6%E3%83%89%E3%82%A4%E3%83%84%E8%88%AA%E7%A9%BA

** http://www.city.kawasaki.jp/en/page/0000038680.html

Supplements

2019 MHLW Grants-in-aid (MHLW Science Special Research Project)

Discussion Group on the Safety Assessment of Transplanted Cells for Implementing Clinical Research Using iPS Cells

Chairperson:

Tsuguya Fukui (St. Luke's International Hospital, St. Luke's International Univ.)

Members:

Tomohiro Akazawa (Juntendo Univ.)
Hiroyuki Aburatani (Univ. of Tokyo)
Toshikazu Ushijima (NCCRI)
Akihiro Umezawa (NCCHD)
Hideyuki Okano (Keio Univ.)
Seishi Ogawa (Kyoto Univ.)
Naoko Kakee (NCCHD)
Hiroko Goto(Chiba Univ.)
Yoji Sato (NIHS)
Yoshiki Sawa (Osaka Univ.)
Takao Hayakawa (NIHS)
Yuji Heike (St. Luke's International Hospital)
Akifumi Matsuyama (Fujita Medical Univ.)
Tomohiro Morio (TMDU)
Teruhide Yamaguchi (Nihon Pharm.Univ.)
Shinya Yamanaka (Kyoto Univ.)

“RM/CT as Medical Care” vs. “Products for RM/CT (& GT)”

	RM/CT as Medical Practices	Products for RM/CT (&GT)
Purpose	Ensuring the safety and validity of **medical treatments AND non-commercial clinical researches using “processed cells”**	Development, manufacturing & marketing of **regenerative medical products (RMPs = CTPs & GTPs)**
Regulatory Framework	**Medical Practitioners Act & Medical Care Act** **Regenerative Medicine Safety Act (RM Safety Act)** **Ordinance for Enforcement of the Act on the Safety of Regenerative Medicine (MHLW Ordinance No. 110 (2014))**	**Pharmaceuticals and Medical Devices Act (PMD Act)** **Guidelines and Standards for Ensuring the Quality/Safety of Cell-Based Therapeutic Products and Gene Therapy Products**
GCTP (GMP) Compliance	**Mandatory (MHLW Ordinance No. 110 (2014))**	**Mandatory (MHLW Ordinance No. 93 (2014))**
GCP Compliance in clinical researches/trials	**NOT necessarily mandatory for the data system** **Mandatory in the ethical procedures (MHLW Ordinance No. 110 (2014))**	**Mandatory in clinical trials of RMPs intended for marketing (MHLW Ordinance No. 89 (2014))**
Review	**Certified Committee for RM [institutional] [for Class 3 RM/CT] or Certified Special Committee for RM [institutional] [for Class 1 & 2 RM/CT]**	**Pharmaceuticals & Medical Devices Agency (PMDA) and Ministry of Health Labour & Welfare (MHLW)**
Advisory	**MHLW Health Science Council [for Class 1 RM/CT]**	
Health Insurance	**NOT covered by public insurance**	**Fully covered by public insurance**