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2023 NIFDS-PMDA-USP Workshop for Advanced Therapies

Session 3-2: Pre-clinical safety study

Assessment of Tumorigenicity and Genomic Instability of Human-Derived Cell Therapy Products

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National Institute of Health Sciences, Japan

DISCLAIMER

The views and opinions expressed in this presentation are those of the presenter and do not necessarily represent the official policy or position of the National Institute of Health Sciences or the Ministry of Health, Labour & Welfare. Also, the presenter has no COI to disclose in connection with this presentation.

AGENDA

- 1. What is tumorigenicity? –The risk of tumorigenesis and its hazards–**
- 2. Development of highly sensitive test methods for the detection of transformed cells in human cell therapy products**
- 3. Development of highly sensitive test methods for the detection of residual pluripotent stem cells in human ES/iPS cell-derived products**
- 4. How much are genomic mutations predictive of abnormal tissue formation from human iPSC-derived products after engraftment?**

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Major Challenges in Regulatory Science of Cell Therapy Products

What should be evaluated?

1. Viral safety (allogeneic vs. autologous)
 2. Characteristics and eligibility of cells to be used as raw materials
 3. Eligibility of ancillary materials of human or animal origin, other than cell substrates
 4. Establishment and management of cell banks as cell substrates
 5. Manufacturing strategy and process validation to achieve reproducibility of the final product quality
 6. Characterization of cells as active ingredients of the final product
 7. Identification and specification of critical quality attributes of the final product (QC of the final product)
 8. Comparability in the quality of products subject to changes in their manufacturing process/cell banks
 9. Design and interpretation of non-clinical safety studies and non-clinical proof-of-concept studies
 10. Design and interpretation of tumorigenicity studies (especially for ESC/iPSC-derived products)
 11. Immunogenicity of the final product
 12. Biodistribution of administered cells *in vivo* and their behavior at the engraftment site
 13. Design and interpretation of clinical trials
 14. Efficacy and safety follow-up
-
- The diagram groups the 14 challenges into four categories using colored brackets on the right side:
- Safety & eligibility of raw materials** (blue bracket): Includes items 1, 2, 3, and 4.
 - Ensuring the quality of the final product** (red bracket): Includes items 5, 6, 7, and 8.
 - Prediction of safety & efficacy in the non-clinical phase** (green bracket): Includes items 9, 10, 11, and 12.
 - Clinical Evaluation** (purple bracket): Includes items 13 and 14.

Major Challenges in Regulatory Science of Cell Therapy Products

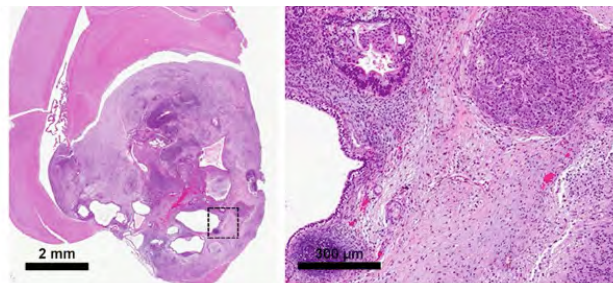
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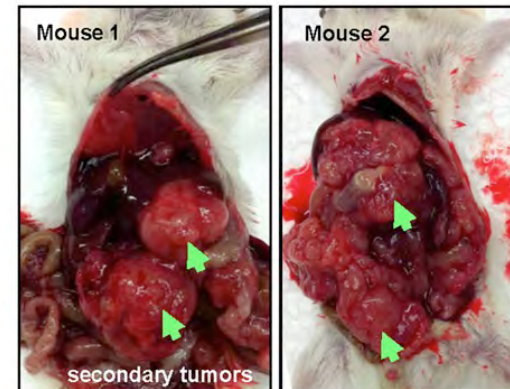
Tumorigenicity

... is one of the major concerns for pluripotent stem cell-derived therapeutic products

- Human pluripotent stem cells (PSC) have the potential to revolutionize regenerative medicine and cell therapy.
- Some clinical trials on pluripotent stem cell-derived products are currently on going, and more trials are expected to start soon in many countries
- However, cells transformed during the manufacturing process and residual undifferentiated PSCs may form tumors in patients.



Ibon Garitaonandi et al. Scientific Reports | 6:34478



MOUSTAFA M et al. STEM CELLS TRANSLATIONALMEDICINE 2016;5:694–702

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

1. Contamination with Tumorigenic Cellular Impurities
 - a. **Malignant Transformed Cells**
 - b. **Residual ES/iPS Cells**
2. Genomic Instability
3. Cancer-Related Genomic Mutations



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

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a. **Malignant Transformed Cells**

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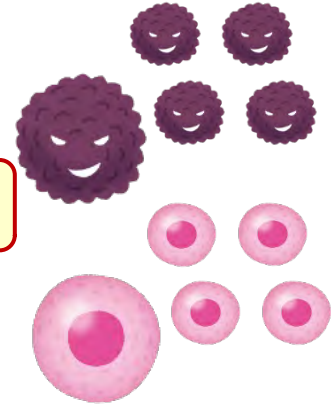
in vitro (or in vivo) tests

in vivo tumorigenicity tests
(& biodistribution studies) using
immunodeficient animals

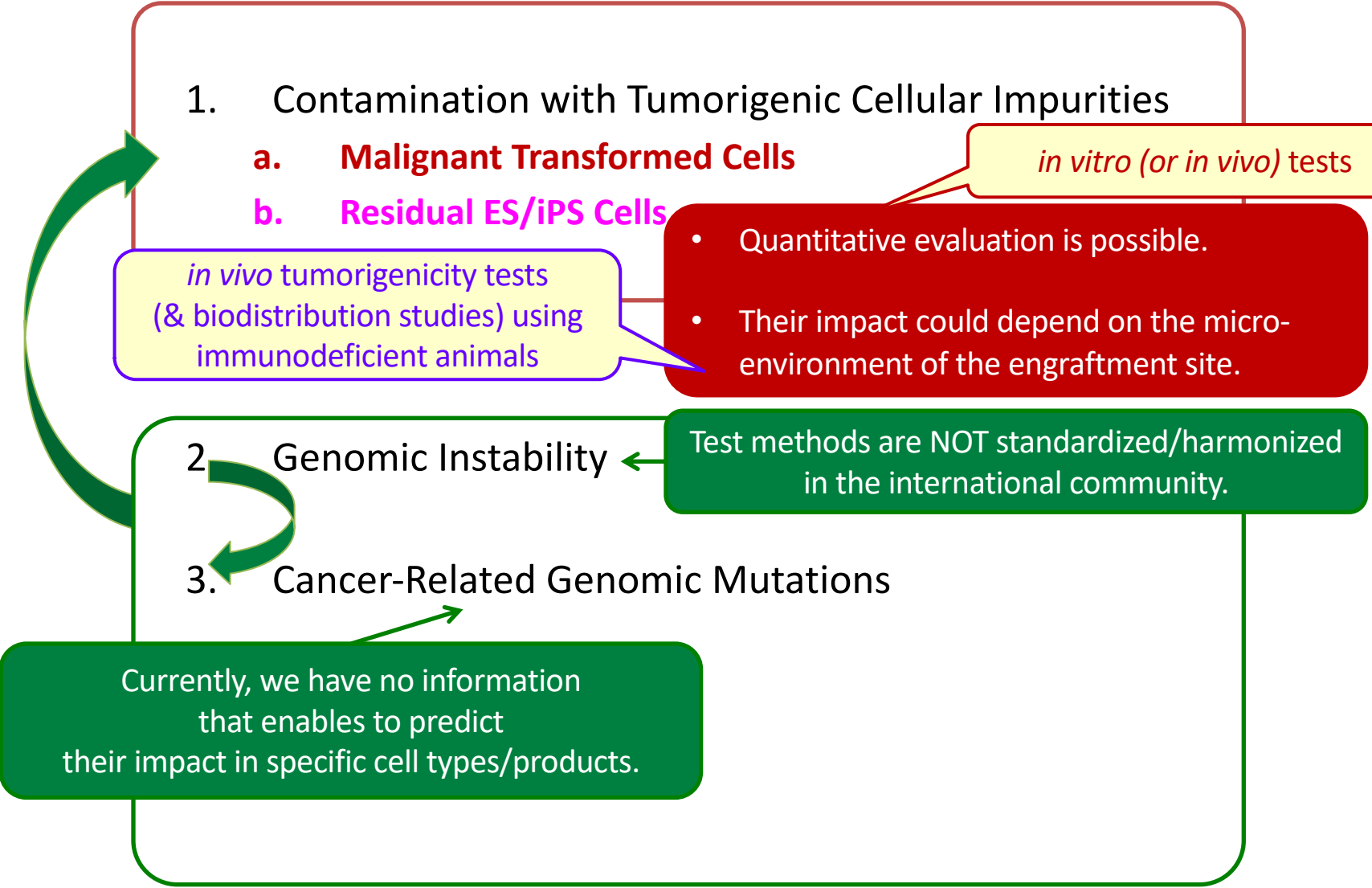
- Quantitative evaluation is possible.
- Their impact could depend on the micro-environment of the engraftment site.

2. Genomic Instability

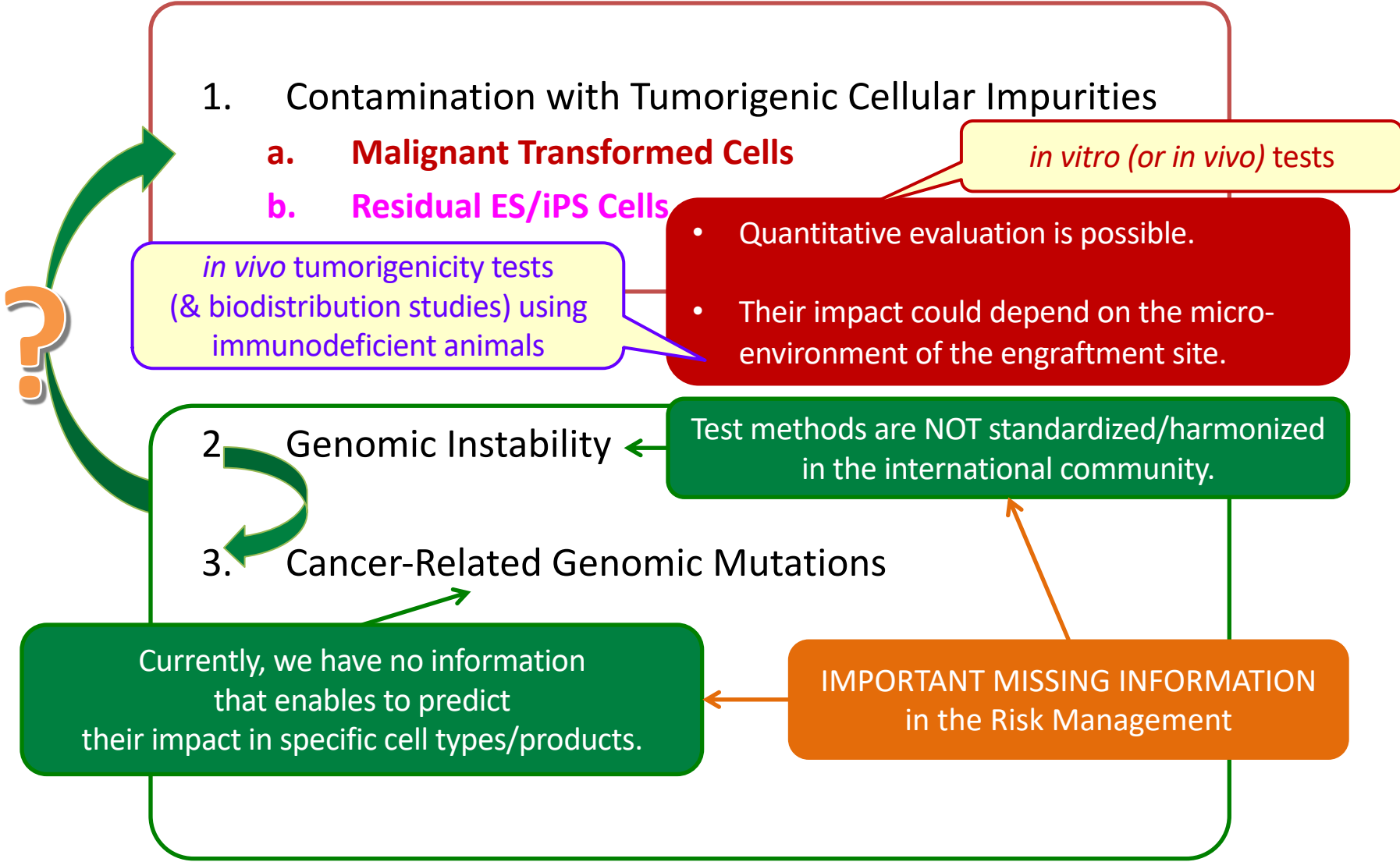
3. Cancer-Related Genomic Mutations



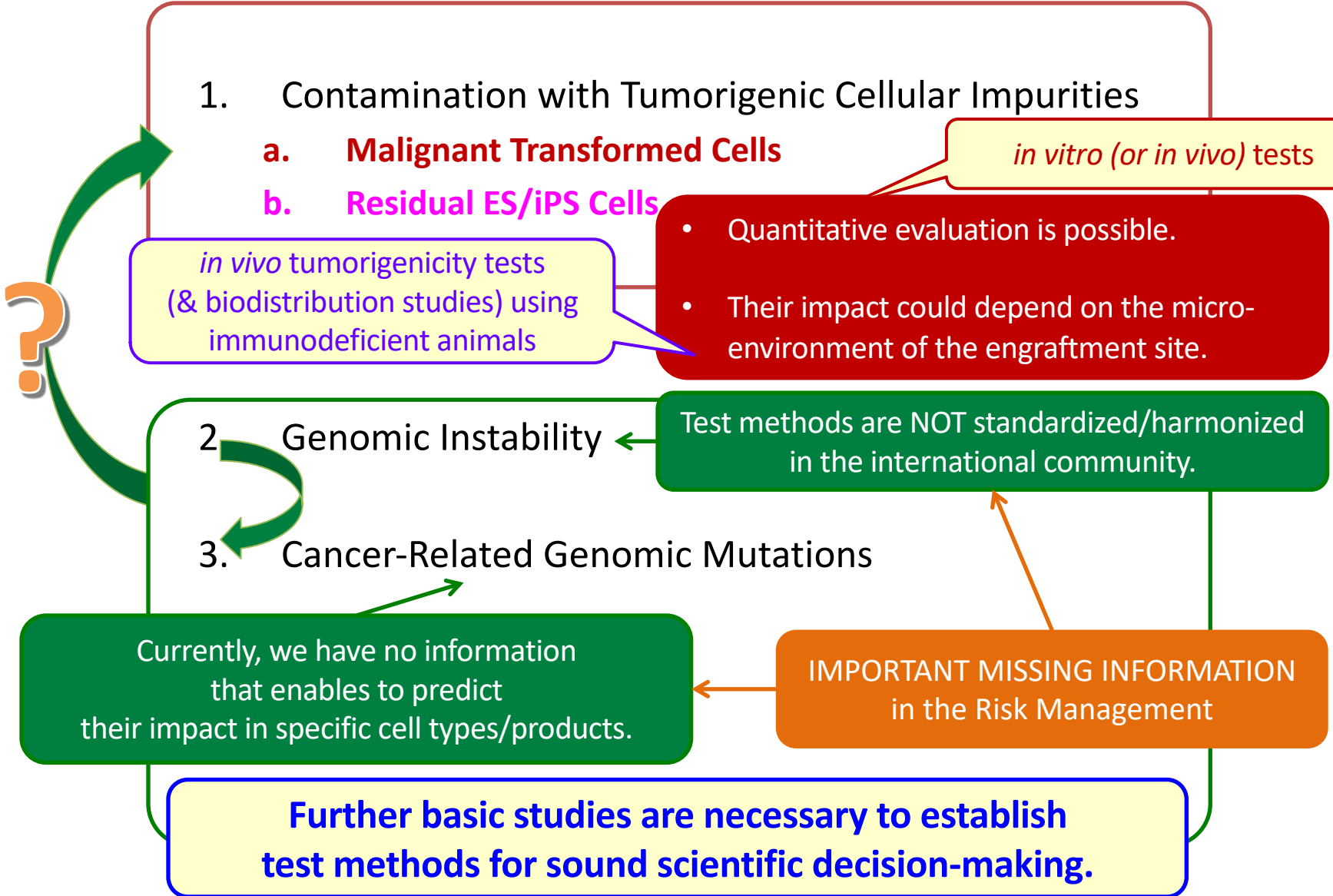
Potential Hazards for the Tumorigenicity Risk of Pluripotent Stem Cell-Derived Therapeutic Products



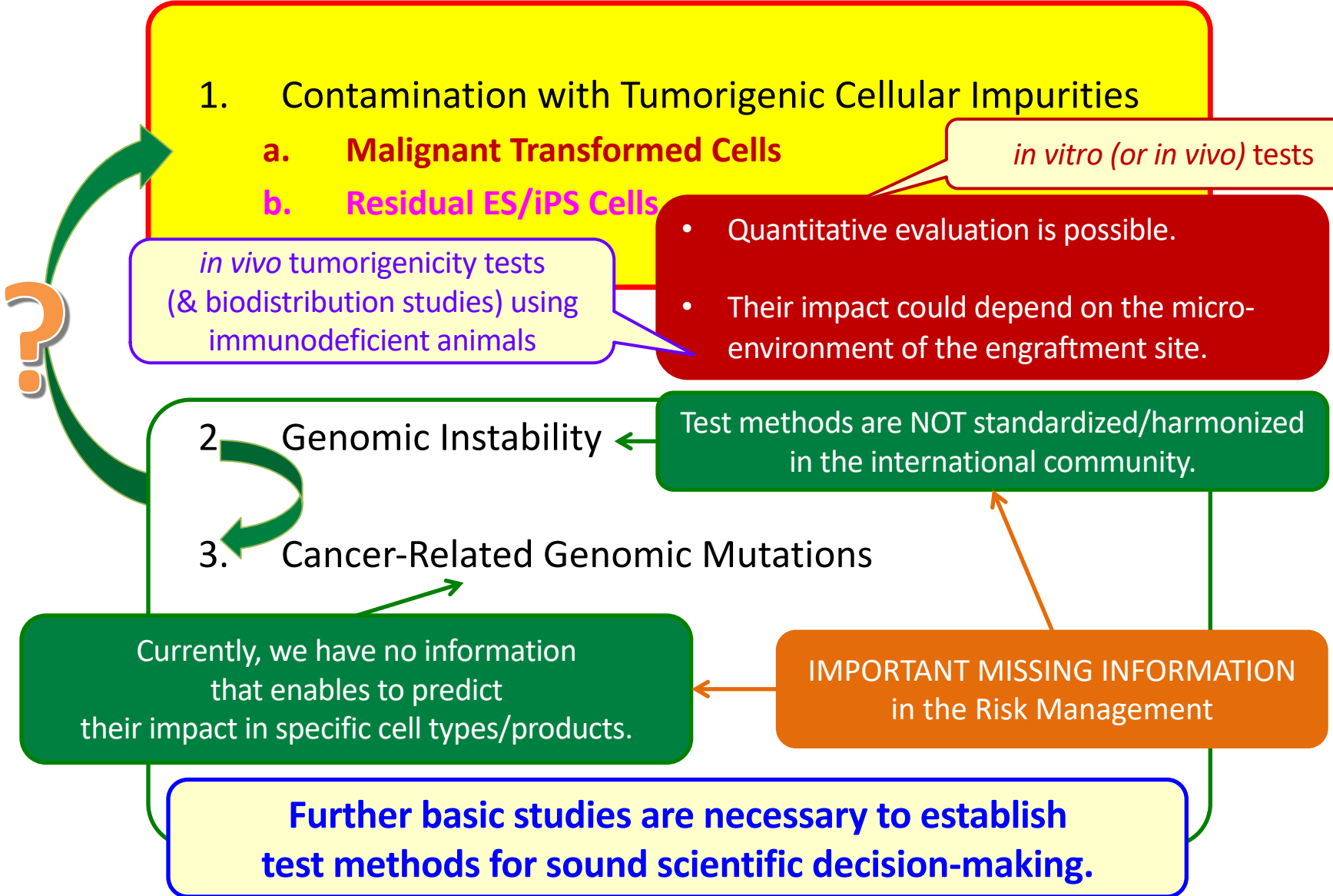
Potential Hazards for the Tumorigenicity Risk of Pluripotent Stem Cell-Derived Therapeutic Products



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



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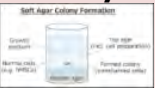


Development of Test Methods for Detection of Transformed Cells



Tumorigenic Cellular Impurities \nearrow
 =Hazards of PSC-Derived Products

In Vitro Assays

In Vivo Assay

Assays/ Platform	Conventional soft agar colony formation 	Digital soft agar colony formation 	Cell growth analysis 
Positive control	HeLa cells	HeLa cells	HeLa cells
Duration	3 to 4 weeks	3 to 4 weeks	4 weeks or more
Assay principle	Conventional SACF assay based on anchorage-independent cell growth	Image-based screening system for the SACF assay using a high-content cell analyzer	The analysis of cell senescence/growth after serial passaging (compare the growth rates of hMSC w/wo positive controls after 5 passages)
Pros	Low cost	High sensitivity	High sensitivity, Low cost
Cons	Low sensitivity	High cost (needs image scanner)	Time-consuming
Sensitivity	0.02%	0.00001%	0.0001%
Reference	Kusakawa et al., Regen Ther. 2015	Kusakawa et al., Sci Rep. 2015	Kono et al., Biologicals. 2015 Hasebe-Takada et al. Regen Ther 2016

Assays/Platform	Tumorigenicity Test
Animals	NOG mice
Route	Subcutaneous transplantation
Positive control	HeLa cells
Duration	>= 16 weeks
Pros	Direct evaluation in micro environment (expected clinical use site)
Cons	High cost, Long time, Especial facility, Low through put, Histopathological evaluation to confirm malignancy of the tumor
Sensitivity	to detect 10 HeLa cells in 10^6 hMSC (0.0001%) at 17% of probability
Reference	Kusakawa et al., Regen Ther. 2015



Development of Test Methods for Detection of Transformed Cells






Tumorigenic Cellular Impurities ↗
= Hazards of PSC-Derived Products

Example 1

In Vitro Assays

In Vivo Assay

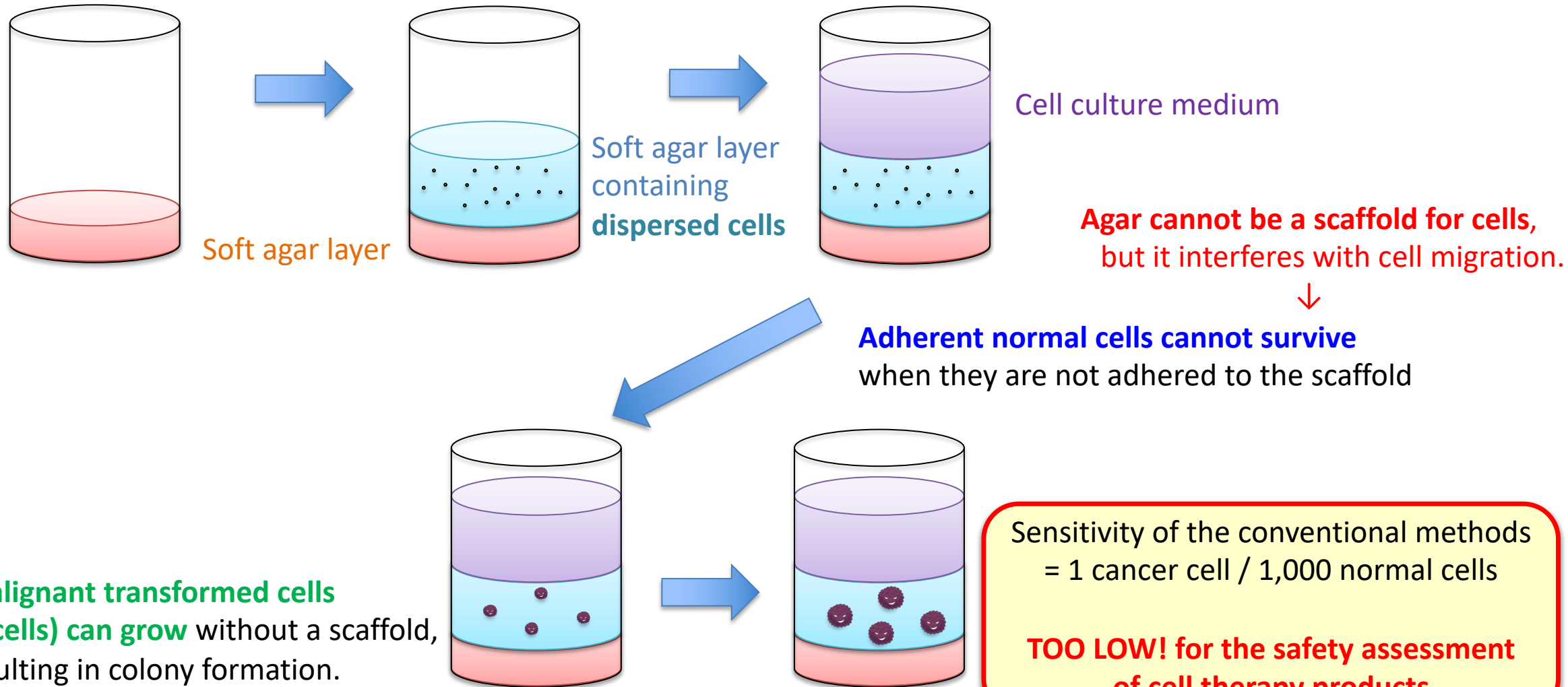
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Conventional Soft Agar Colony Formation Assay

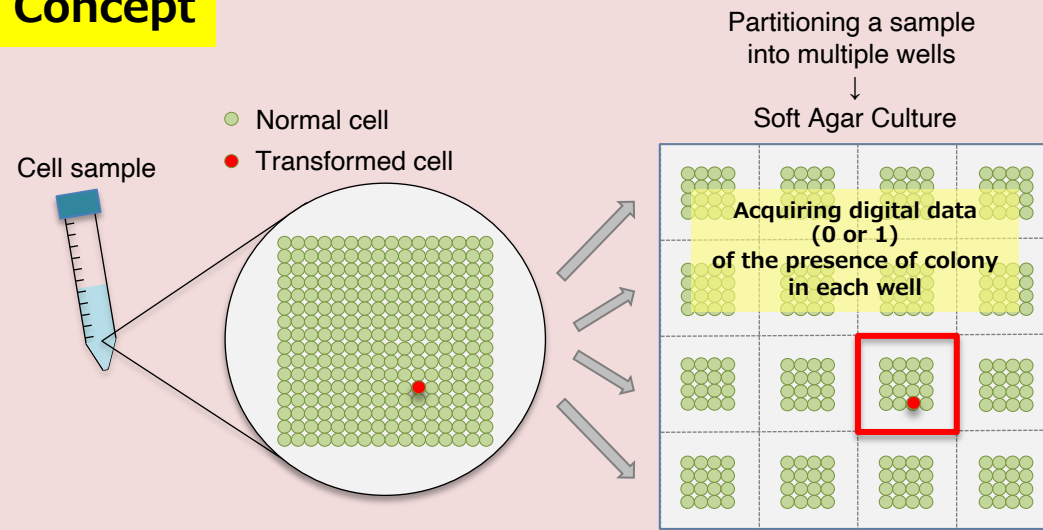
Purpose: Detection of scaffold-independent proliferation (= **malignant transformed cells**)





Digital Soft-Agar Colony Formation Assay

Concept



Partitioning a cell sample into multiple wells of culture plates enables digital readout of the presence of colony in each well and elevates the sensitivity for their detection.

Low S/N ratio

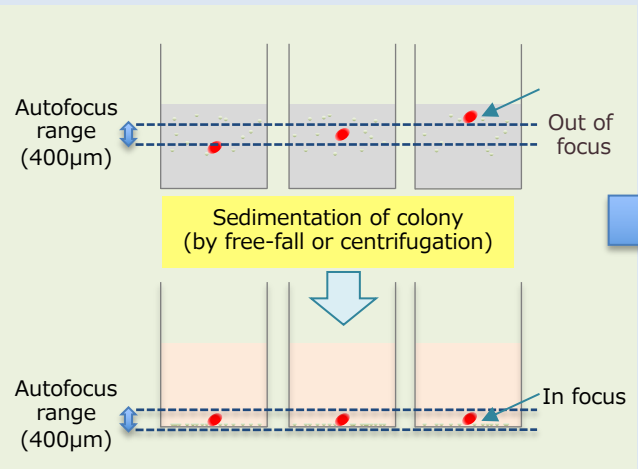
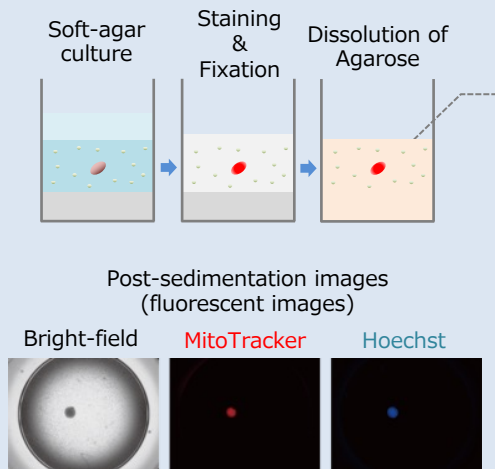


High S/N ratio

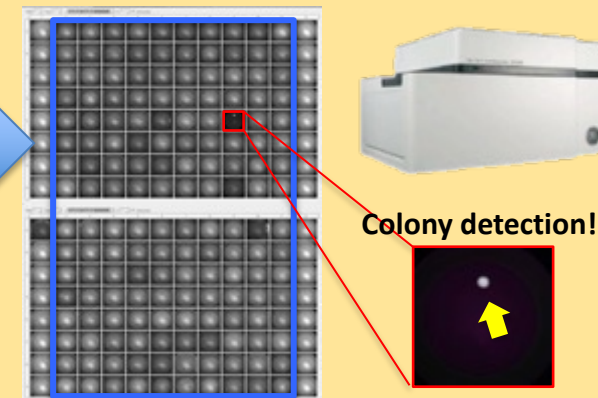


Procedures

Soft-agar culture & sample preparation

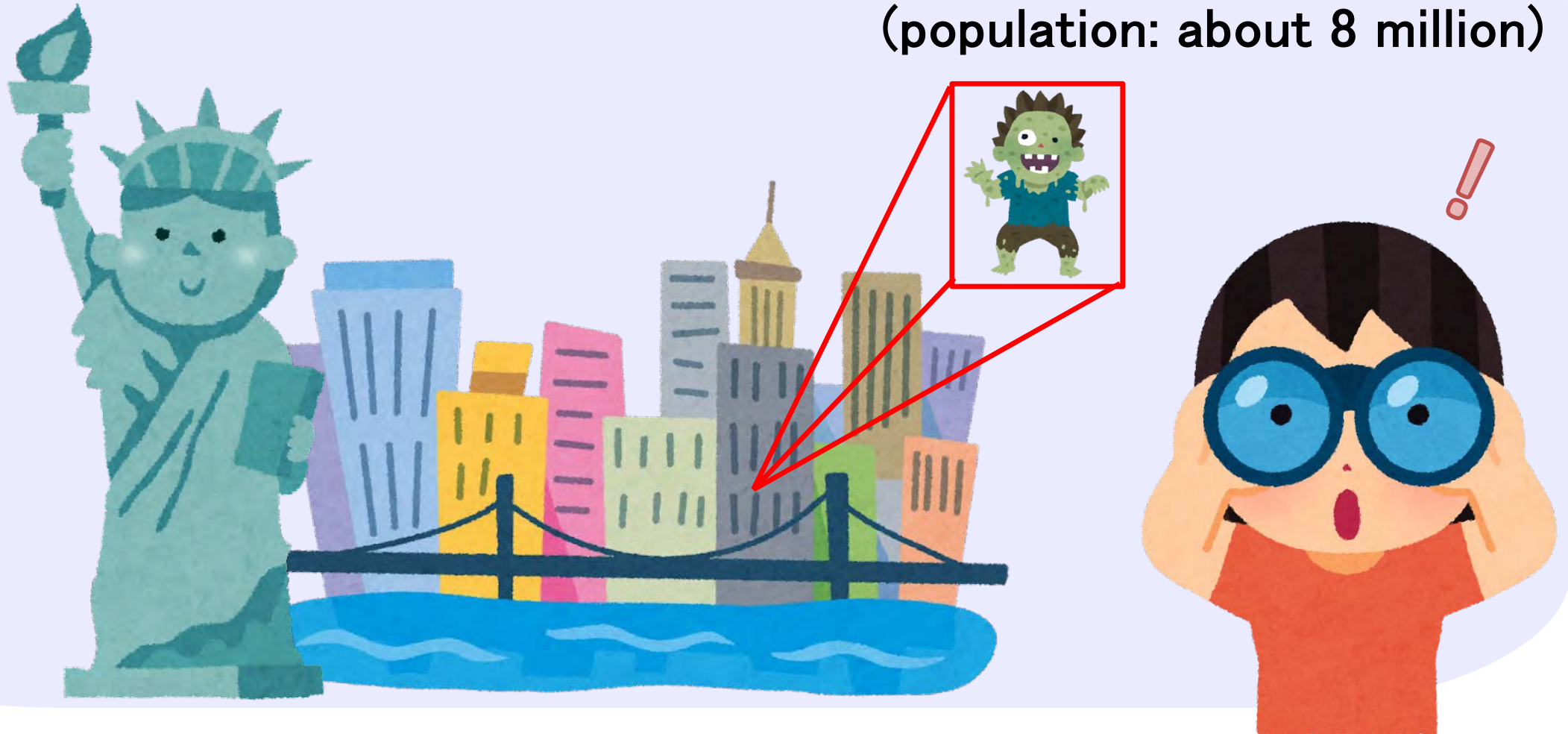


High-throughput screening of colony formation using an imaging cytometer



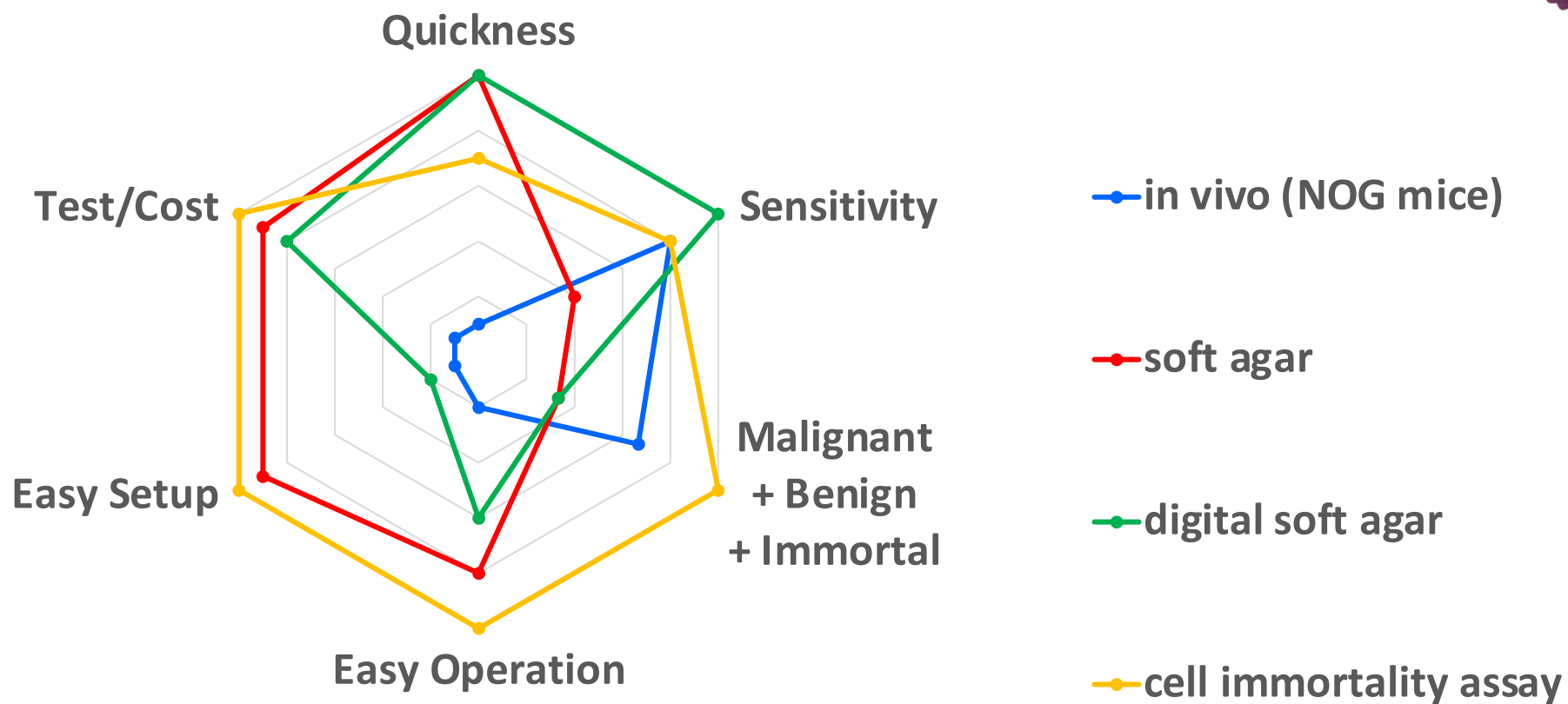
Digital Soft-Agar Colony Formation Assay has achieved the ability to detect cancer cells in normal cells at a ratio of **1 in 10 million**

Comparable to the ability to find one zombie in New York City
(population: about 8 million)



Qualitative Comparisons of Test Methods for Detection of Transformed Cells

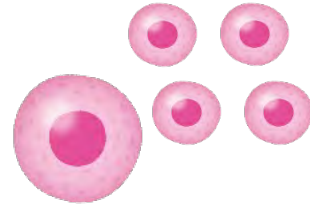
(based on our validation studies and past literature)



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Development of Test Methods for Detection of Residual Undifferentiated PSCs



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In Vitro Assays

In Vivo Assay

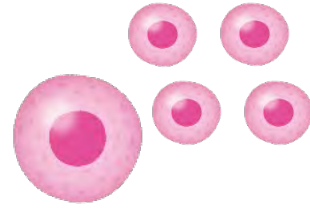
Assays/ Platform	Flow cytometry 	qRT-PCR 	Droplet Digital PCR 	Direct detection using a highly efficient amplification method*
Positive control	iPS cells	iPS cells	iPS cells	iPS cells
Duration	1 day	6 hours	a few hours	about a week
Marker	TRA-1-60 etc	Lin28	Lin28	-
Pros	Simple/quick	Simple/quick, High sensitivity	Simple/quick, High sensitivity	Direct detection, High sensitivity
Cons	Low sensitivity, Indirect detection, Difficulty in the manual selection of marker thresholds	Indirect detection, Lin28 expression is noted in some differentiated cells	Indirect detection, Lin28 expression is noted in some differentiated cells	Time-consuming, Low throughput
Sensitivity	0.1%	0.002%	0.001%	0.01-0.001%
Reference	Kuroda et al., PLoS ONE. 2012	Kuroda et al., PLoS ONE. 2012	Kuroda et al., Regen Ther. 2015	Tano et al., PLoS ONE. 2014

Assays/Platform	Tumorigenicity Test
Animals	NOG mice
Route	Subcutaneous transplantation
Positive control	iPS cells
Duration	17-30 weeks
Pros	Direct evaluation in micro environment (expected clinical use site)
Cons	High cost, Long time, Especial facility, Low through put, Histopathological evaluation to confirm tumor origin from whether residual undifferentiated iPS cells or transformed cells
Sensitivity	to detect 1000 hiPS cells in 2.5/10 ⁵ hRPE with 50% probability
Reference	Kanemura et al., Sci Rep. 2013; Kawamata et al., J Clin Med. 2015



*: eg. cultured on laminin-521 in Essential 8 medium

Development of Test Methods for Detection of Residual Undifferentiated PSCs






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Example 2

In Vitro Assays

In Vivo Assay

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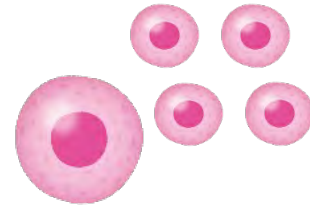
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Highly-Efficient Culture (HEC) Assay

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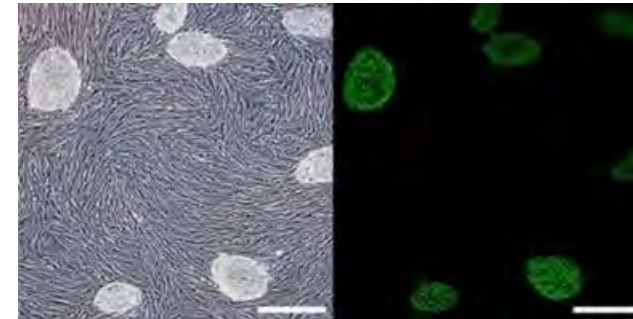
detects **residual undifferentiated pluripotent stem cells (PSCs)** in cell therapy products using highly efficient culture system which favors the growth of PSCs



Assays/ Platform	Highly efficient culture assay
Positive control	iPS cells <i>etc</i>
Duration	about a week
Marker	TRA-1-60 <i>etc</i>
Pros	Direct detection, High sensitivity
Cons	Time-consuming, Low throughput
Sensitivity	1/10,000 - 1/100,000
Reference	Tano et al., PLoS ONE. 2014 Garitaonandia et al., Scientific Reports. 2016

This assay ...

- ✓ is able to directly detect a trace amount of undifferentiated PSCs by measuring the number of colonies originated from a single PSC.



Tano et al., PLoS ONE. 2014

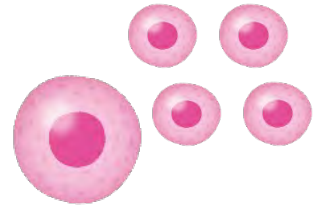
- ✓ is quite sensitive and has a potential to become more sensitive by improving culture system /colony detection method.



Highly-Efficient Culture (HEC) Assay

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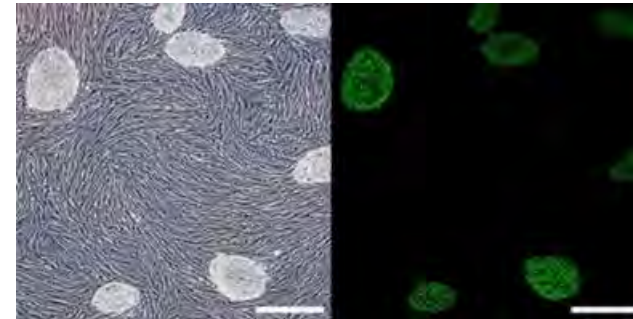
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Improvement of detection method for residual undifferentiated iPS cells (tumorigenic cells) in differentiated cells derived from human iPS cells

Cytotherapy 23 (2021) 176–183

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ISCT
Cell & Gene Therapy®

journal homepage: www.isct-cytotherapy.org

FULL-LENGTH ARTICLE

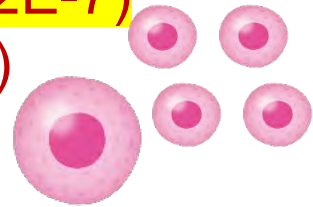
Regulatory Policies

Multisite studies for validation and improvement of a highly efficient culture assay for detection of undifferentiated human pluripotent stem cells intermingled in cell therapy products

Takeshi Watanabe^{1,2,*}, Satoshi Yasuda³, Shinji Kusakawa³, Takuya Kuroda³, Mayumi Futamura^{2,4}, Mitsuhide Ogawa^{2,5}, Hidemi Mochizuki^{2,6}, Eri Kikkawa^{2,7}, Hatsue Furukawa^{2,8}, Masato Nagaoka^{2,9}, Yoji Sato³

¹ Drug Safety Research and Evaluation, Takeda Pharmaceutical Company Limited, Fujisawa, Japan
² The Committee for Non-Clinical Safety Evaluation of Pluripotent Stem Cell-Derived Product, Forum for Innovative Regenerative Medicine, Tokyo, Japan
³ Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, Kawasaki, Japan
⁴ Drug Discovery Support Division, Tsukuba Research Institute, BoZo Research Center Inc, Tsukuba, Japan
⁵ CMIC Bioresearch Center, CMIC Pharma Science Co, Ltd, Hokuto, Japan
⁶ Research Planning Section, Ina Research Inc, Ina-shi, Japan
⁷ Research Division, HEALIOS K.K., Kobe, Japan
⁸ Integrated & Translational Science, Axcelead Drug Discovery Partners, Inc, Fujisawa, Japan
⁹ Life Science Research Laboratory, Tosoh Corporation, Ayase-shi, Japan

Detection of iPS cells in differentiated cells at a ratio of 1 in 5 million (**2E-7**) (WORLD RECORD!!)



ABSTRACT

Background aims: The Multisite Evaluation Study on Analytical Methods for Non-Clinical Safety Assessment of Human-Derived Regenerative Medical Products (MEASURE) is a Japanese experimental public-private partnership initiative, which aims to standardize methodology for tumorigenicity evaluation of human pluripotent stem cell (hPSC)-derived cell therapy products (CTPs). Undifferentiated hPSCs possess tumorigenic potential, and thus residual undifferentiated hPSCs are one of the major hazards for the risk of tumor formation from hPSC-derived CTPs. Among currently available assays, a highly efficient culture (HEC) assay is reported to be one of the most sensitive for the detection of residual undifferentiated hPSCs.

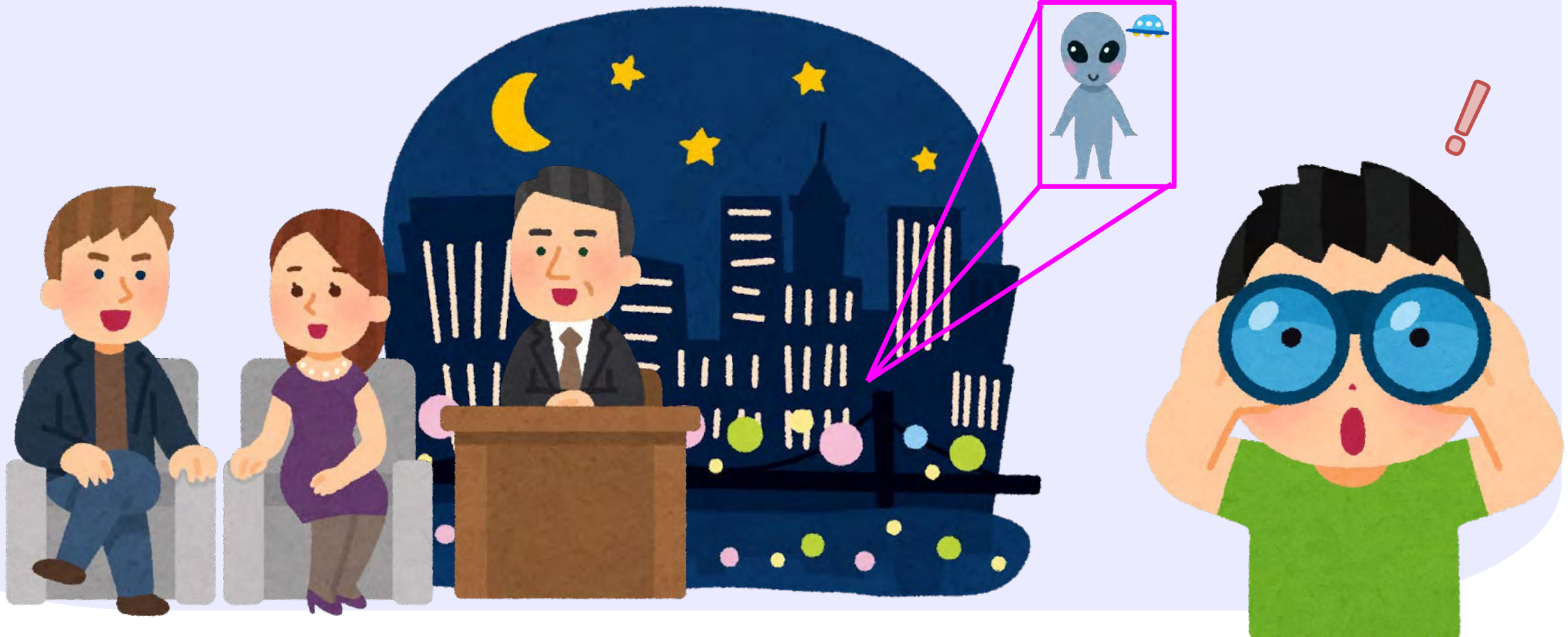
Methods: MEASURE first validated the detection sensitivity of HEC assay and then investigated the feasibility of magnetic-activated cell sorting (MACS) to improve sensitivity.

Results: The multisite experiments confirmed that the lower limit of detection under various conditions to which the human induced pluripotent stem cell lines and culture medium/substrate were subjected was 0.001%. In addition, MACS concentrated cells expressing undifferentiated cell markers and consequently achieved a detection sensitivity of 0.00002%.

Conclusions: These results indicate that HEC assay is highly sensitive and robust and that the application of MACS on this assay is a promising tool for further mitigation of the potential tumorigenicity risk of hPSC-derived CTPs.

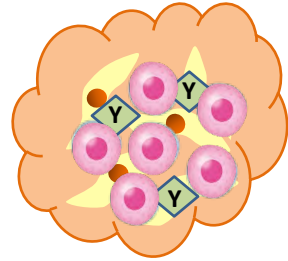
The improved Highly-Efficient Culture (HEC) Assay has achieved the ability to detect residual iPSCs in differentiated cells at a ratio of **1 in 5 million**

Comparable to the ability to find one alien in Los Angeles
(population: about 4 million)

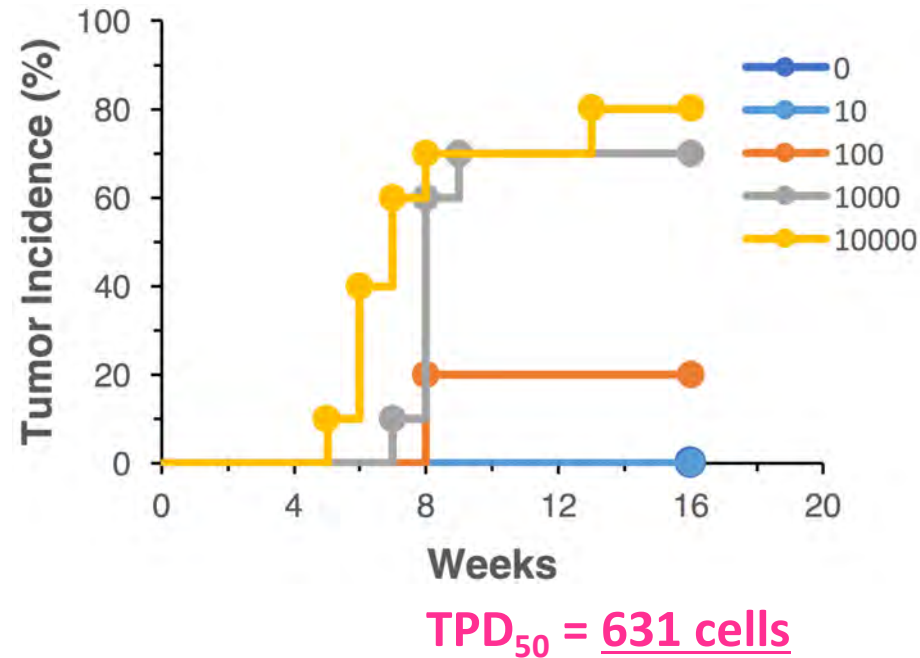


In vivo Tumorigenicity Test using NOG mice subcutaneously transplanted with iPSCs

Yasuda et al.,
PLoS One 2018



iPS cells (single cell-dissociated)
+Matrigel
+feeder cells
+ROCK inhibitor



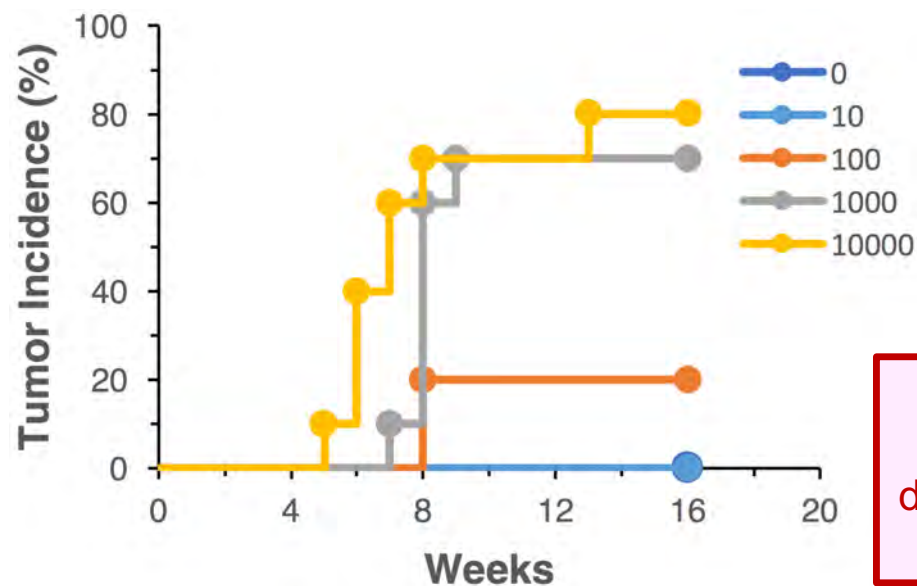
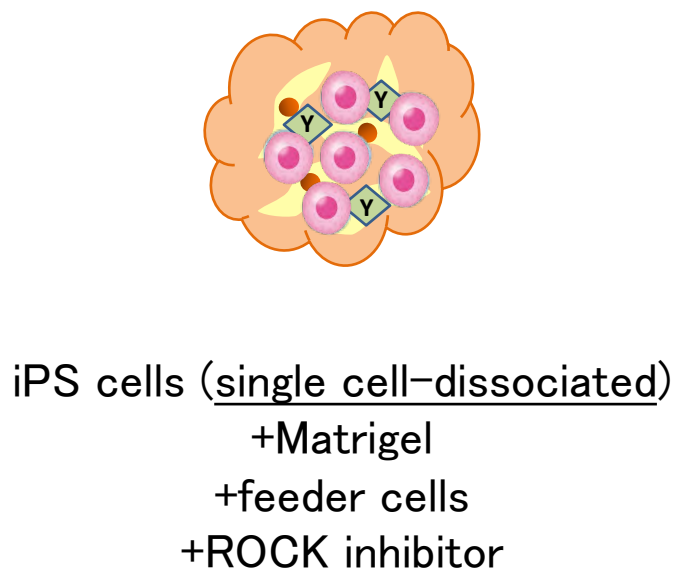
When iPSC cells were most efficiently engrafted in severely immunodeficient mice, TPD₅₀ was 631 cells.

If 10^6 and 10^7 cells are injected, TPD₅₀ = 631 would correspond to:

0.06% ($6E-4$) and 0.006% ($6E-5$), respectively.

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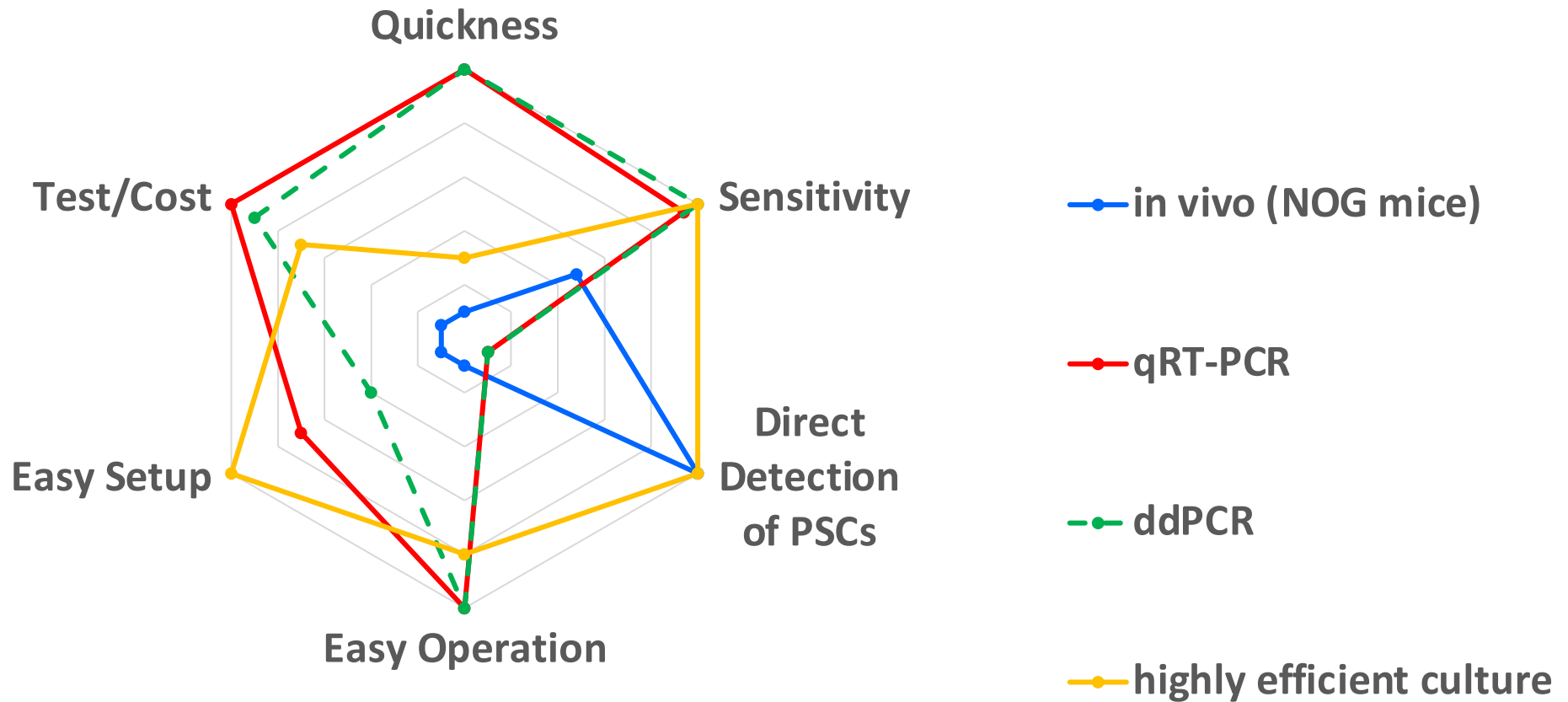
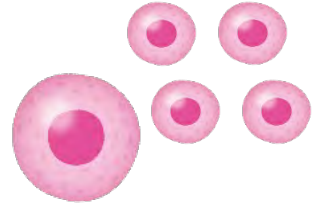
$TPD_{50} = 631 \text{ cells}$

HEC Assay (*in vitro*) detects iPSC cells in differentiated cells at a ratio of 1 in 5 million ($2E-7$)

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Qualitative Comparisons of Test Methods for Detection of Residual PSCs

(based on our validation studies and past literature)



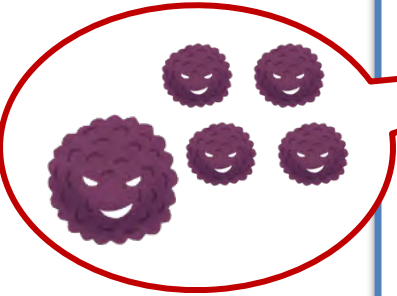
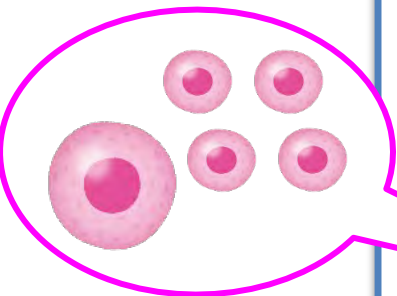
“Points to Consider for Detection of Undifferentiated Pluripotent Stem Cells/Transformed Cells, Tumorigenicity Testing and Genomic Stability Evaluation of Human Cell-Processed Products” *[in Japanese]*

(Annex of Notification No. 0627-1 Issued on June 27, 2019, Pharmaceutical and Food Safety Bureau, MHLW)

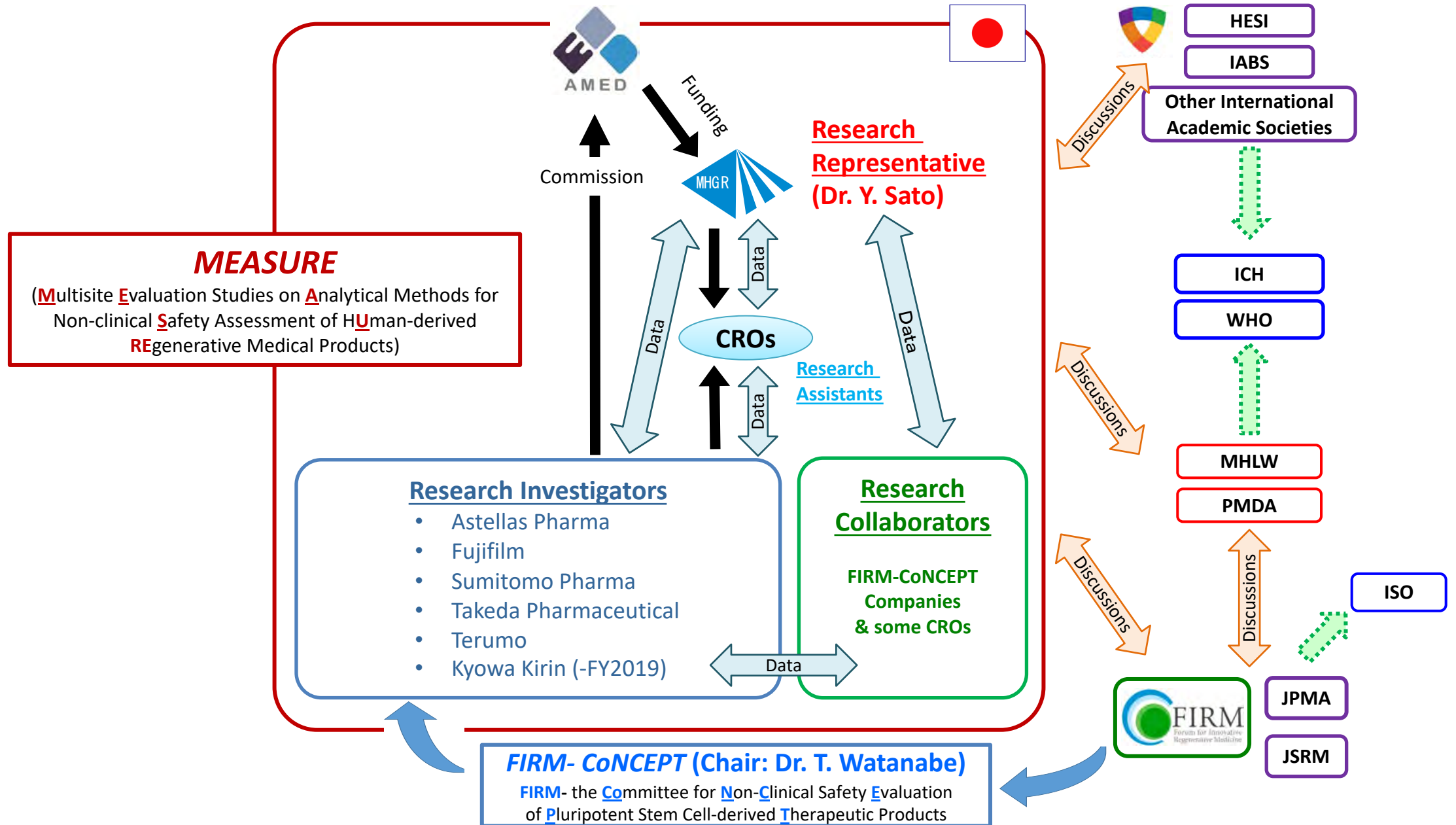


Contents

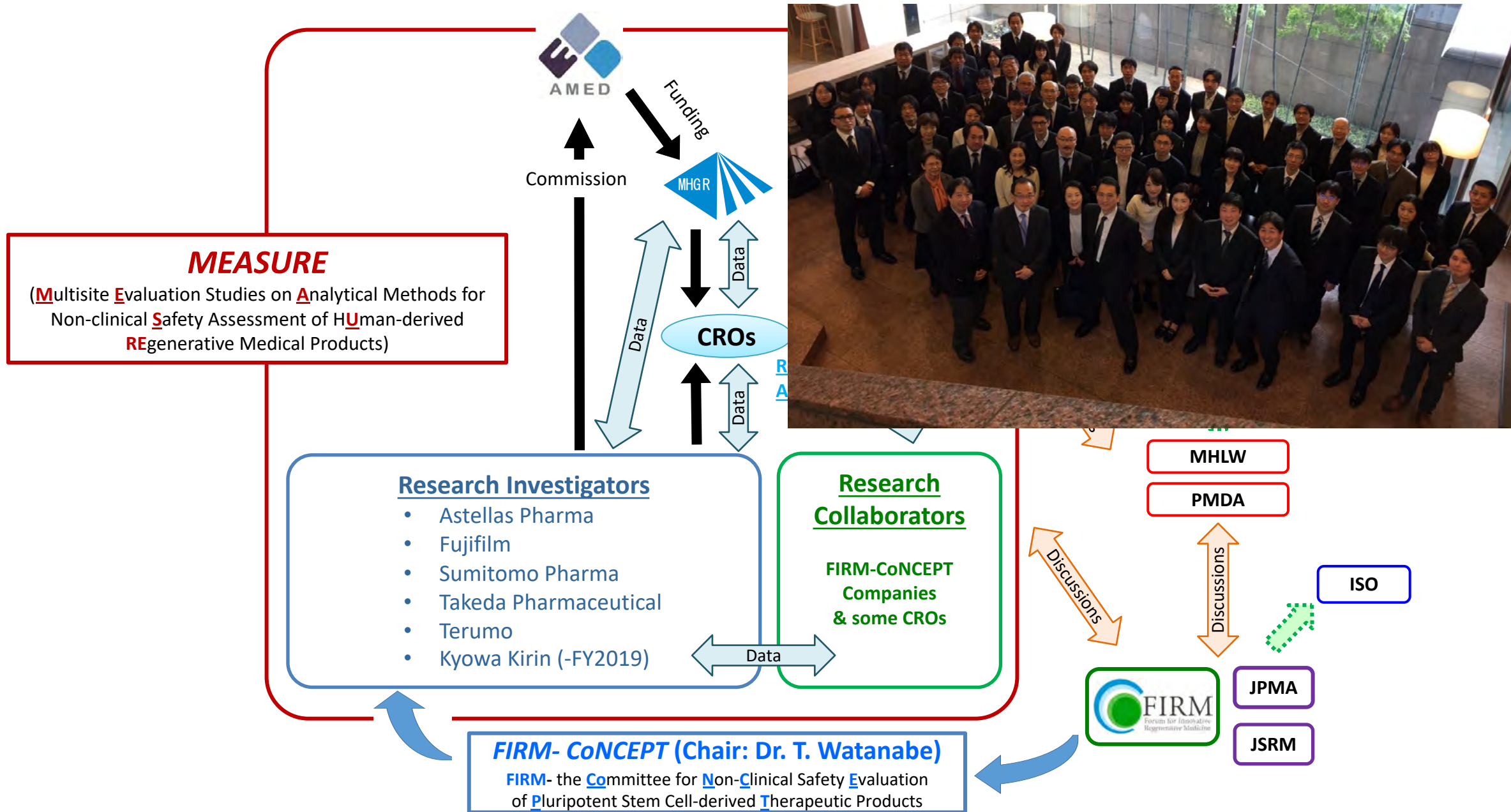
1. Introduction
 2. Position of This Document
 3. Glossaries
 4. General Considerations
 5. Tumorigenicity Tests for Human ES/iPS Cell-Processed Products
 - 5.1 Tumorigenicity Tests for **Quality Characterization of Starting Cell Substrate**
 - 5.2 Tests for **Quantification of Tumorigenic Cells in Intermediate or Final Products**
 - 5.2.1. Tests for **detection of undifferentiated pluripotent stem cells** in intermediate or final products
 - 5.2.1.1. **In vitro studies**
 - 5.2.1.2. **In vivo studies**
 - 5.2.2. Tests for **detection of transformed cells** in intermediate or final products
 - 5.2.2.1. **In vitro studies**
 - 5.2.2.2. **In vivo studies**
 - 5.3 Tests to **Evaluate the Tumorigenic Potential of Cells in the Final Products at the Site of Engraftment in Humans**
 - 5.3.1. Selection of test animals
 - 5.3.2. Selection of control cells
 - 5.3.3. Number of test animals
 - 5.3.4. Site, repeat number and mode of cell administration
 - 5.3.5. Duration of observation
 - 5.3.6. Observation of the site of administration
 - 5.3.7. Pathological evaluation of the site of administration
 - 5.3.8. Interpretation of the results
 6. Tumorigenicity-related Studies for Human Somatic Cell-processed/Somatic Stem Cell-processed Products
 - 6.1. Tumorigenicity Tests for Quality Characterization of Starting Cell Substrate
 - 6.2. Considerations for Tumorigenicity Testing for Final Products
 7. General Considerations for Genomic Stability
- Reference literature
Tables Details of detection methods for residual undifferentiated iPS/ES cells and malignant transformed cells
Reference information (experimental protocols of the test methods)



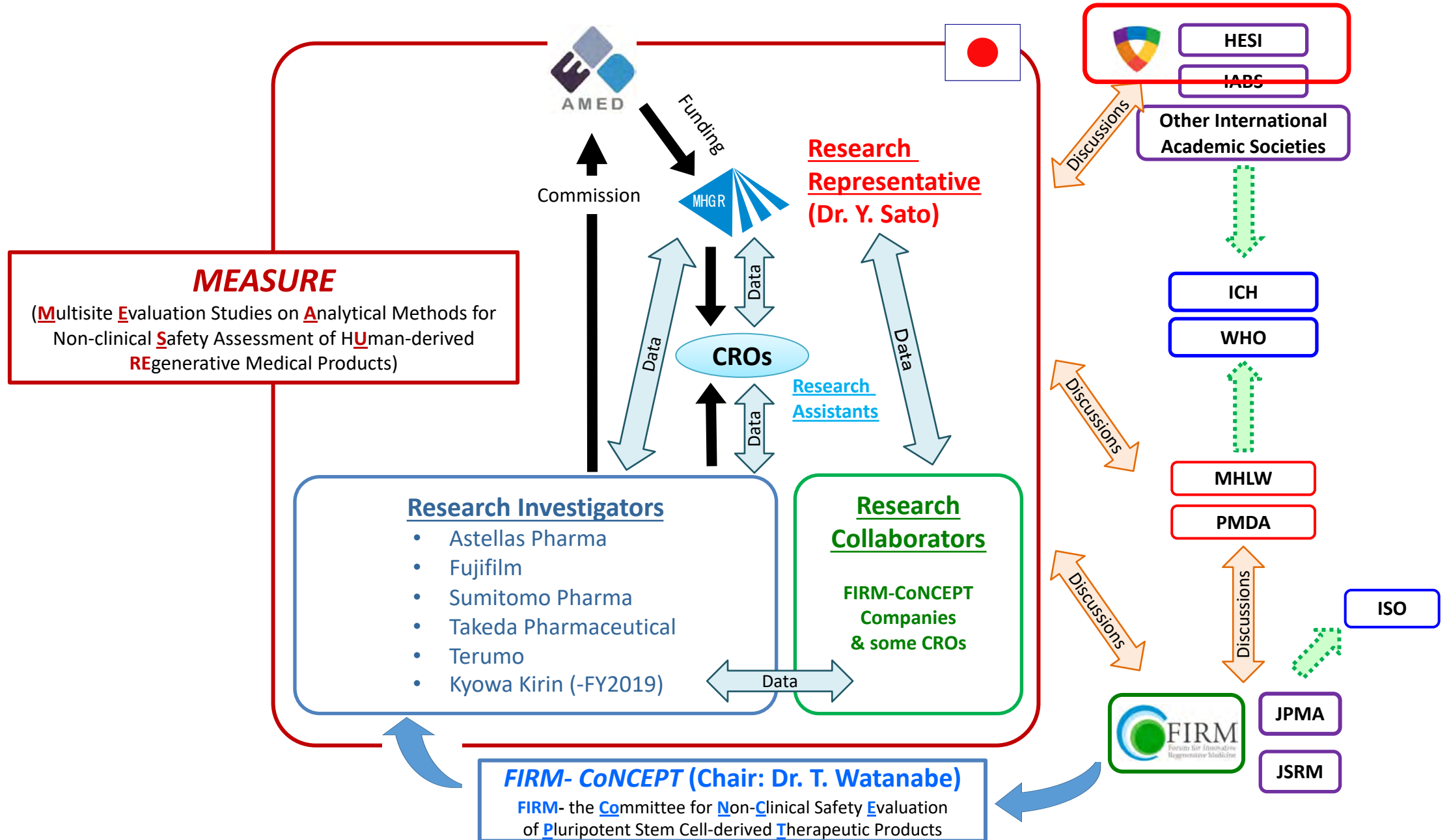
Multisite Validation Studies on the Test Methods for Tumorigenicity Assessment of PSC-derived Products



Multisite Validation Studies on the Test Methods for Tumorigenicity Assessment of PSC-derived Products



Multisite Validation Studies on the Test Methods for Tumorigenicity Assessment of PSC-derived Products



NGOs / Consortia:



European infrastructure for translational medicine



CT-TRACS Members

HESI. (2022 data)

Universities/ Research Centers:



Memorial Sloan Kettering Cancer Center



The University of Sheffield.



Universiteit Leiden



WAGENINGEN UNIVERSITY & RESEARCH



THE UNIVERSITY OF SYDNEY



>100 Participants

>30 Organizations

Government & Regulatory bodies:



National Institutes of Health



National Institute of Standards and Technology



janssen



NOVARTIS



SANOFI

SONOMA BIOTHERAPEUTICS



- **Public-Private Collaborative effort**
- >100 participants
- >35 organizations

CT-TRACS (Cell Therapy: TRacking, Circulation and Safety) Committee

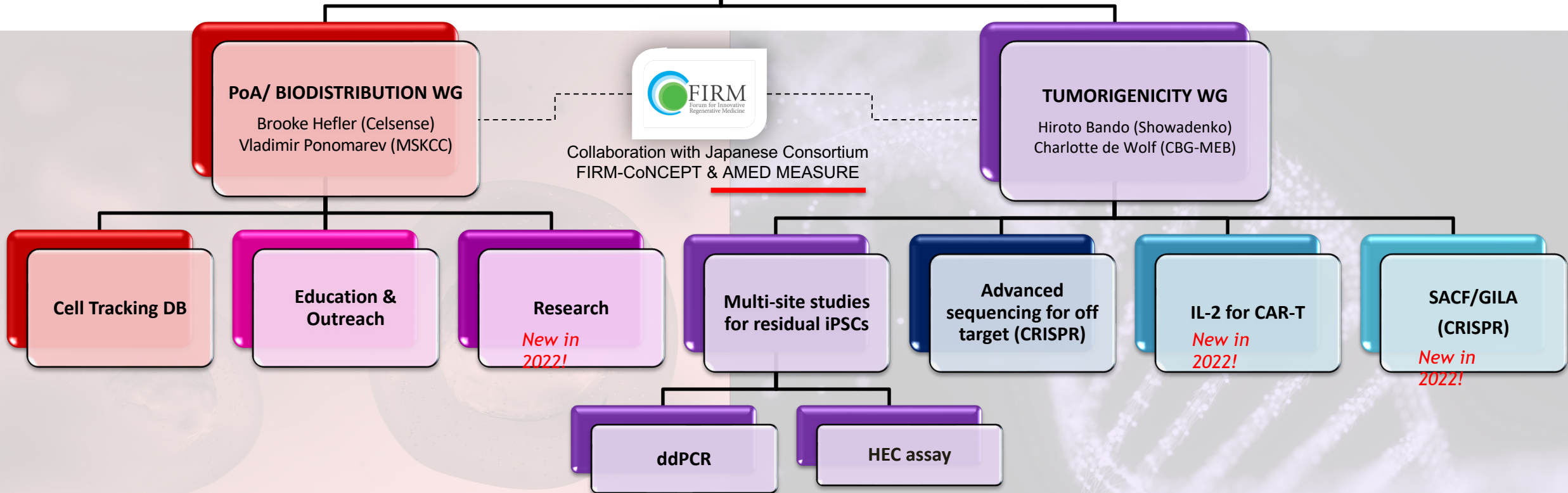
*To facilitate the translation of cell-based therapies to the clinic by driving the development of **tools, methods and knowledge** required to evaluate safety and fate of therapeutic cells.*

Co-Chairs

- Mick Fellows (AstraZeneca)
- Tineke van der Hoorn (CBG-MEB)

HESI Staff

- Lucilia Mouriès
- Connie Chen



- **Public-Private Collaborative effort**
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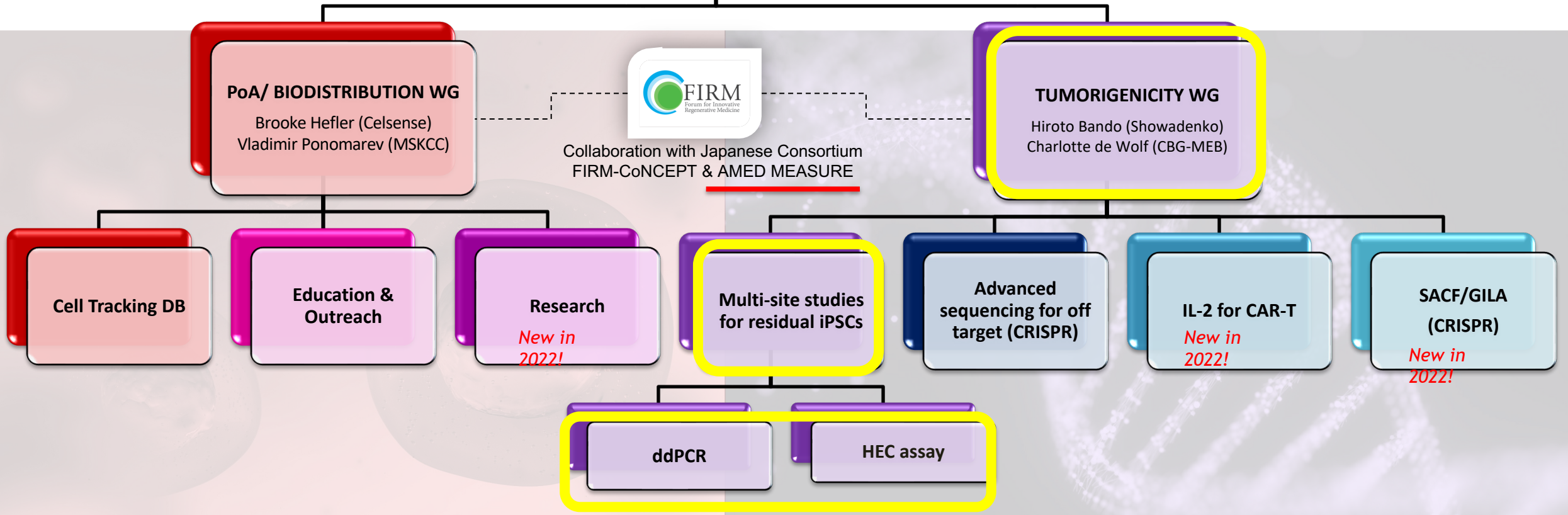
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Position Paper of HESI CT-TRACS Tumorigenicity WG

Addressing Challenges & Needs



Cytotherapy. 2019;21:1095-1111

Cytotherapy, 2019; 21: 1095–1111



REVIEW

International Society
ISCT
Cell & Gene Therapy

Tumorigenicity assessment of cell therapy products: The need for global consensus and points to consider

Y. SATO¹, H. BANDO^{2,*}, M. DI PIAZZA³, G. GOWING⁴, C. HERBERTS^{5,†}, S. JACKMAN⁶,
G. LEONI⁷, S. LIBERTINI⁸, T. MACLACHLAN⁹, J.W. MCBLANE¹⁰,
L. PEREIRA MOURIÈS¹¹, M. SHARPE⁷, W. SHINGLETON^{12,†}, B. SURMACZ-CORDLE⁷,
K. YAMAMOTO¹³ & J.W. VAN DER LAAN^{5,*}

¹Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, Kawasaki, Japan, ²FUJIFILM Corporation, Tokyo, Japan, ³Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut, USA, ⁴FUJIFILM Cellular Dynamics, Inc., Madison, Wisconsin, USA, ⁵Medicines Evaluation Board, Utrecht, The Netherlands, ⁶Charles River Laboratories, Horsham, Pennsylvania, USA, ⁷Cell and Gene Therapy Catapult, London, UK, ⁸Novartis Institutes for BioMedical Research, Basel, Switzerland, ⁹Novartis Institutes for BioMedical Research, Cambridge, Massachusetts, USA, ¹⁰Medicines & Healthcare Products Regulatory Agency, London, UK, ¹¹Health and Environmental Sciences Institute (HESI), Washington, DC, USA, ¹²GE Healthcare, Cambridge, UK, and ¹³Takeda Pharmaceutical Company Limited, Tokyo, Japan

Chair of the EMA/CHMP Safety Working Party
(at the time of publication)

Abstract

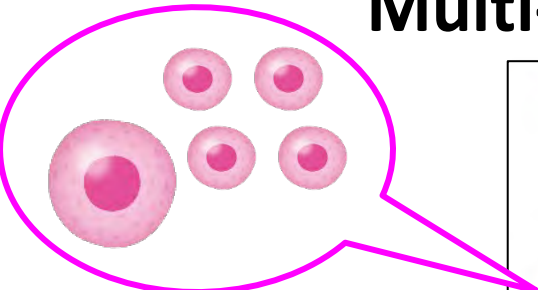
“[...] Here, we critically review currently available *in vivo* and *in vitro* testing methods for tumorigenicity evaluation against expectations in international regulatory guidelines. We discuss the value of those approaches, in particular the limitations of *in vivo* methods, and comment on challenges and future directions. In addition, we note the need for an internationally harmonized procedure for tumorigenicity assessment of cell therapy products from both regulatory and technological perspectives”.

[https://www.isct-cytotherapy.org/article/S1465-3249\(19\)30861-8/fulltext](https://www.isct-cytotherapy.org/article/S1465-3249(19)30861-8/fulltext)



HESI CT-TRACS Tumorigenicity WG

International Experimental Consortium for Multi-site Validation Studies on the *In Vitro* Test Methods



Short Communication

For reprint orders, please contact reprints@futuremedicine.com



International evaluation study of a highly efficient culture assay for detection of residual human pluripotent stem cells in cell therapies

Takeshi Watanabe^{*1}, Satoshi Yasuda², Connie L Chen³, Louise Delsing⁴, Mick D Fellows⁵, Gabor Foldes^{6,7}, Shinji Kusakawa², Lucilia Pereira Mouries³ & Yoji Sato²

¹Drug Safety Research & Evaluation, Takeda Pharmaceutical Company Limited, 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa, 251-8555, Japan

²Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa, 210-9501, Japan

³Health & Environmental Sciences Institute, 740 Fifteenth Street NW, Suite 600, Washington, DC 20005, USA

⁴CVRM Safety, Clinical Pharmacology & Safety Science, R&D, AstraZeneca, Pepparedsleden 1, Mölndal, 43150, Sweden

⁵CVRM Safety, Clinical Pharmacology & Safety Science, R&D, AstraZeneca, Darwin Building 310, Milton Science Park, Cambridge, CB4 0WG, UK

⁶National Heart & Lung Institute, Imperial College London, London, W120NN, UK

⁷Current address, BioPharmaceuticals R&D, AstraZeneca, Milstein Building, Granta Park, Cambridge, CB21 6GH, UK

* Author for correspondence: takeshi.watanabe@takeda.com

Aim & methods: The Health and Environmental Sciences Institute Cell Therapy-TRACKing, Circulation & Safety Technical Committee launched an international, multisite study to evaluate the sensitivity and reproducibility of the highly efficient culture (HEC) assay, an *in vitro* assay to detect residual undifferentiated human pluripotent stem cells (hPSCs) in cell therapy products. **Results:** All facilities detected colonies of human induced pluripotent stem cells (hiPSCs) when five hiPSCs were spiked into 1 million hiPSC-derived cardiomyocytes. Spiking with a trace amount of hiPSCs revealed that repeatability accounts for the majority of reproducibility while the true positive rate was high. **Conclusion:** The results indicate that the HEC assay is highly sensitive and robust and can be generally applicable for tumorigenicity evaluation of hPSC-derived cell therapy products.

First draft submitted: 7 December 2022; Accepted for publication: 23 January 2023; Published online: 28 February 2023



HESI



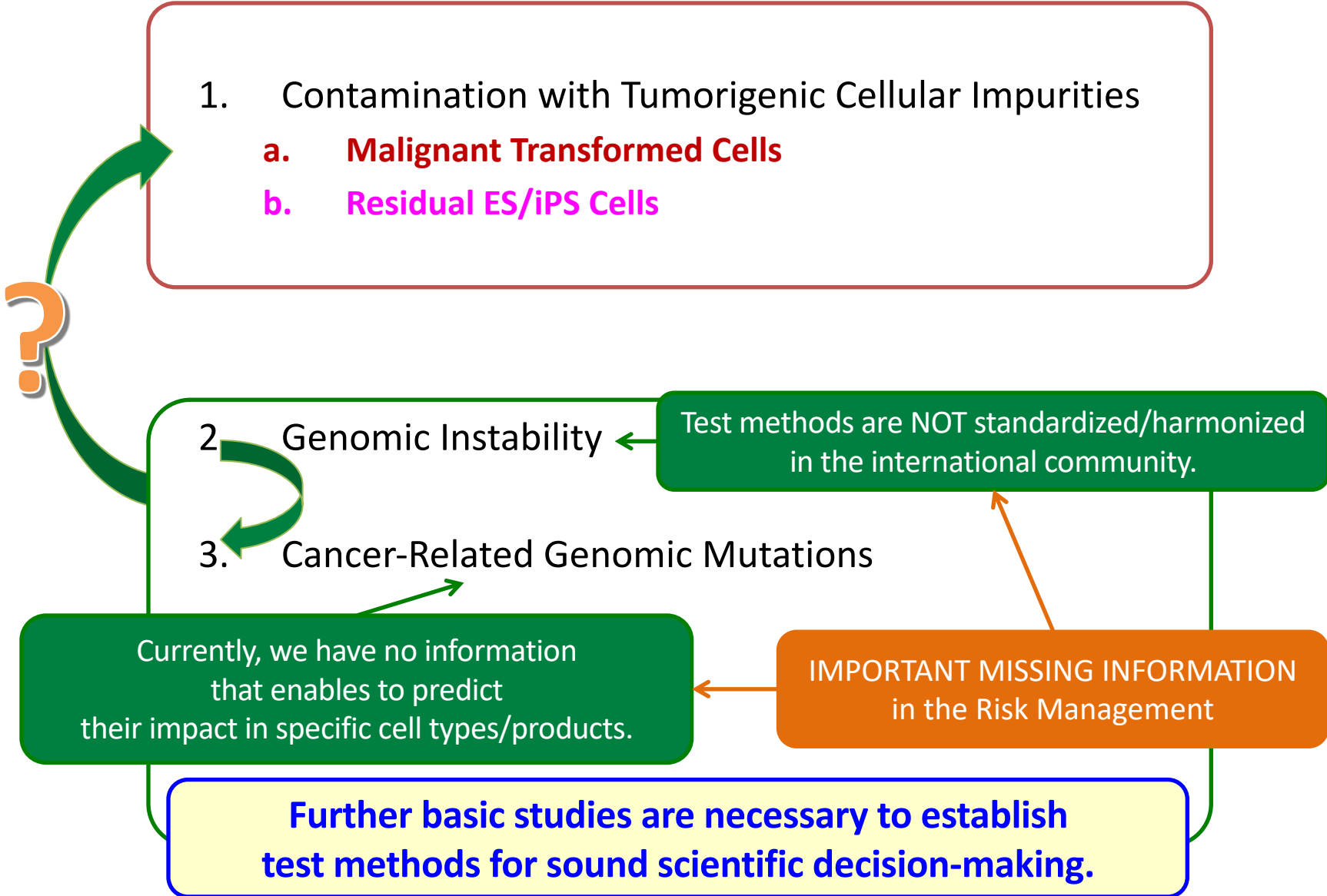
Imperial College London

... More papers on the *in vitro* test methods to be published by the HESI CT-TRACS Experimental Consortium

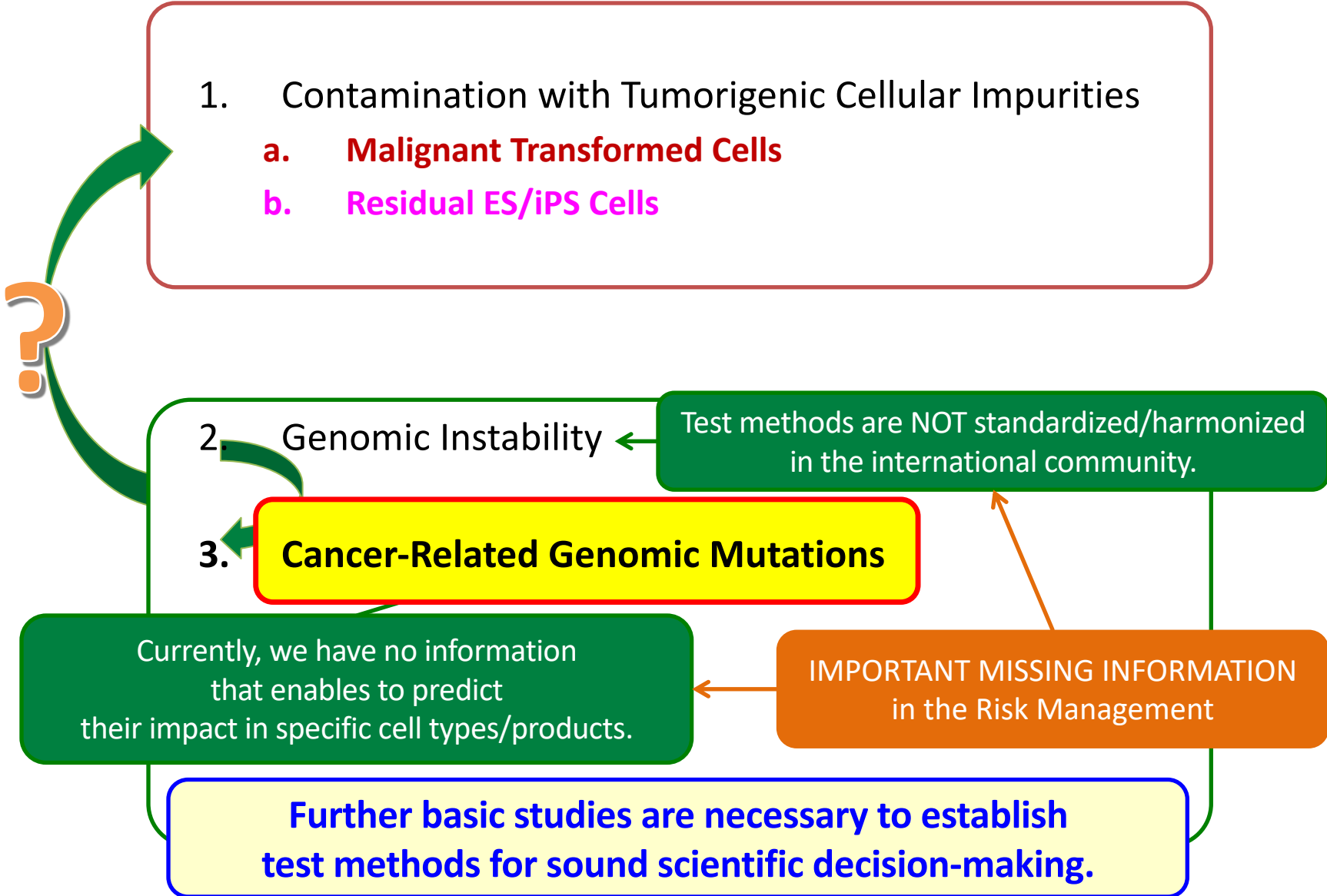
AGENDA

- 1. What is tumorigenicity? –The risk of tumorigenesis and its hazards–**
- 2. Development of highly sensitive test methods for the detection of transformed cells in human cell therapy products**
- 3. Development of highly sensitive test methods for the detection of residual pluripotent stem cells in human ES/iPS cell-derived products**
- 4. How much are genomic mutations predictive of abnormal tissue formation from human iPSC-derived products after engraftment?**

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



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



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 Yizhak¹, François Aguet¹, Jaegil Kim¹, Julian M. Hess¹, Kirsten Kübler^{1,2,3}, Jonna Grimsby¹, Ruslana Frazer¹, Hailei Zhang¹, Nicholas J. Haradhvala^{1,2}, Daniel Rosebrock¹, Dimitri Livitz¹, Xiao Li¹, Eila Arich-Landkof^{1,2}, Noam Shores¹, Chip Stewart¹, Ayellet V. Segrè^{1,3,4}, Philip A. Branton⁵, Paz Polak⁶, Kristin G. Ardlie¹, Gad Getz^{1,2,3,7,*}

¹Broad Institute of MIT and Harvard, Cambridge, MA, USA.

²Center for Cancer Research, Massachusetts General Hospital, Boston, MA, USA.

³Harvard Medical School, Boston, MA, USA.

⁴Ocular Genomics Institute, Department of Ophthalmology, Massachusetts Eye and Ear, Boston, MA, USA.

⁵Biorepositories and Biospecimen Research Branch, Cancer Diagnosis Program, National Cancer Institute, Bethesda, MD, USA.

⁶Oncological Sciences, Icahn School of Medicine at Mount Sinai Hospital, New York, NY, USA.

⁷Department of Pathology, Massachusetts General Hospital, Boston, MA, USA.

*Corresponding author. Email: gadgetz@broadinstitute.org

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Science 07 Jun 2019;
Vol. 364, Issue 6444, eaaw0726
DOI: 10.1126/science.aaw0726

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.

“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



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...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



“Points to Consider for Detection of Undifferentiated Pluripotent Stem Cells/Transformed Cells, Tumorigenicity Testing and Genomic Stability Evaluation of Human Cell-Processed Products” *[in Japanese]*

(Annex of Notification No. 0627-1 Issued on June 27, 2019, Pharmaceutical and Food Safety Bureau, MHLW)

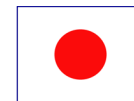
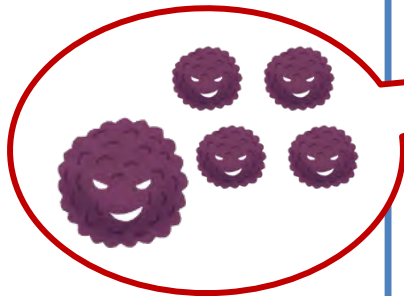
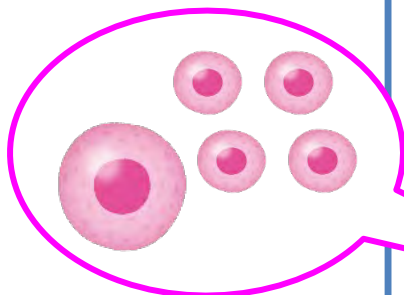
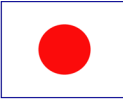


Table of Contents

1. Introduction
2. Position of This Document
3. Glossaries
4. General Considerations
5. **Tumorigenicity Tests for Human ES/iPS Cell-Processed Products**
 - 5.1 Tumorigenicity Tests for Quality Characterization of Starting Cell Substrate
 - 5.2 Tests for Quantification of Tumorigenic Cells in Intermediate or Final Products
 - 5.2.1. Tests for detection of undifferentiated pluripotent stem cells in intermediate or final products
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 - 5.2.1.2. In vivo studies
 - 5.2.2. Tests for detection of transformed cells in intermediate or final products
 - 5.2.2.1. In vitro studies
 - 5.2.2.2. In vivo studies
 - 5.3 Tests to Evaluate the Tumorigenic Potential of End-product Cells at the Site of Engraftment in Human
 - 5.3.1. Selection of test animals
 - 5.3.2. Selection of control cells
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 - 5.3.4. Site, repeat number and mode of cell administration
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 - 5.3.6. Observation of the site of administration
 - 5.3.7. Pathological evaluation of the site of administration
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6. **Tumorigenicity-related Studies for Human Somatic Cell-processed/Somatic Stem Cell-processed Products**
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 - 6.2. Considerations for Tumorigenicity Testing for Final Products
7. **General Considerations for Genomic Stability**
 - Reference literature
 - Tables Details of detection methods for residual undifferentiated iPS/ES cells and malignant transformed cells
 - Reference information (experimental protocols of the test methods)





7. General Considerations for Genomic Stability

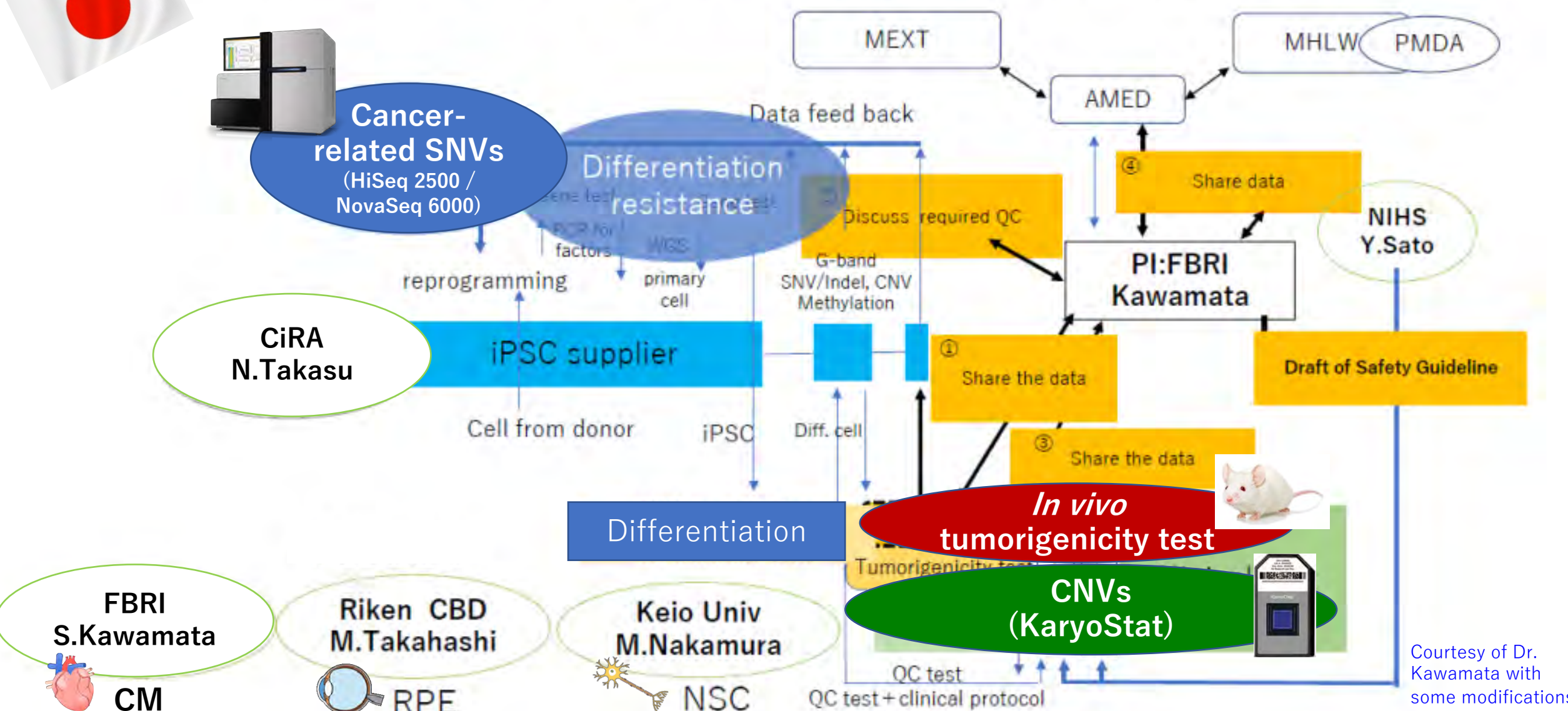
“Reduced genetic stability is a potential hazard with respect to tumorigenic risk because it is presumed to increase the probability of transformed cells through the increased probability of karyotypic abnormalities and genetic mutations.

....

Information from FISH and next-generation sequencing should be scientifically validated for relevance to tumorigenicity and evaluated for appropriateness for use as a test method, while the sensitivity of detection to genetic changes (type of mutation and its allele frequency) and the availability of appropriate controls should be considered as issues.”

Study on the correlation between genomic variation in human iPS cell-derived products and abnormal tissue formation after implantation into immunodeficient animals

AMED RS Research Grants (2017-2022) PI: Shin Kawamata FBRI



FBRI S.Kawamata
CM

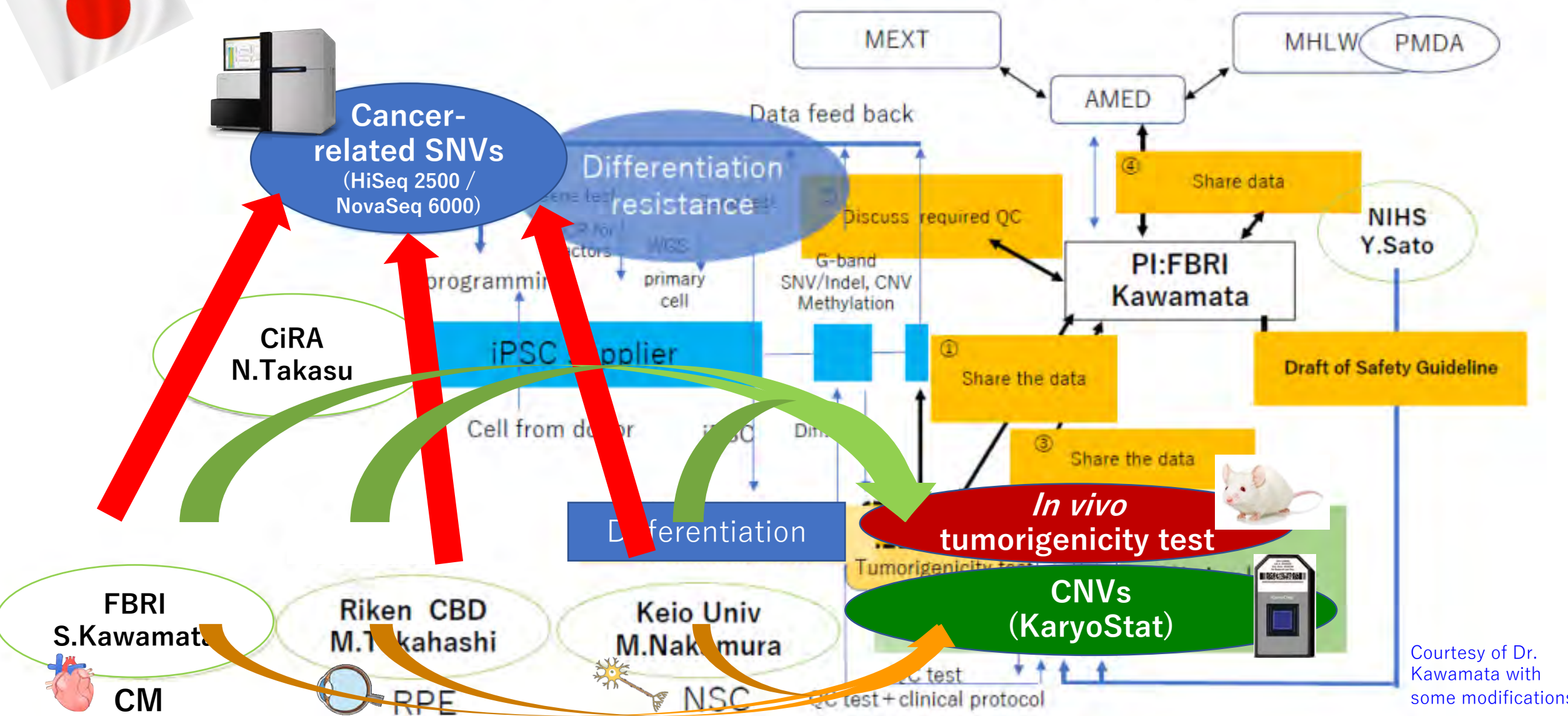
Riken CBD M.Takahashi
RPE

Keio Univ M.Nakamura
NSC

Courtesy of Dr. Kawamata with some modifications

Study on the correlation between genomic variation in human iPS cell-derived products and abnormal tissue formation after implantation into immunodeficient animals

AMED RS Research Grants (2017-2022) PI: Shin Kawamata FBRI



Courtesy of Dr. Kawamata with some modifications

Study on the correlation between genomic variation in human iPS cell-derived products and abnormal tissue formation after implantation into immunodeficient animals

A.

Explanatory variables in PSC-derivatives				Outcome variable
Cell line	Cell typing	SNV	CNV	Histological finding
16E84	RPEs	SNV(-)	CNV(+)	Abnormal
16E84	CMs	SNV(+)	CNV(+)	Normal
16E85	RPEs	SNV(-)	CNV(+)	Normal
16E85	CMs	SNV(+)	CNV(-)	Normal
16H12	RPEs	SNV(+)	CNV(-)	Normal
16H12	non-CMs	SNV(+)	CNV(-)	Normal
15M38	RPEs	SNV(-)	CNV(+)	Abnormal
15M38	non-CMs	SNV(-)	CNV(+)	Abnormal
1210B2	NSCs	SNV(+)	CNV(-)	Normal
Ff-WJ	NSCs	SNV(-)	CNV(-)	Normal
Ff-I01	RPEs	SNV(-)	CNV(+)	Abnormal
Ff-I01	NSCs	SNV(-)	CNV(+)	Abnormal
H9	RPEs	SNV(-)	CNV(-)	Normal
H9	CMs	SNV(-)	CNV(-)	Normal

B. Explanatory variable: SNV (in COSMIC Cancer Gene Census or Shibata's List)

Explanatory variable		SNV(-)	SNV(+)	Discriminative ratio	Overall predictability
Expectancy		Normal	Abnormal		
Outcome variable	Normal	4	5	44% (Specificity)	29%
	Abnormal	5	0	0% (Sensitivity)	
Predictivity		44%	0%	Correlation ratio η : 0.56	
Overall Predictivity		29%			
Likelihood ratio for abnormal outcome		2.3	0.0		

C. Explanatory variable: CNV (- : CNV \leq 3; + : CNV >4)

Explanatory variable		CNV(-)	CNV(+)	Discriminative ratio	Overall predictability
Expectancy		Normal	Abnormal		
Outcome variable	Normal	7	2	78% (Specificity)	86%
	Abnormal	0	5	100% (Sensitivity)	
Predictivity		100%	71%	Correlation ratio η : 0.75	
Overall predictivity		86%			
Likelihood ratio for abnormal outcome		0.0	4.5		

Yamamoto T, et al.,
Stem Cells Transl Med. 2022;11:527-538.

Study on the correlation between genomic variation in human iPS cell-derived products and abnormal tissue formation after implantation into immunodeficient animals

A.

Explanatory variables in PSC-derivatives				Outcome variable
Cell line	Cell typing	SNV	CNV	Histological finding
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16H12	RPEs	SNV(+)	CNV(-)	Normal
16H12	non-CMs	SNV(+)	CNV(-)	Normal
15M38	RPEs	SNV(-)	CNV(+)	Abnormal
15M38	non-CMs	SNV(-)	CNV(+)	Abnormal
1210B2	NSCs	SNV(+)	CNV(-)	Normal
Ff-WJ	NSCs	SNV(-)	CNV(-)	Normal
Ff-I01	RPEs	SNV(-)	CNV(+)	Abnormal
Ff-I01	NSCs	SNV(-)	CNV(+)	Abnormal
H9	RPEs	SNV(-)	CNV(-)	Normal
H9	CMs	SNV(-)	CNV(-)	Normal

B. Explanatory variable: SNV (in COSMIC Cancer Gene Census or Shibata's List)

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Predictivity		44%	0%	Correlation ratio η : 0.56	
Overall Predictivity		29%			
Likelihood ratio for abnormal outcome		2.3	0.0		

CNVs may help predict abnormal tissue formation, including tumorigenesis, after product implantation.

C. Explanatory variable: CNV (- : CNV \leq 3; + : CNV >4)

Explanatory variable		CNV(-)	CNV(+)	Discriminative ratio	Overall predictability
Expectancy		Normal	Abnormal		
Outcome variable	Normal	7	2	78% (Specificity)	86%
	Abnormal	0	5	100% (Sensitivity)	
Predictivity		100%	71%	Correlation ratio η : 0.75	
Overall predictivity		86%			
Likelihood ratio for abnormal outcome		0.0	4.5		

Yamamoto T, et al.,
Stem Cells Transl Med. 2022;11:527-538.

Clinical Applications of iPSC/ESC-Derived Products in Japan

in Non-Commercial Clinical Researches under **the RM Safety Act** and Commercial Clinical Trials under **the PMD ACT**



As of October 21, 2023; ** According to a newspaper report

Final Product	Starting Cells	Target Disease	Institution(s)	Type of Clinical Trial	IMP Approval	FIH Trial
Retinal pigment epithelial cells	<i>Autologous iPSCs</i>	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	<i>Autologous iPSCs</i>	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	<i>ESCs (Allogeneic)</i>	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
Neural progenitor cells	Allogeneic iPSCs	Subacute spinal cord injury	Keio Univ. etc.	Non-commercial clinical research under the RM Safety Act	2019	2021
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
Cartilage	Allogeneic iPSCs	Knee articular cartilage injury	Kyoto Univ.	Non-commercial clinical research under the RM Safety Act	2020	(2021)**
Retinal pigment epithelial cells	Allogeneic iPSCs	Retinal pigment epithelial insufficiency	Kobe City Eye Hospital	Non-commercial clinical research under the RM Safety Act	2021	2021
Innate lymphoid Cells/NK cells Expressing GPC3-CAR	Allogeneic iPSCs	Ovarian cancer	Kyoto Univ., NCRI	Clinical trial under the PMD Act	2021	2021
Platelets	Allogeneic iPSCs	Thrombocytopenia	Megakaryon, Kyoto Univ., CiRA-F	Clinical trial under the PMD Act	2021	2022
Corneal endothelial cells	Allogeneic iPSCs	Bullous keratopathy	Keio Univ.	Non-commercial clinical research under the RM Safety Act	2021	2023
Cardiomyocytes	Allogeneic iPSCs	Ischemic Cardiomyopathy	Heartseed, Novo Nordisk	Clinical trial under the PMD Act	2021	2023

Clinical Applications of iPSC/ESC-Derived Products in Japan

in Non-Commercial Clinical Researches under **the RM Safety Act** and Commercial Clinical Trials under **the PMD Act**



As of October 21, 2023; ** According to a newspaper report

Final Product	Starting Cells	Target Disease	Institution(s)	Type of Clinical Trial	IMP Approval	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	
Dopaminergic neural progenitor cells	Allogeneic iPSCs	Parkinson's disease	Kyoto Univ.	Clinical trial under the PMD Act	2018	
Platelets	Autologous iPSCs	Aplastic anemia	Kyoto Univ.	Non-commercial clinical research under the RM Safety Act	2018	
Corneal epithelial cells	Allogeneic iPSCs	Corneal epithelial stem cell exhaustion	Osaka Univ.	Non-commercial clinical research under the RM Safety Act	2019	
Hepatocytes	ESCs (Allogeneic)	Congenital urea cycle disorder	NCCHD	Clinical trial under the PMD Act	2019	
Cardiomyocytes	Allogeneic iPSCs	Ischemic cardiomyopathy	Osaka Univ.	Clinical trial under the PMD Act	2019	
Neural progenitor cells	Allogeneic iPSCs	Subacute spinal cord injury	Keio Univ. etc.	Non-commercial clinical research under the RM Safety Act	2019	2021
Retinal photoreceptor cells	Allogeneic iPSCs	Retinitis pigmentosa	Kobe City Eye Hospital	Non-commercial clinical research under the RM Safety Act	2020	
NKT cells	Allogeneic iPSCs	Recurrent or advanced head and neck cancer	Chiba Univ., RIKEN	Clinical trial under the PMD Act	2020	
Cartilage	Allogeneic iPSCs	Knee articular cartilage injury	Kyoto Univ.	Non-commercial clinical research under the RM Safety Act	2020	
Retinal pigment epithelial cells	Allogeneic iPSCs	Retinal pigment epithelial insufficiency	Kobe City Eye Hospital	Non-commercial clinical research under the RM Safety Act	2021	
Lymphoid Cells/NK cells expressing GPC3-CAR	Allogeneic iPSCs	Ovarian cancer	Kyoto Univ., NCRI	Clinical trial under the PMD Act	2021	
Platelets	Allogeneic iPSCs	Thrombocytopenia	Megakaryon, Kyoto Univ., CiRA-F	Clinical trial under the PMD Act	2021	2022
Corneal endothelial cells	Allogeneic iPSCs	Bullous keratopathy	Keio Univ.	Non-commercial clinical research under the RM Safety Act	2021	2023
Cardiomyocytes	Allogeneic iPSCs	Ischemic Cardiomyopathy	Heartseed, Novo Nordisk	Clinical trial under the PMD Act	2021	2023



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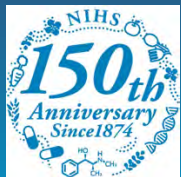


Our research has contributed to clinical applications of PSC-derived products through the development of test methods for the assessment of their quality and safety.

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- The Secretariat of the Forum for Innovation in Regenerative Medicine (**FIRM**)
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- **PMDA** Regenerative Medicine Products Review Division
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Thank you for your attention!

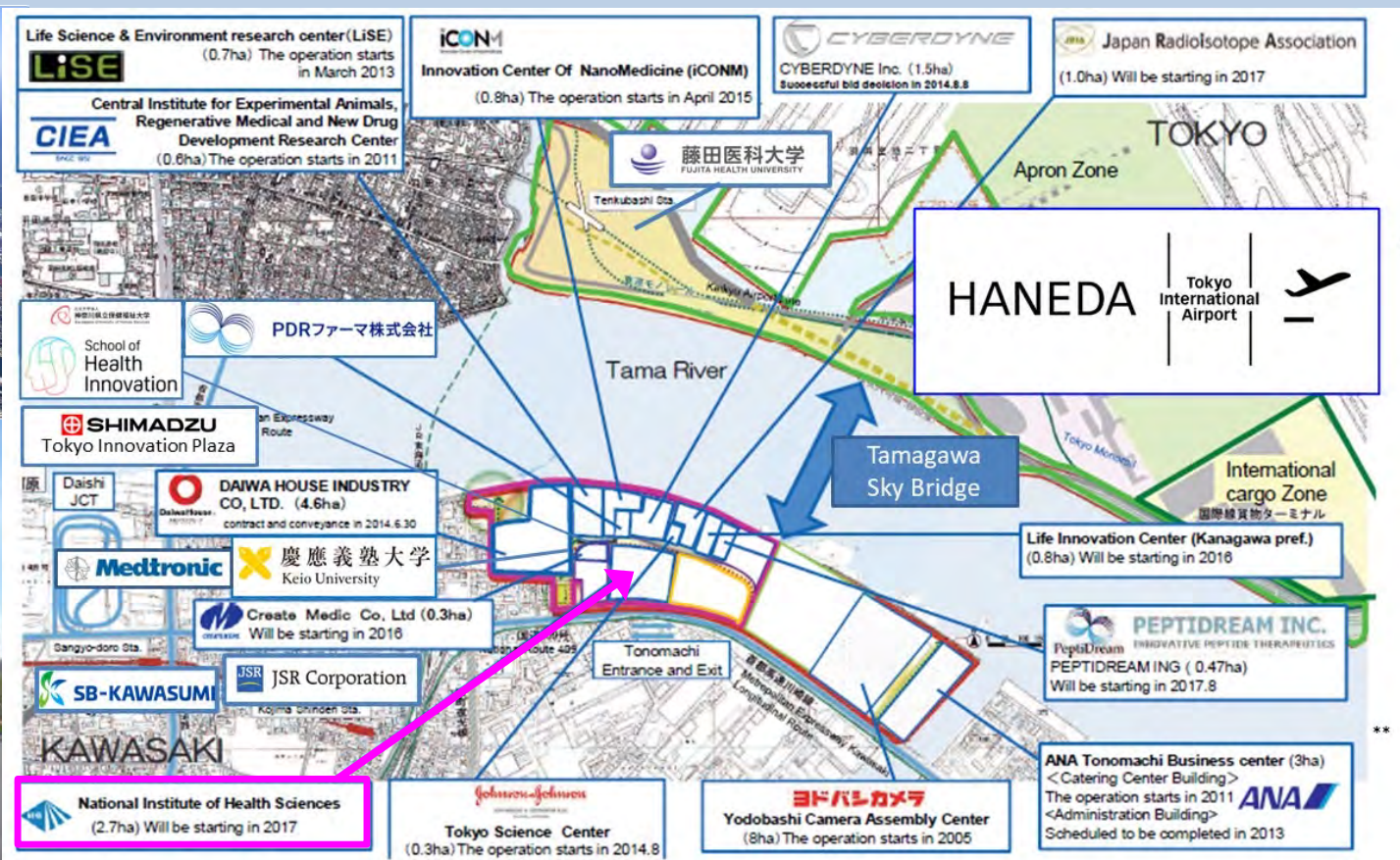
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* <https://www.oag.com/hubs/air-canada-787.jpg>

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