NIHS
Since 1874

October 15, 2016
The Jackson Lab, Bar Harbor, Maine, USA

ISCI Workshop: Origins & Implications of Pluripotent Stem Cell (Epi)Genetic Instability and A symposium: to honor the work of Leroy Stevens

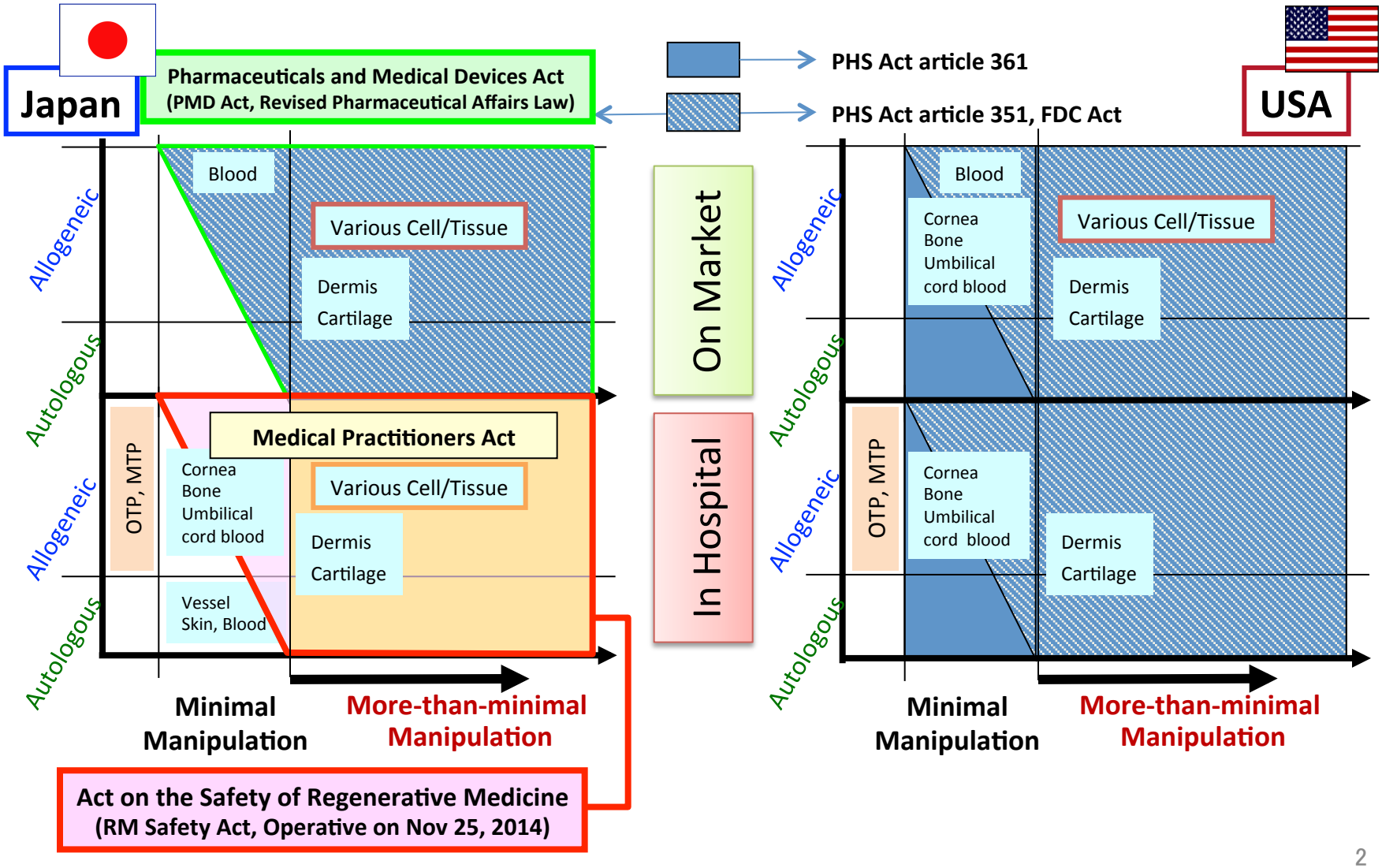
Current regulatory issues on tumorigenicity assessment of human pluripotent stem cell-derived products in Japan

Yoji SATO, PhD

Head, Division of Cell-Based Therapeutic Products

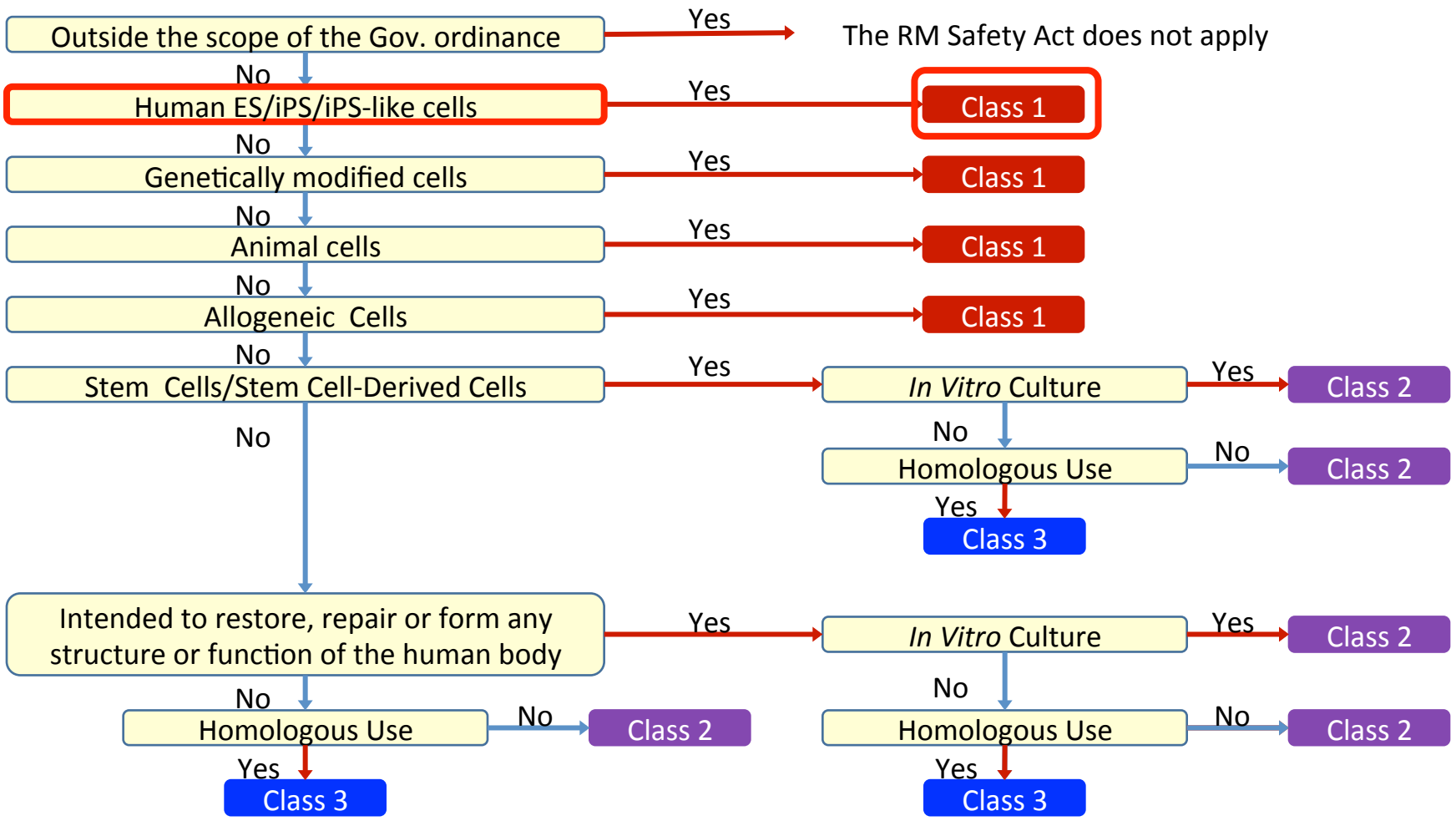
National Institute of Health Sciences, Tokyo, JAPAN

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



Classification of RM/CT under the RM Safety Act

Class 1, High Risk; Class 2, Middle Risk; Class 3, Low Risk.



“RM/CT as Medical Practice” vs. “Products for RM/CT”

	RM/CT as Medical Practice	Products for RM/CT
Purpose	Development & Provision of the Medical Treatment	Development, Manufacturing & Marketing of the Products
Regulatory Framework	**Act on the Safety of Regenerative Medicine (RM Safety Act, enacted on Nov 25, 2014)** The Standards for the Provision of Regenerative Medicine (MHLW Ministerial Ordinance No.110 (2014)) Ethical GLs for Medical Researches on Human Subjects (MEXT/MHLW Notification No.3 (2014)) GLs for Gene Therapy Clinical Research (MHLW & MEXT Notification No.2 (2004)) [in vivo gene therapy]	**Pharmaceuticals and Medical Devices Act (PMD Act, Revised Pharmaceutical Affairs Law, enacted on Nov 25,2014)** GLs and Standards for Assuring the Quality/Safety of Cell-Based Therapeutic Products and Gene Therapy Products
GCP Compliance	**Not Mandatory**	**Mandatory**
GCTP Compliance	**Mandatory**	**Mandatory**
Review	Certified Committee for RM [for Class 3 RM/CT] Certified Special Committee for RM [for Class 1 & 2 RM/CT] Ministry of Health Labour & Welfare (MHLW) [for Class 1 RM/CT and *in vivo* gene therapy]	Pharmaceuticals & Medical Devices Agency (PMDA) MHLW
Health Insurance	**Not or Partly Covered by the Public Insurance**	**Fully Covered by the Public Insurance**
Demarcation of MHLW	**Research and Development Division, Health Policy Bureau**	**Pharmaceutical Safety and Environmental Health Bureau**

Annex of Notification 0613-3 issued June 13, 2016

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

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"

Target:

Certified special committees for regenerative medicine

Contents:

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

2015 MHLW Grants-in-aid (MHLW Science Special Research Project)

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

Chairperson:

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

Members:

Tomohiro Akazawa (Tokyo Medical and Dental University)
Hiroyuki Aburatani (The University of Tokyo)
Toshikazu Ushijima (National Cancer Center Research Institute)
Akihiro Umezawa (National Center for Child Health and Development)
Hideyuki Okano (Keio University)
Seishi Ogawa (Kyoto University)
Naoko Kakee (National Center for Child Health and Development)
Hiroko Goto(Chiba University)
Yoji Sato (National Institute of Health Sciences)
Yoshiki Sawa (Osaka University)
Ryozo Nagai (Jichi Medical University)
Takao Hayakawa (Kindai University)
Akifumi Matsuyama (National Institutes of Biomedical Innovation, Health and Nutrition)
Tomohiro Morio (Tokyo Medical and Dental University)
Teruhide Yamaguchi (Nihon Pharmaceutical University)
Shinya Yamanaka (Kyoto University)

Advisors:

Yuji Heike (St. Luke's International Hospital)
Satoshi Tsunoda (PMDA)
Mazago Minami (The Yomiuri Shimbun, Tokyo Head Office)

Annex of Notification 0613-3 issued June 13, 2016

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

"The requirements for non-clinical study necessary for assessing the risk of pluripotent stem cell-derived cell products 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 products to be delivered to patients as safely and quickly as possible."

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

Annex of Notification 0613-3 issued June 13, 2016

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

"Confirm:

- Chromosomal abnormalities (conventional or G-band)
- Structural abnormalities including SNV/Indel of tumor-related genes (Cosmic census http://cancer.sanger.ac.uk/census &
  "Shibata's list" https://www.pmda.go.jp/files/000152599.pdf)
  and copy number variants (CNV)
- Significant residual external factors that may promote tumors

approx. 600 genes in total

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. 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."

Genomic indicators that cannot rule out tumorigenicity in pluripotent stem cells to be used **as raw material** (cont'd)

Appropriateness of Clinical Use

"To minimize the risk to target patients, pluripotent stem cells that have been determined as having no abnormalities related to the above items (the previous slide) 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."

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

Annex of Notification 0613-3 issued June 13, 2016

In vitro study of the **final product**

"Confirm:

- A) **Chromosomal abnormalities** (conventional or G-band),
  B) **structural abnormalities** including SNV/Indel of tumor-related genes (Cosmic census + Shibata list) and copy number variants (CNV),
  C) 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. The explanation document upon consent to target patients should be confirmed to obtain a clear explanation about the risks and benefits."

In vitro study of the **final product** (cont'd)

Risk-benefit assessment

"To minimize the risk to target patients, pluripotent stem cell-derived products that have been determined as having no abnormalities related to the above items (the previous slide) 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 products, if there is a possibility that health benefits to target patients exceed the risk, use of pluripotent stem cell-derived products 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 tumor growth, and whether cell observation after transplantation is easy."

Annex of Notification 0613-3 issued June 13, 2016

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

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."

Reference Information (cont'd)

"If some 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 genetic 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 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.

It's 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 genetic mutation, and confirm the above points (1) to (3). The judgment on clinical administration of pluripotent stem cell-derived 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."

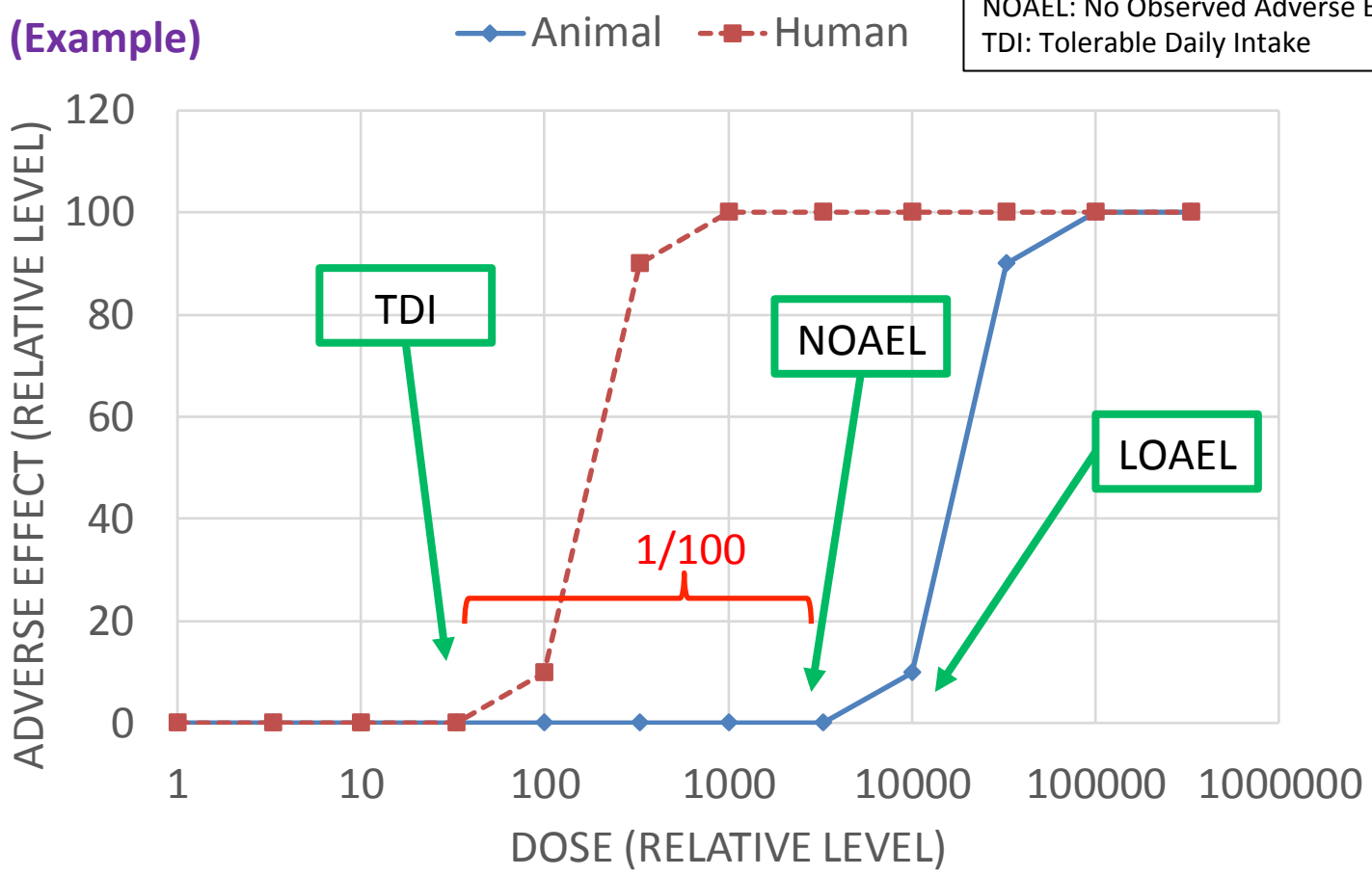
"Analytical science" plays a critical role .

Why is (epi)genomic analysis “for reassurance”, not “a requirement”?

…Is is because of the Principle of Non-Clinical Toxicology

Chronic toxicity assessment of a chemical compound

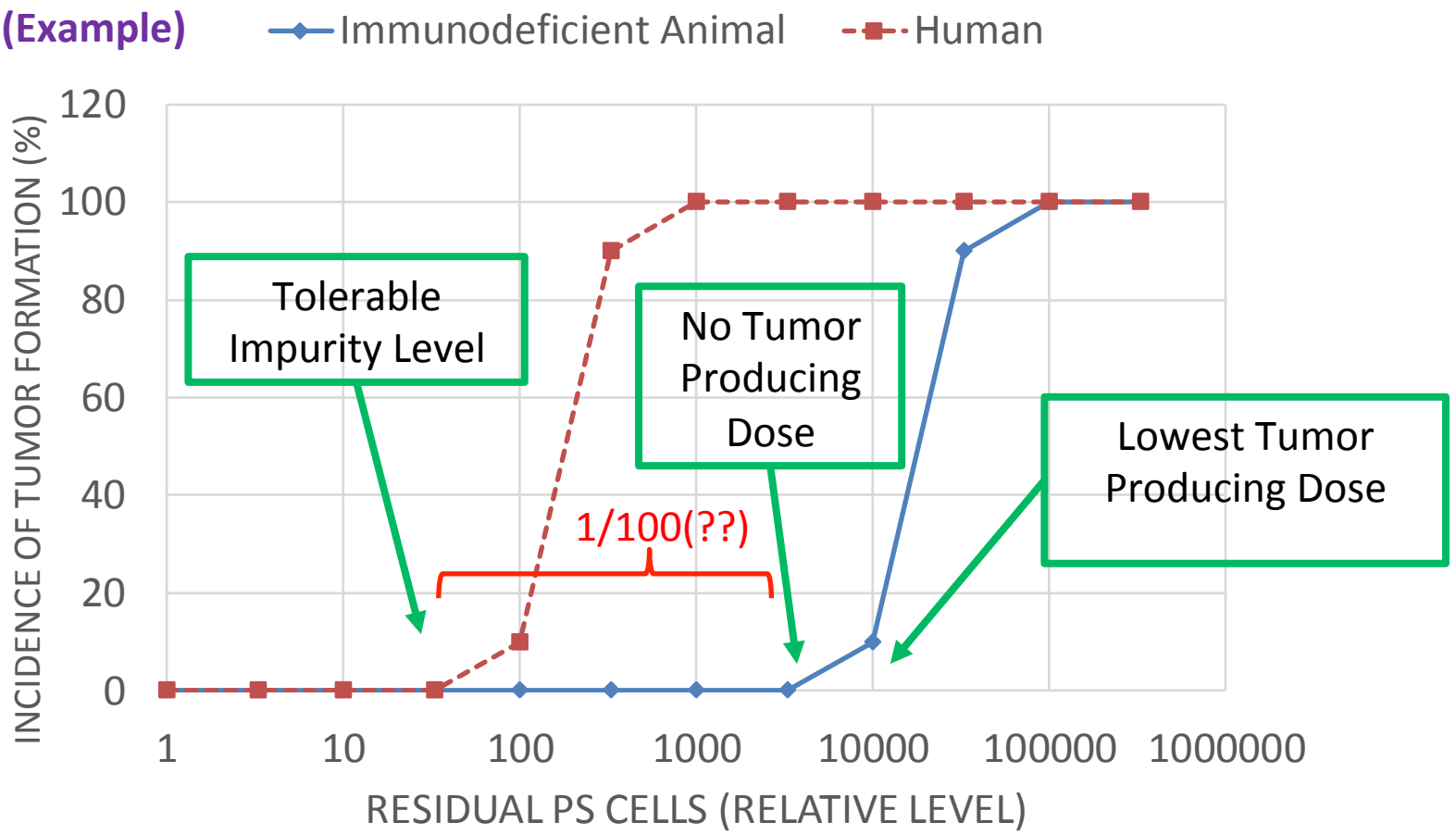
LOAEL: Lowest Observed Adverse Effect Level
NOAEL: No Observed Adverse Effect Level
TDI: Tolerable Daily Intake



Why is (epi)genomic analysis “for reassurance”, not “a requirement”?

…Theoretically, the similar approach could be applied to

Assessment of tumorigenicity derived from residual PSCs in CTPs



Why is (epi)genomic analysis “for reassurance”, not “a requirement”?

…But, in case of

Assessment of tumorigenicity derived from (epi)genomic abnormalities in CTPs

We have no information about the dose-response relationship between a specific (epi)genomic abnormality in CTPs and their tumorigenicity both in immunodeficient animals and human. We also have no information about the condition for the specific (epi)genomic abnormality to elicit tumor formation in a specific target microenvironment of human body.

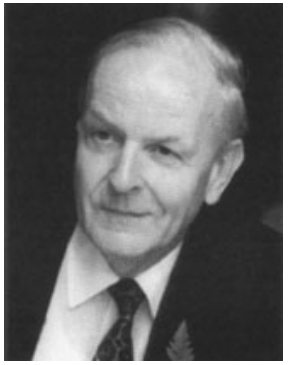
- We cannot figure out the tolerable level/condition of the (epi)genomic abnormality in human.
- We don’t know whether and how much the (epi)genomic abnormality is hazardous in a specific type of cells in a specific target microenvironment of human body.

For the moment, (epi)genomic analysis is **not necessarily mandatory but encouraged** to be performed prior to the FIH of hPSC-derived products under the RM Safety Act.

Quotes from Gerhard Zbinden (arguably the father of modern toxicology)
with Supplements by Robert Hamlin, DVM (Ohio State Univ.)

From Zbinden

1. Do not do something just because you can.
2. Do not do something just because it has always been done.
3. Do not do something just because others do it.



http://www.forschung3r.ch/de/publications/bu3.html

Supplements by Hamlin

4. Do not do something because (you believe) it is expected.
5. Do not do something the results of which cannot be interpreted.
6. Do something because there is a reasonable expectation it will provide knowledge necessary for an accurate decision.

https://vet.osu.edu/hamlin-robert

Hamlin RL, Toxicologic Pathology, 34:75–80, 2006

Thank you for your attention

Contact Information

Yoji Sato, Ph.D.
Head, Division of Cell-Based Therapeutic Products
National Institute of Health Sciences
1-18-1 Kami-Yoga, Setagaya, Tokyo 158-8501, Japan

E-mail: yoji@nihs.go.jp