PLURIPOTENT STEM CELL CONFERENCE 2023

Stem Cell Manufacturing: Current experiences with stem cell-derived products and organoids

Session 2: Genetic stability and tumorigenicity assays

In Vitro Assays of Product Tumorigenicity

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DISCLAIMER

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

AGENDA

1. Regulatory science on emerging S&Q issues for PSC-derived products

2. Development and validation of test methods for tumorigenicity assessment of PSC-derived products

3. Study on the correlation between genomic variations in PSC-derived products and abnormal tissue formation

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"Regulatory Science"

...is the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of all FDA-regulated products.
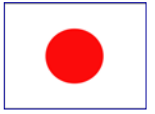
The Act to Promote Healthcare and Medical Strategy

(promulgated in Japan on May 30, 2014)

Regulatory Science!

"The national government **shall take necessary measures for the promotion of science** related to **the prompt and sound scientific prediction, evaluation and decision-making of the quality, efficacy and safety of the deliverables of medical research and development**, which include the development of systems, securing, training and improving the quality of human resources."

The promotion of regulatory science is a government obligation in Japan.

Why is regulatory science necessary for the development of advanced therapeutic products?

- It is because **the development of evaluation methods often do not catch up with the rapid development of new types of products** (e.g., cell therapy products), which emerge as a result of technological advances.

- It is also because even when **new types of analytical tools** (e.g., next-generation sequencers) are developed as a result of technological advances, **their capabilities and limitations** when used to evaluate the quality and safety of therapeutic products **are unknown**.

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

Safety & eligibility of raw materials

Ensuring the quality of the final product

Prediction of safety & efficacy in the non-clinical phase

Clinical Evaluation

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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.
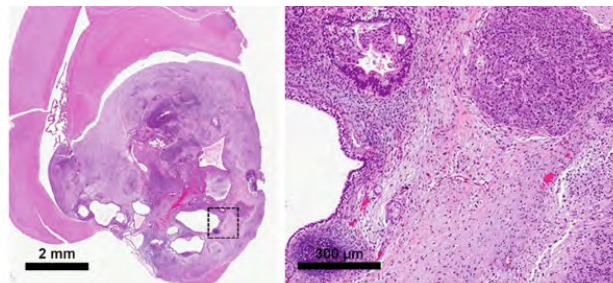
Ibon Garitaonandi et al. Scientific Reports | 6:34478
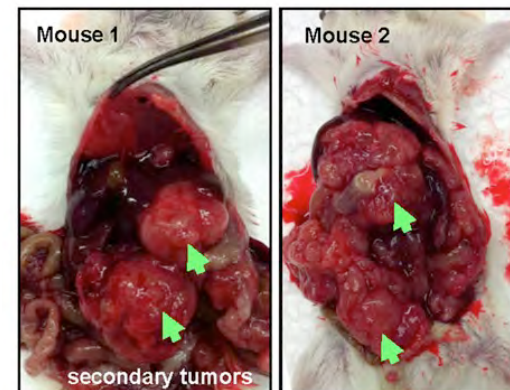
MOUSTAFA M et al. STEM CELLS TRANSLATIONALMEDICINE 2016;5:694–702

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

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

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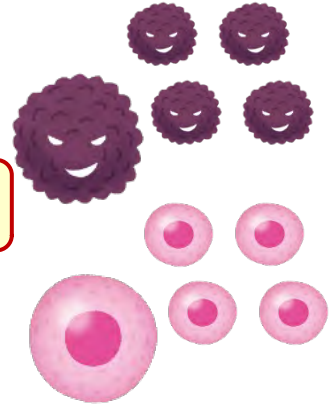   a.   **Malignant Transformed Cells**

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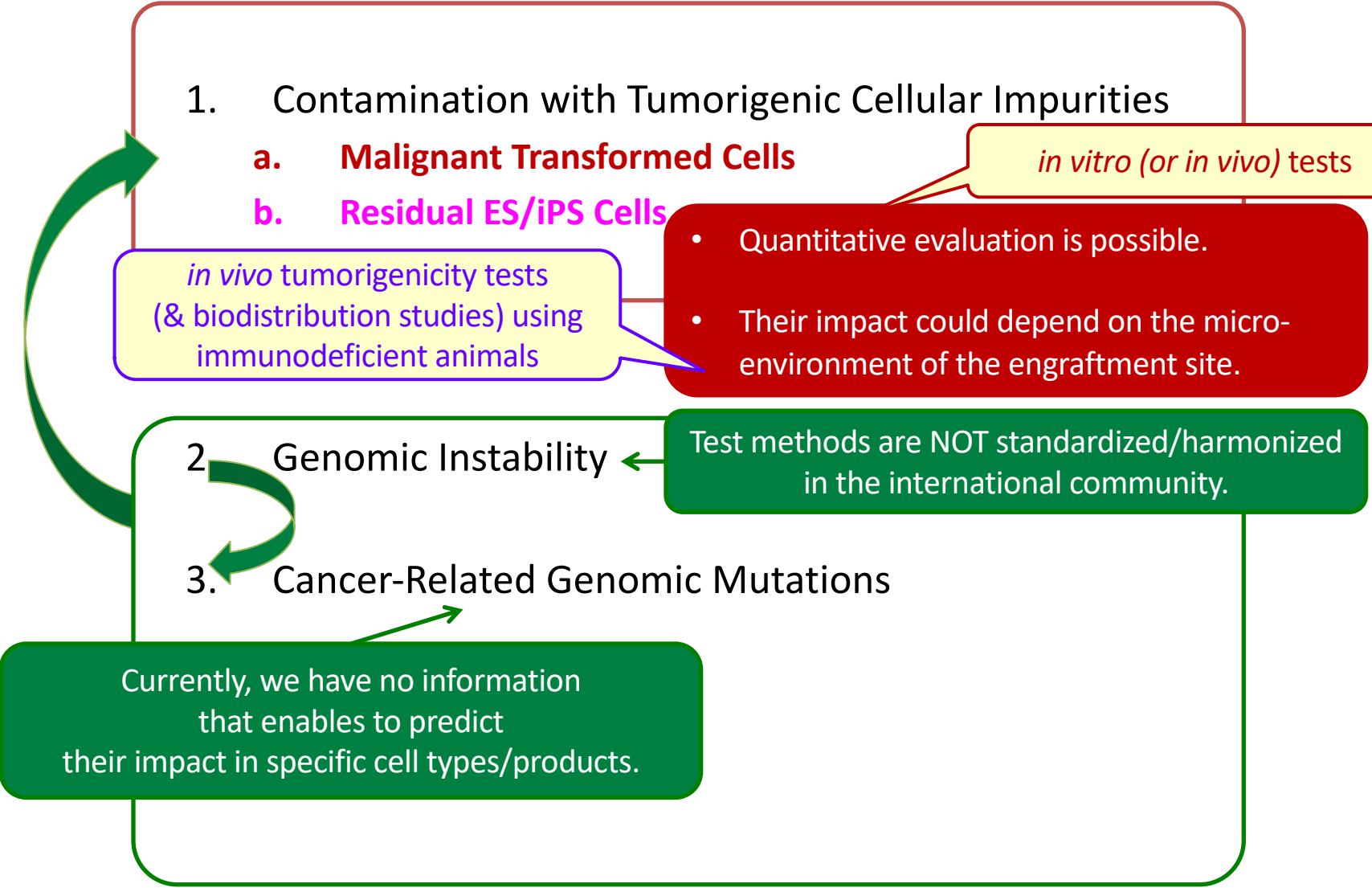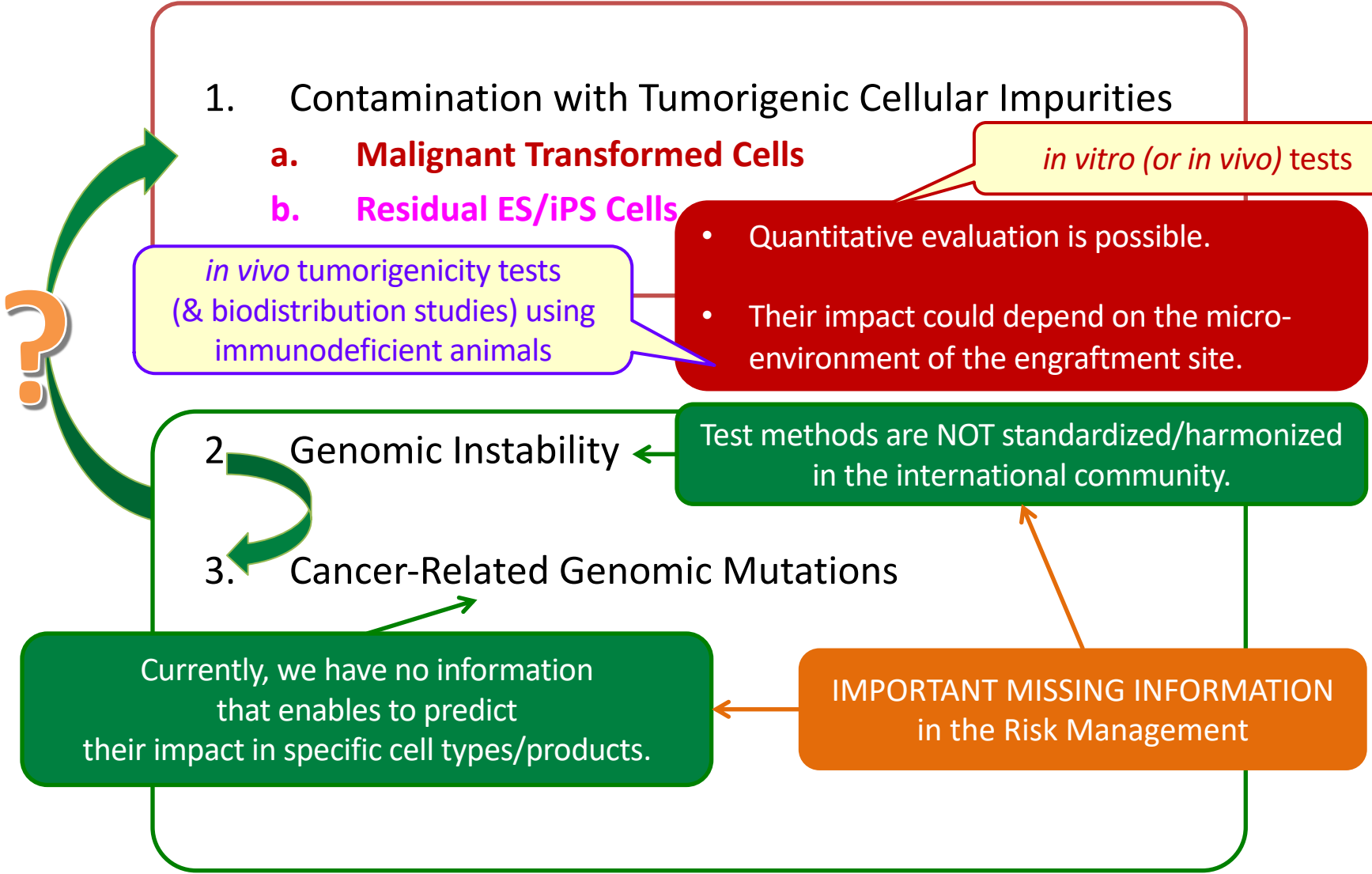   a. **Malignant Transformed Cells**
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in vitro (or in vivo) tests

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

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

2. Genomic Instability ← Test methods are NOT standardized/harmonized in the international community.

3. Cancer-Related Genomic Mutations

Currently, we have no information that enables to predict their impact in specific cell types/products.
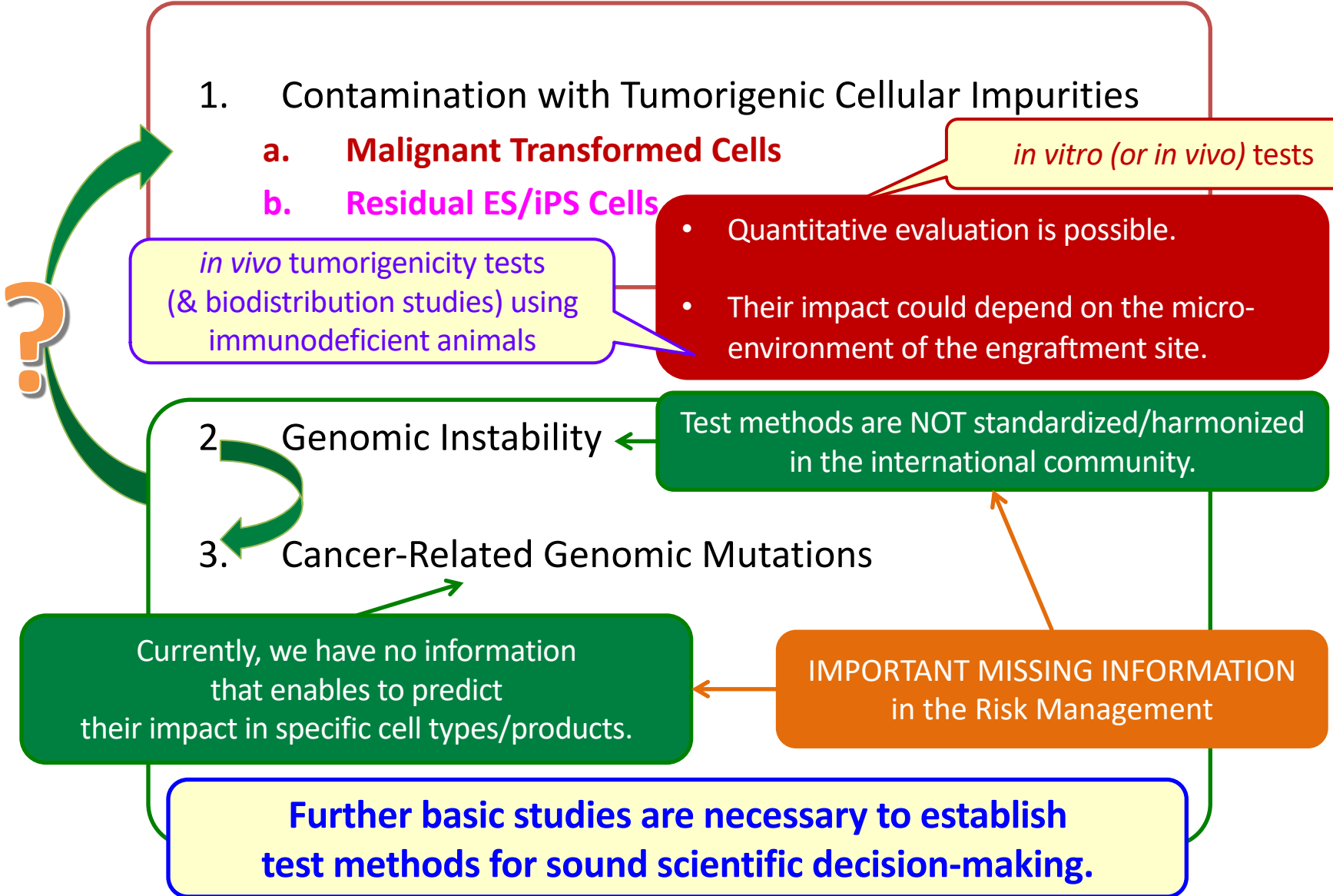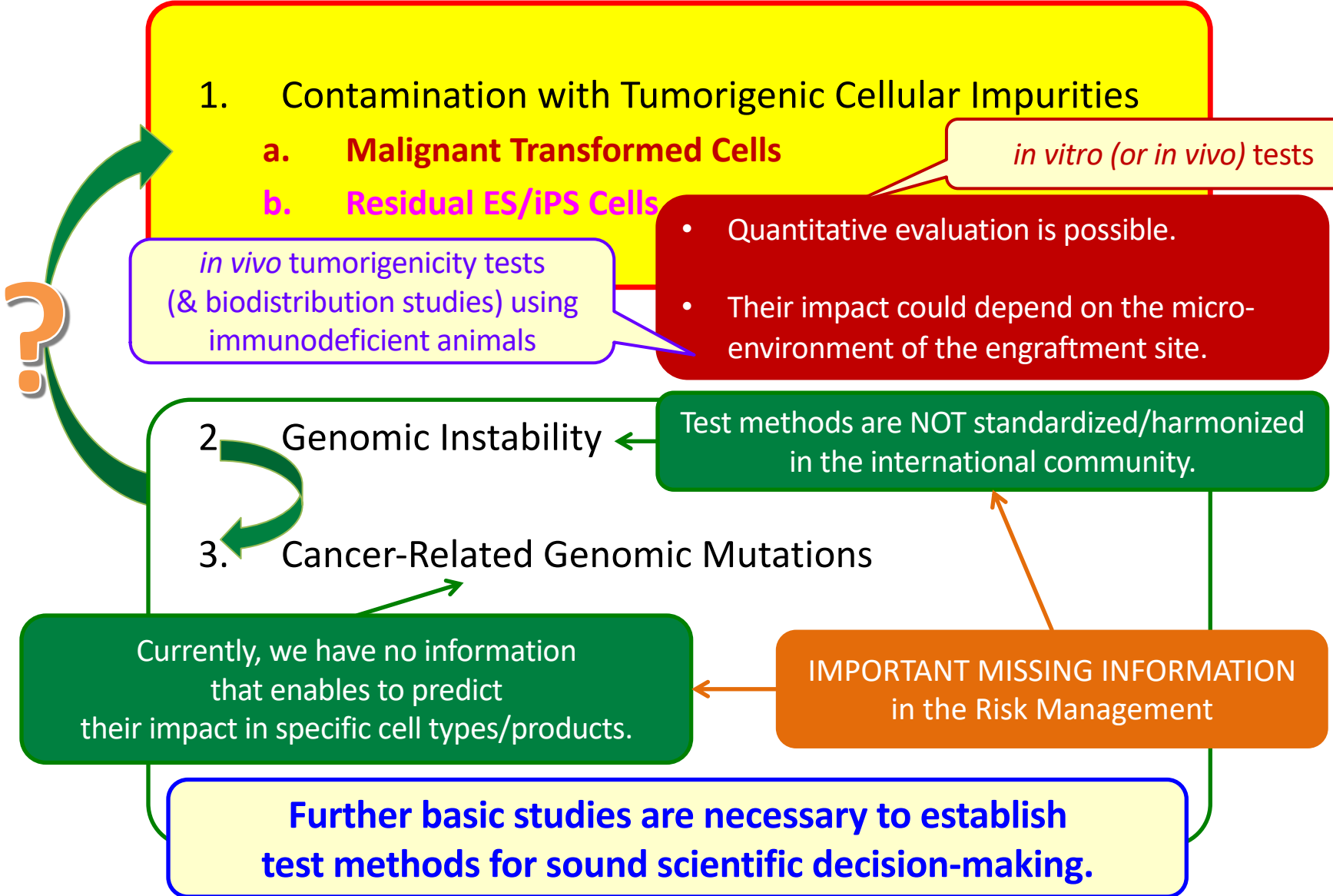
IMPORTANT MISSING INFORMATION in the Risk Management

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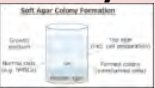
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IMPORTANT MISSING INFORMATION in the Risk Management

Further basic studies are necessary to establish test methods for sound scientific decision-making.

Development of Test Methods for Detection of Transformed Cells

Tumorigenic Cellular Impurities
=Hazards of PSC-Derived Products

In Vitro Assays

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

In Vivo Assay

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
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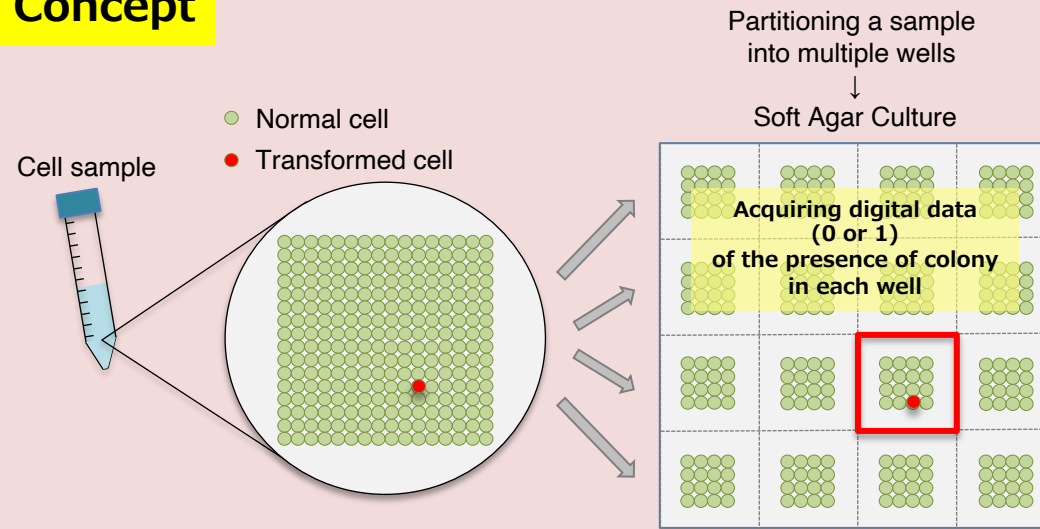# Conventional Soft Agar Colony Formation Assay

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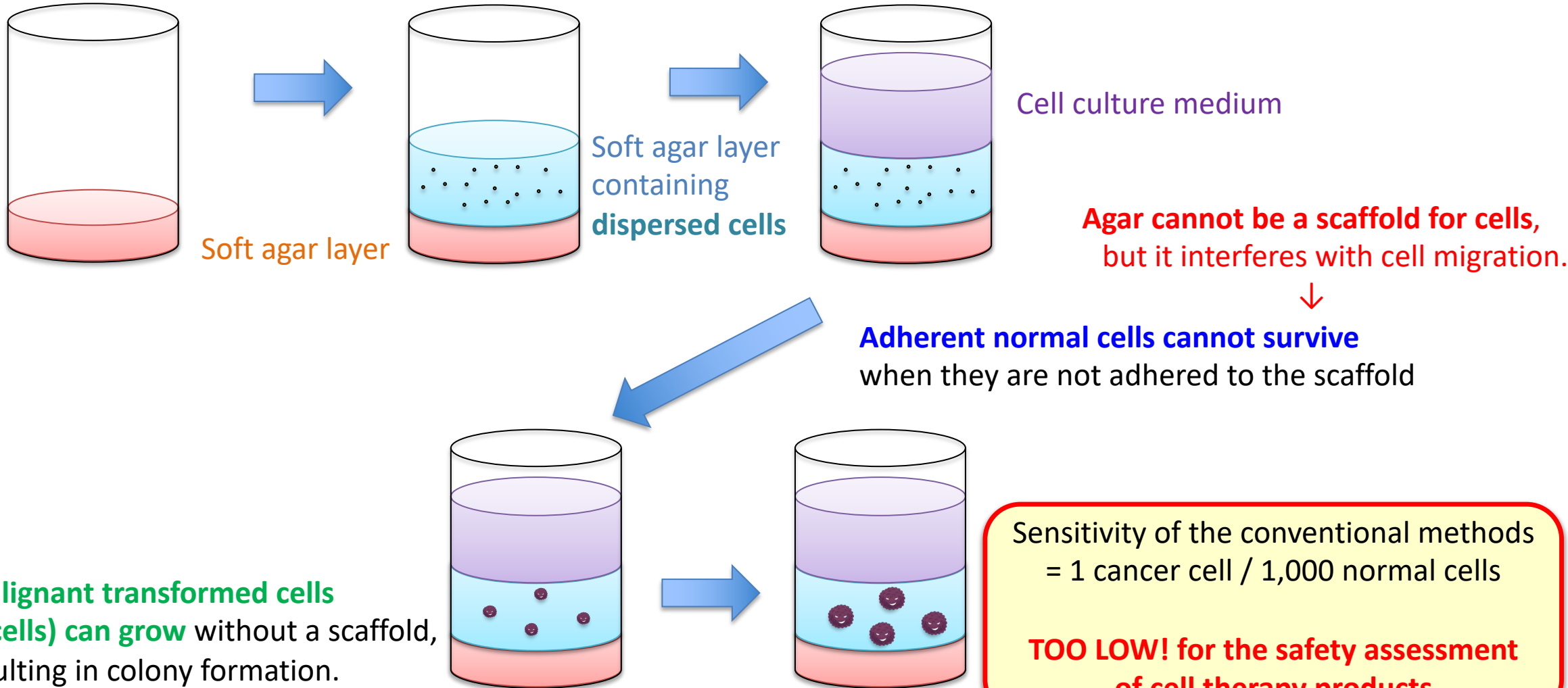
Soft agar layer

Soft agar layer containing **dispersed cells**

Cell culture medium

Agar cannot be a scaffold for cells, but it interferes with cell migration.
↓
Adherent normal cells cannot survive when they are not adhered to the scaffold

Malignant transformed cells (= cancer cells) can grow without a scaffold, resulting in colony formation.

Sensitivity of the conventional methods = 1 cancer cell / 1,000 normal cells

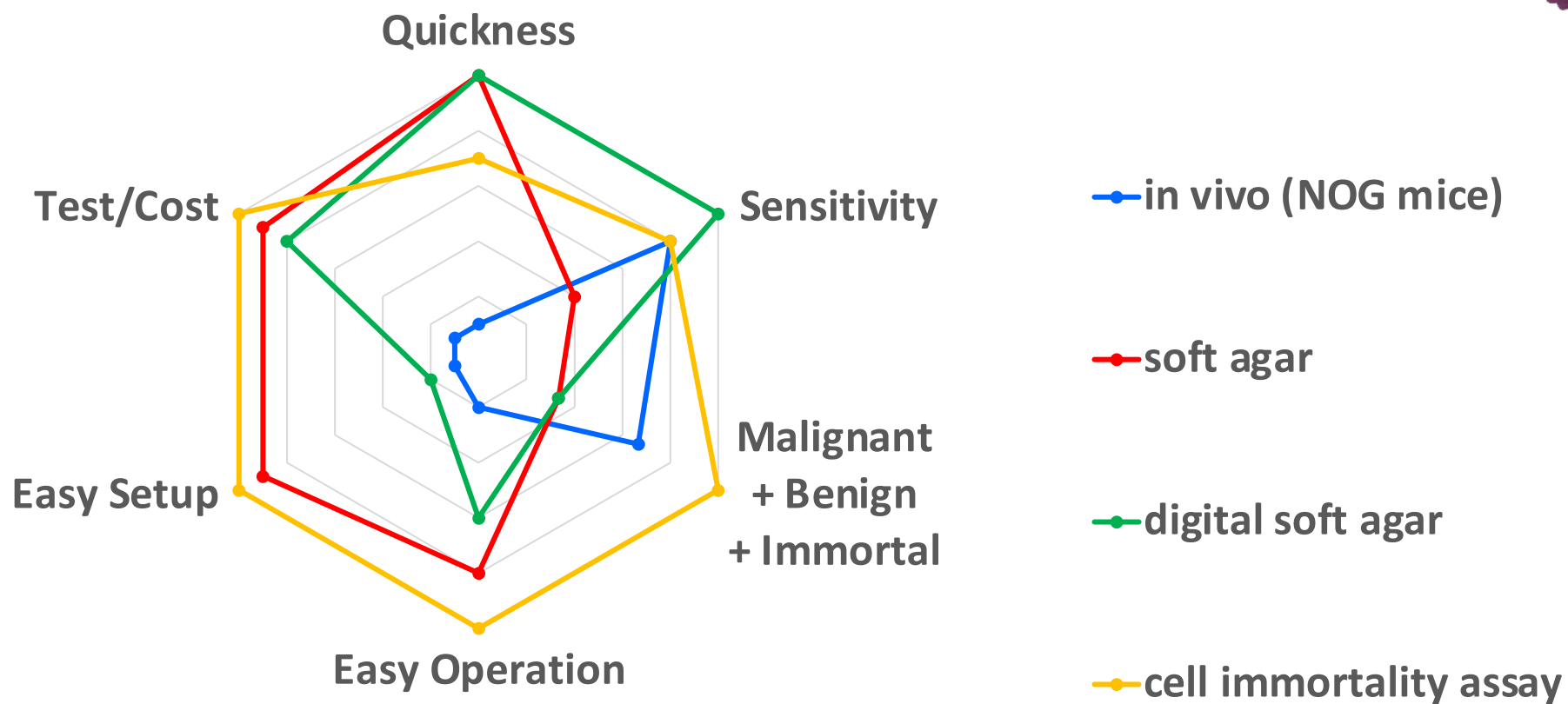**TOO LOW! for the safety assessment of cell therapy products**
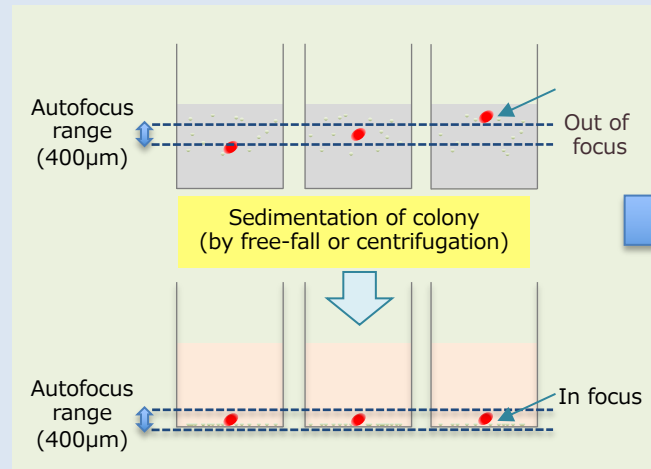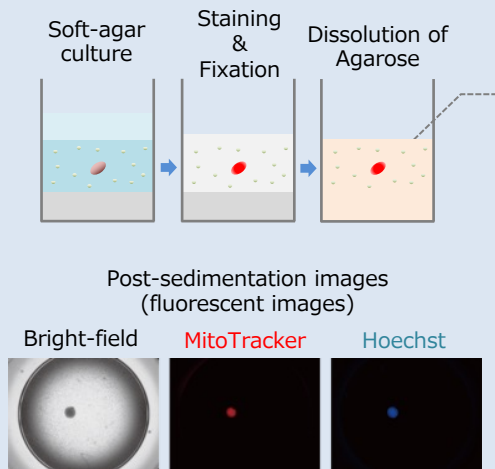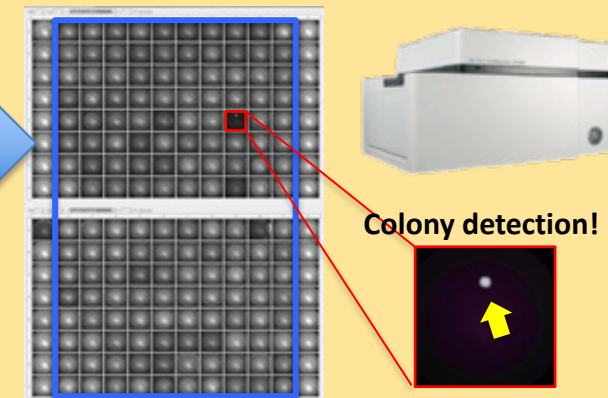
Digital Soft-Agar Colony Formation Assay has achieved the ability to detect cancer cells in normal cells at a ratio of 1 in 10 million

Comparable to the ability to find one zombie in New York City
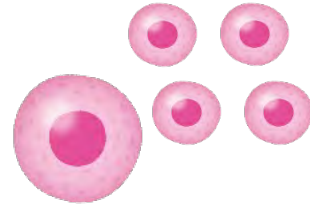(population: about 8 million)
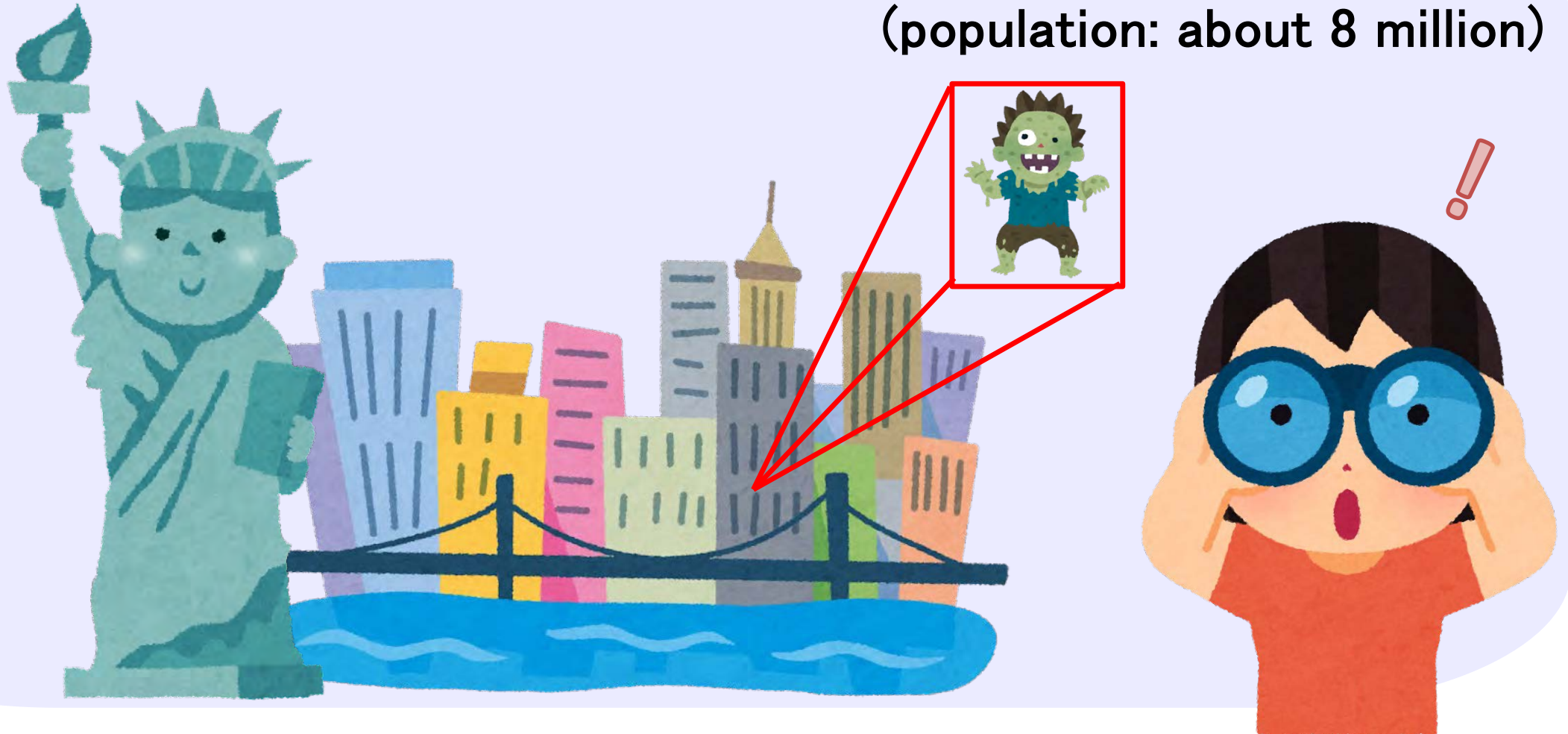
Qualitative Comparisons of Test Methods for Detection of Transformed Cells
(based on our validation studies and past literature)

Quickness

Sensitivity

Malignant + Benign + Immortal

Easy Operation

Easy Setup

Test/Cost

- in vivo (NOG mice)
- soft agar
- digital soft agar
- cell immortality assay

Development of Test Methods for Detection of Residual Undiffrentiated PSCs

Tumorigenic Cellular Impurities
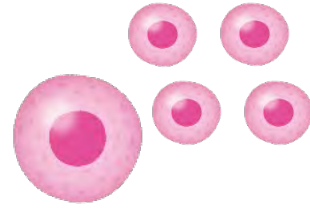=Hazards of PSC-Derived Products

In Vitro Assays

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

In Vivo Assay

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 Undiffrentiated PSCs

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Tumorigenic Cellular Impurities
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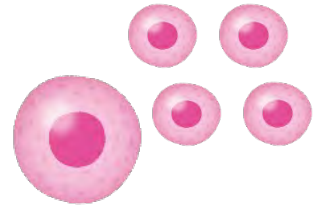| Assays/Platform	Tumorigenicity Test
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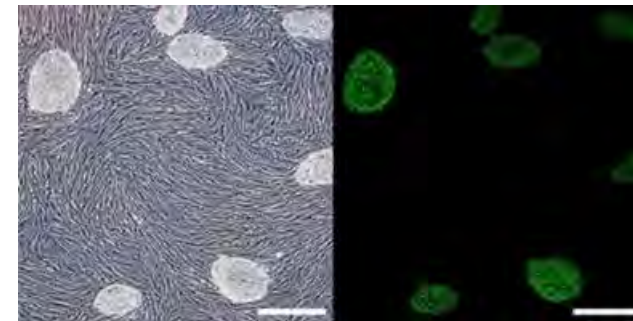# Highly-Efficient Culture (HEC) Assay

Example 2

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 *etc*
Duration	about a week
Marker	TRA-1-60 *etc*
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.
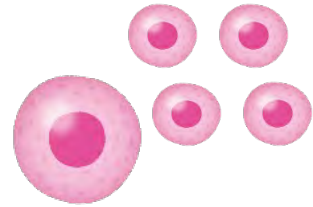

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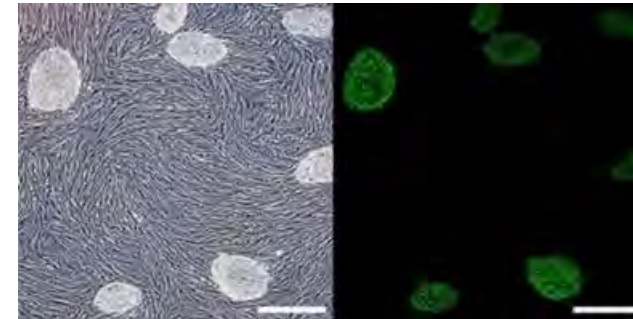# Highly-Efficient Culture (HEC) Assay

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

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 Yasuda[3], Shinji Kusakawa[3], Takuya Kuroda[3], 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 Sato[3]

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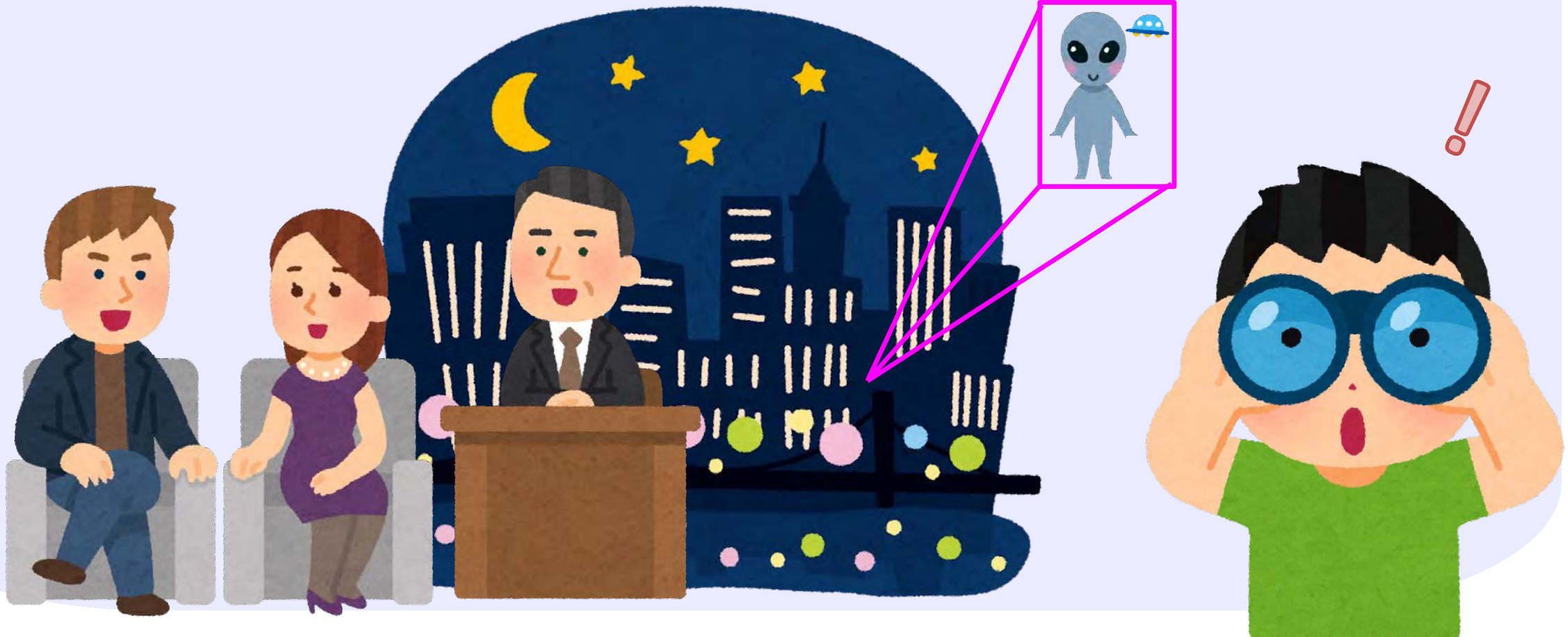
Methods: MEASURE first validated the detection sensitivity of HEC assay and then investigated the feasibility of magnetic-activated cell sorting (MACS) to improve sensitivity.

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.
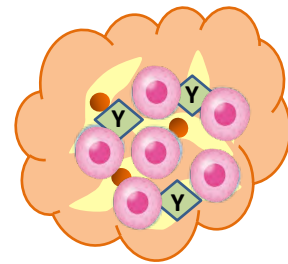
In vivo Tumorgenicity Test using NOG mice subcutaneously transplanted with iPSCs

iPS cells （201B7）   Feeder cells （Human fibroblast）   Matrigel   ROCK inhibitor （Y-27632）   NOG mice

iPS cells （single cell-dissociated）
+Matrigel
+feeder cells
+ROCK inhibitor
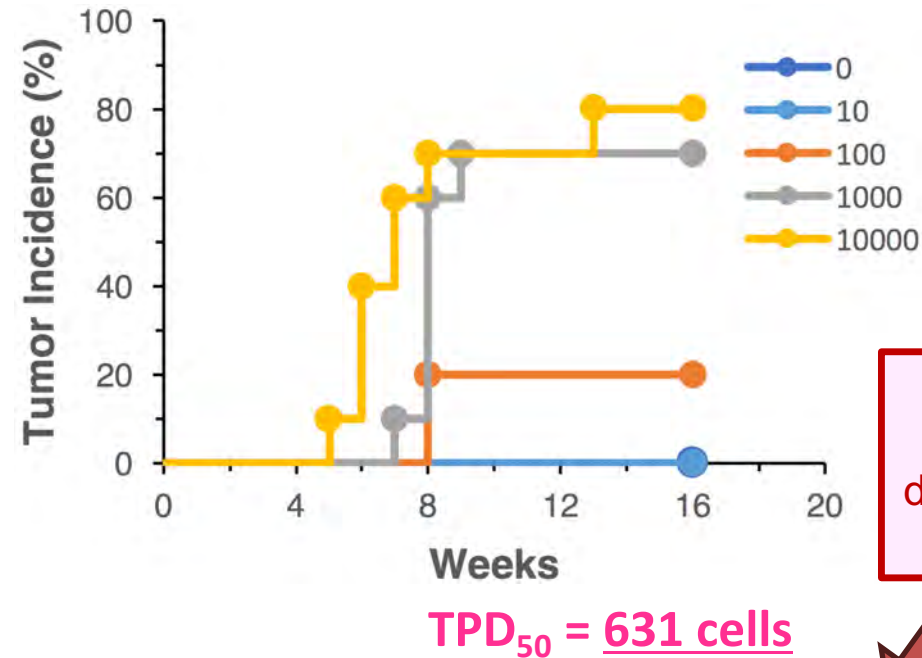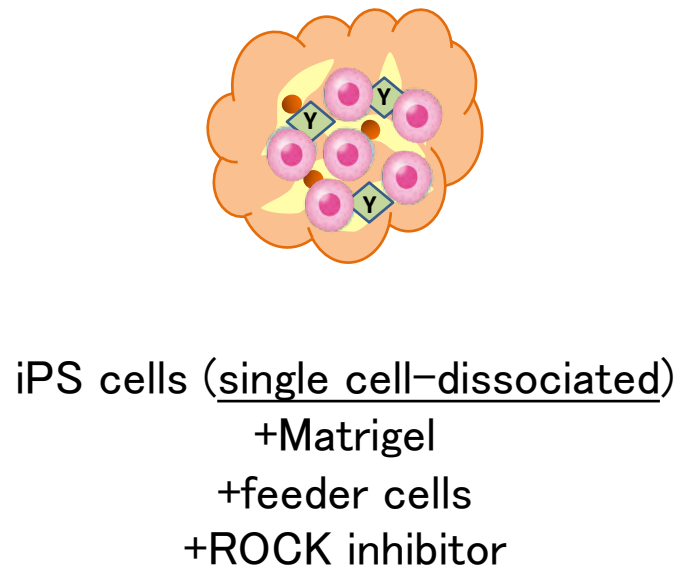
TPD_{50} = 631 cells

When iPS cells were most efficiently engrafted in severely immunodeficient mice, TPD_{50} was 631 cells.
If 10^6 and 10^7 cells are injected, TPD_{50} = 631 would correspond to:
0.06% (6E-4) and 0.006% (6E-5), respectively.

In vivo Tumorgenicity Test using NOG mice subcutaneously transplanted with iPSCs

Yasuda et al., PLoS One 2018

iPS cells (201B7)　　Feeder cells (Human fibroblast)　　Matrigel　　ROCK inhibitor (Y-27632)　　NOG mice

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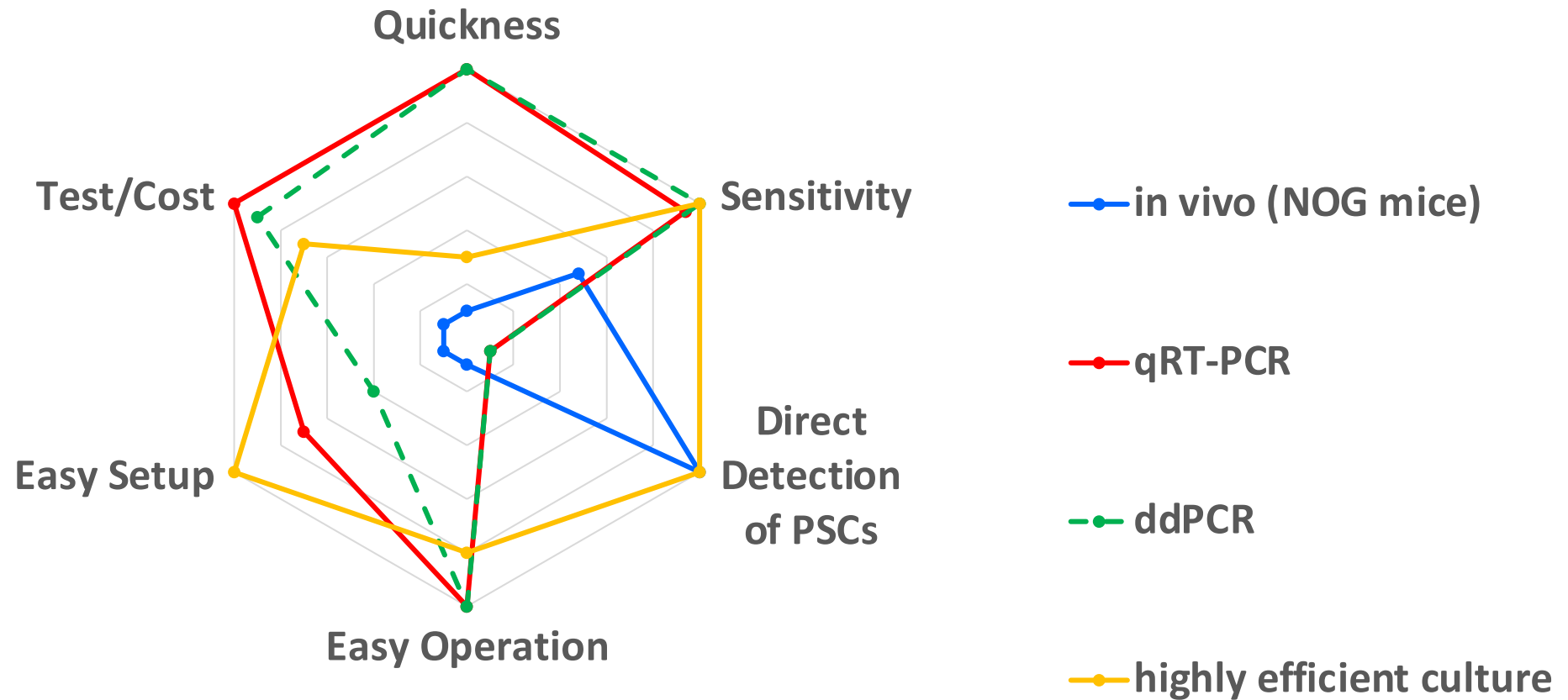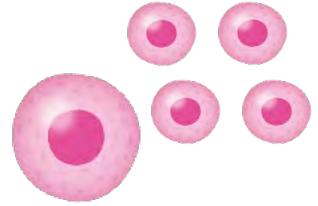+Matrigel
+feeder cells
+ROCK inhibitor

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

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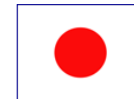0.06% (6E-4) and 0.006% (6E-5), respectively.

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for Detection of Residual PSCs
(based on our validation studies and past literature)**

Quickness

Sensitivity

Test/Cost

Direct Detection of PSCs

Easy Setup

Easy Operation

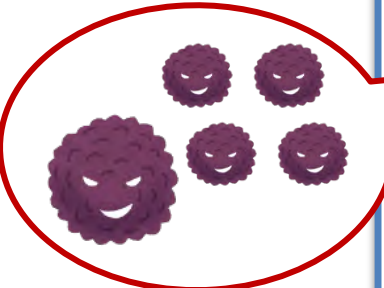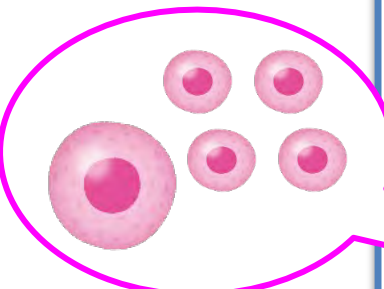in vivo (NOG mice)

qRT-PCR

ddPCR

highly efficient culture

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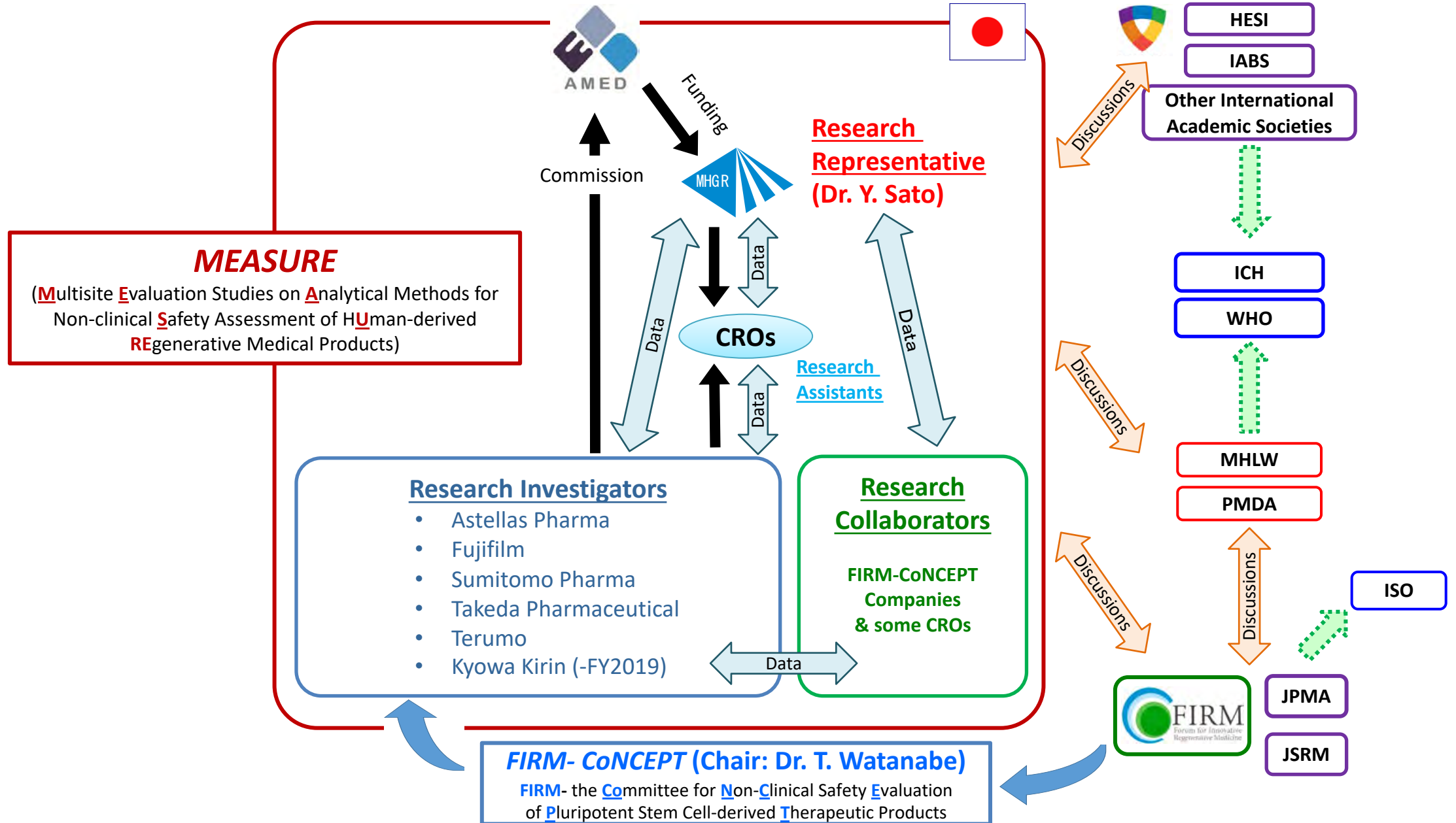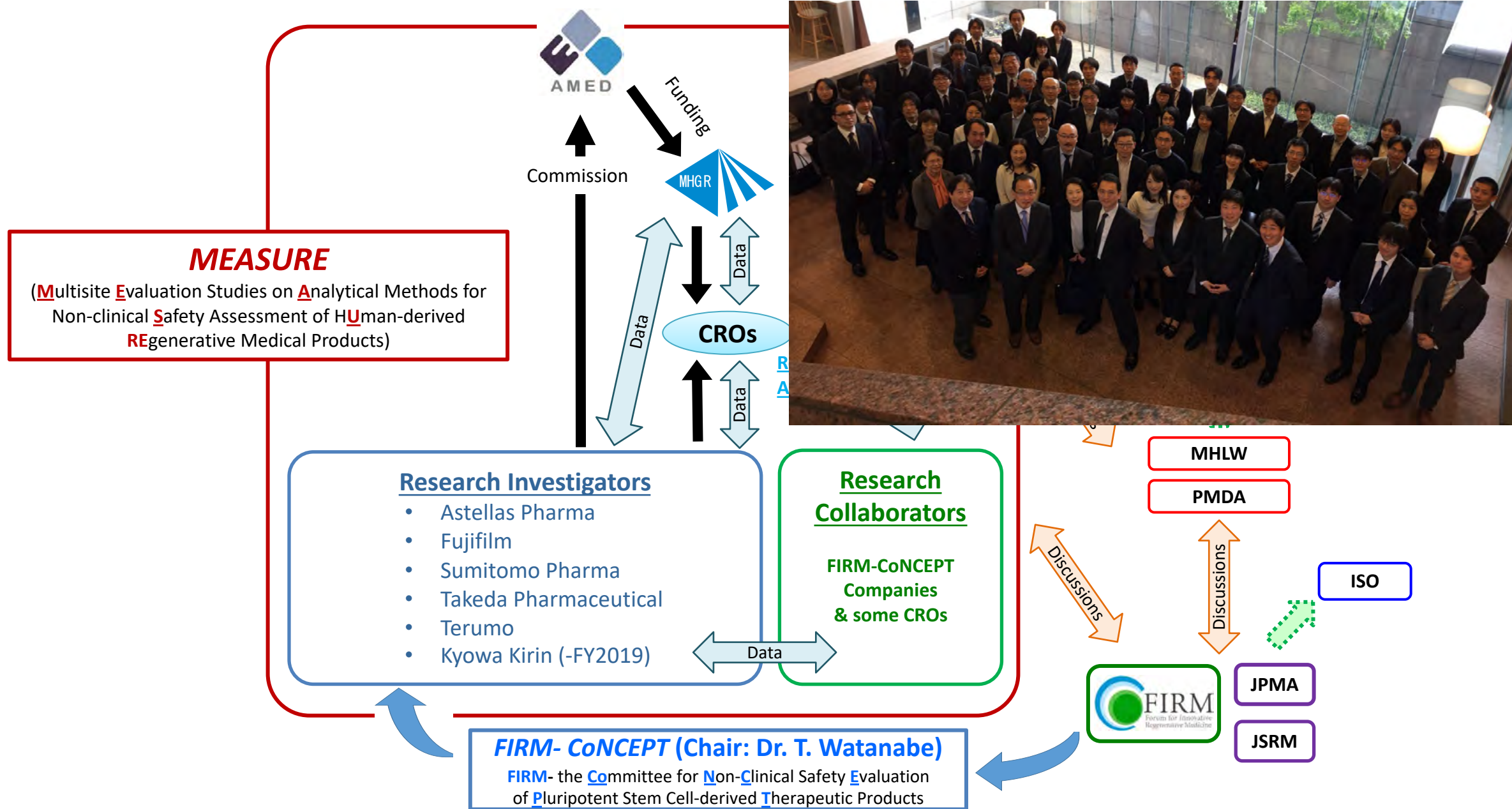## (Annex of Notification No. 0627-1 Issued on June 27, 2019, Pharmaceutical and Food Safety Bureau, MHLW)

Contents

https://www.clea-japan.com/promotion/japanese/nextgenerationnog

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

AMED

Funding

Commission

MHGR

Data

Data

CROs

Data

MEASURE

(**M**ultisite **E**valuation Studies on **A**nalytical Methods for Non-clinical **S**afety Assessment of H**U**man-derived **RE**generative Medical Products)

Research Investigators
- Astellas Pharma
- Fujifilm
- Sumitomo Pharma
- Takeda Pharmaceutical
- Terumo
- Kyowa Kirin (-FY2019)

Data

Research Collaborators

FIRM-CoNCEPT Companies & some CROs

MHLW

PMDA

ISO

Discussions

Discussions

JPMA

FIRM

JSRM

FIRM- CoNCEPT (Chair: Dr. T. Watanabe)

FIRM- the **C**ommittee for **N**on-**C**linical Safety **E**valuation of **P**luripotent Stem Cell-derived **T**herapeutic Products

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

NGOs / Consortia:

Universities/ Research Centers:

Government & Regulatory bodies:

CT-TRACS Members *(2022 data)*

>100 Participants

>30 Organizations

Courtesy of Dr. Lucilia Mouriès, HESI

Health and Environmental Sciences Institute, www.hesiglobal.org

- **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

PoA/ BIODISTRIBUTION WG

Brooke Hefler (Celsense)
Vladimir Ponomarev (MSKCC)

FIRM
Forum for Innovative Regenerative Medicine

Collaboration with Japanese Consortium
FIRM-CoNCEPT & AMED MEASURE

TUMORIGENICITY WG

Hiroto Bando (Showadenko)
Charlotte de Wolf (CBG-MEB)

Cell Tracking DB

Education & Outreach

Research

New in 2022!

Multi-site studies for residual iPSCs

Advanced sequencing for off target (CRISPR)

IL-2 for CAR-T

New in 2022!

SACF/GILA (CRISPR)

New in 2022!

ddPCR

HEC assay

https://hesiglobal.org/cell-therapy-tracking-circulation-safety-ct-tracs/

Courtesy of Dr. Lucilia Mouriès, HESI

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- >35 organizations

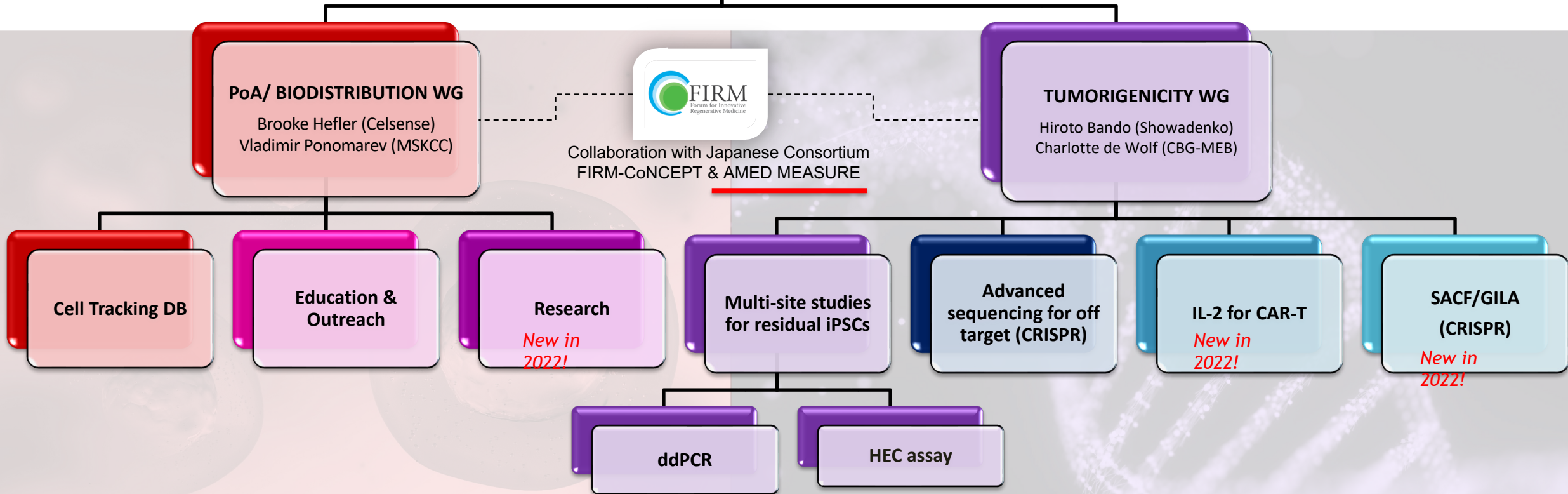**CT-TRACS (Cell Therapy: TRAcking, Circulation and Safety) Committee**

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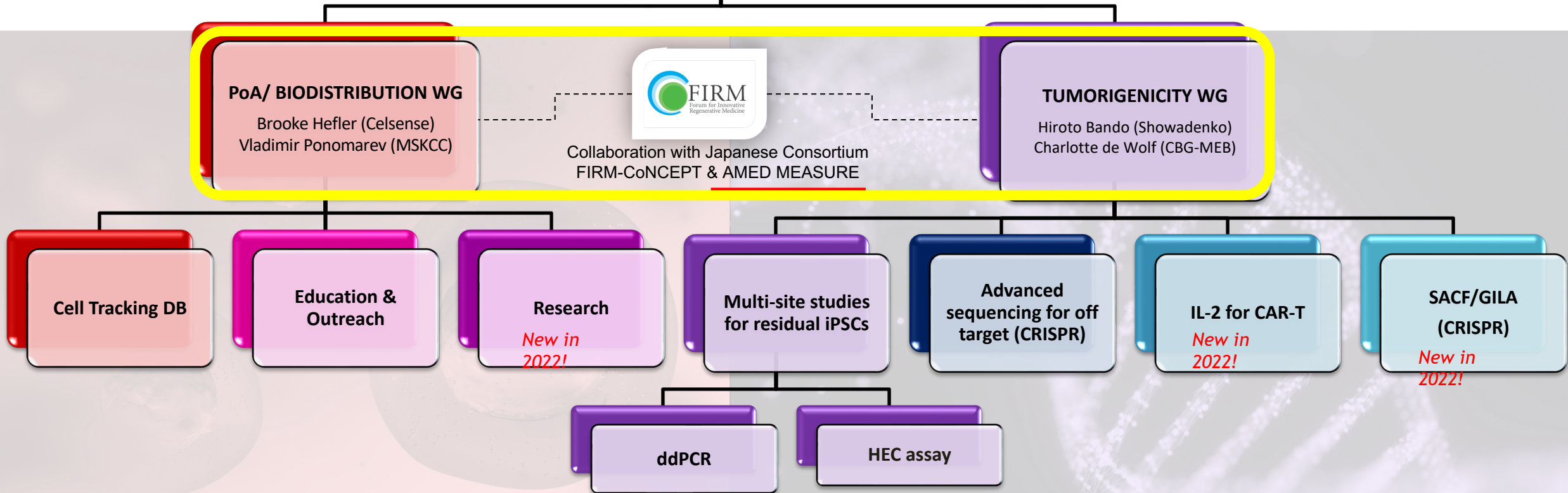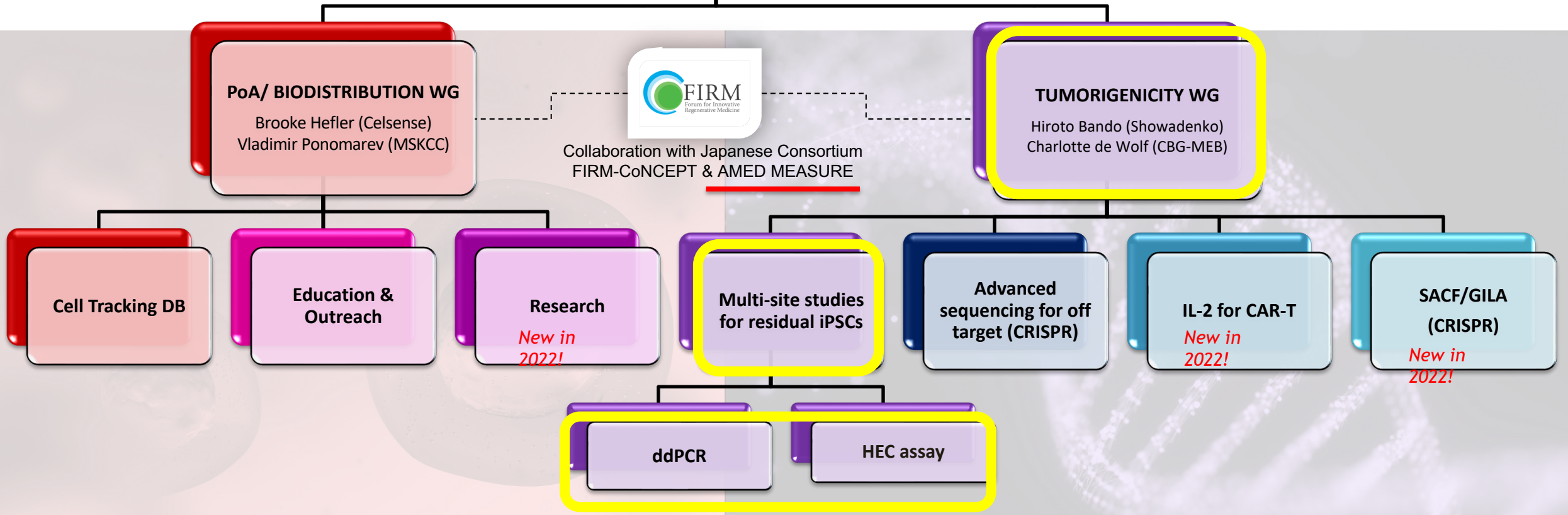**CT-TRACS (Cell Therapy: TRAcking, Circulation and Safety) Committee**

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Addressing Challenges & Needs

HESI.

International Society
ISCT
Cell & Gene Therapy

Cytotherapy, 2019; 21: 1095–1111

REVIEW

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

Y. SATO[1], H. BANDO[2,*], M. DI PIAZZA[3], G. GOWING[4], C. HERBERTS[5,|], S. JACKMAN[6], G. LEONI[7], S. LIBERTINI[8], T. MACLACHLAN[9], J.W. MCBLANE[10], L. PEREIRA MOURIES[11], M. SHARPE[7], W. SHINGLETON[12,|], B. SURMACZ-CORDLE[7], K. YAMAMOTO[13] & J.W. VAN DER LAAN[5,*]

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

HESI CT-TRACS Tumorigenicity WG

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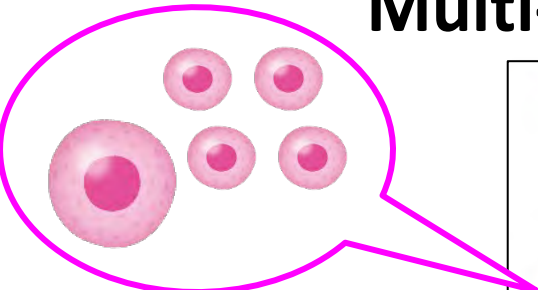
Short Communication

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Regenerative Medicine

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 Foldes6,7, Shinji Kusakawa2, Lucilia Pereira Mouriès3 & 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.

Takeda NIHS
HESI AstraZeneca
Imperial College London

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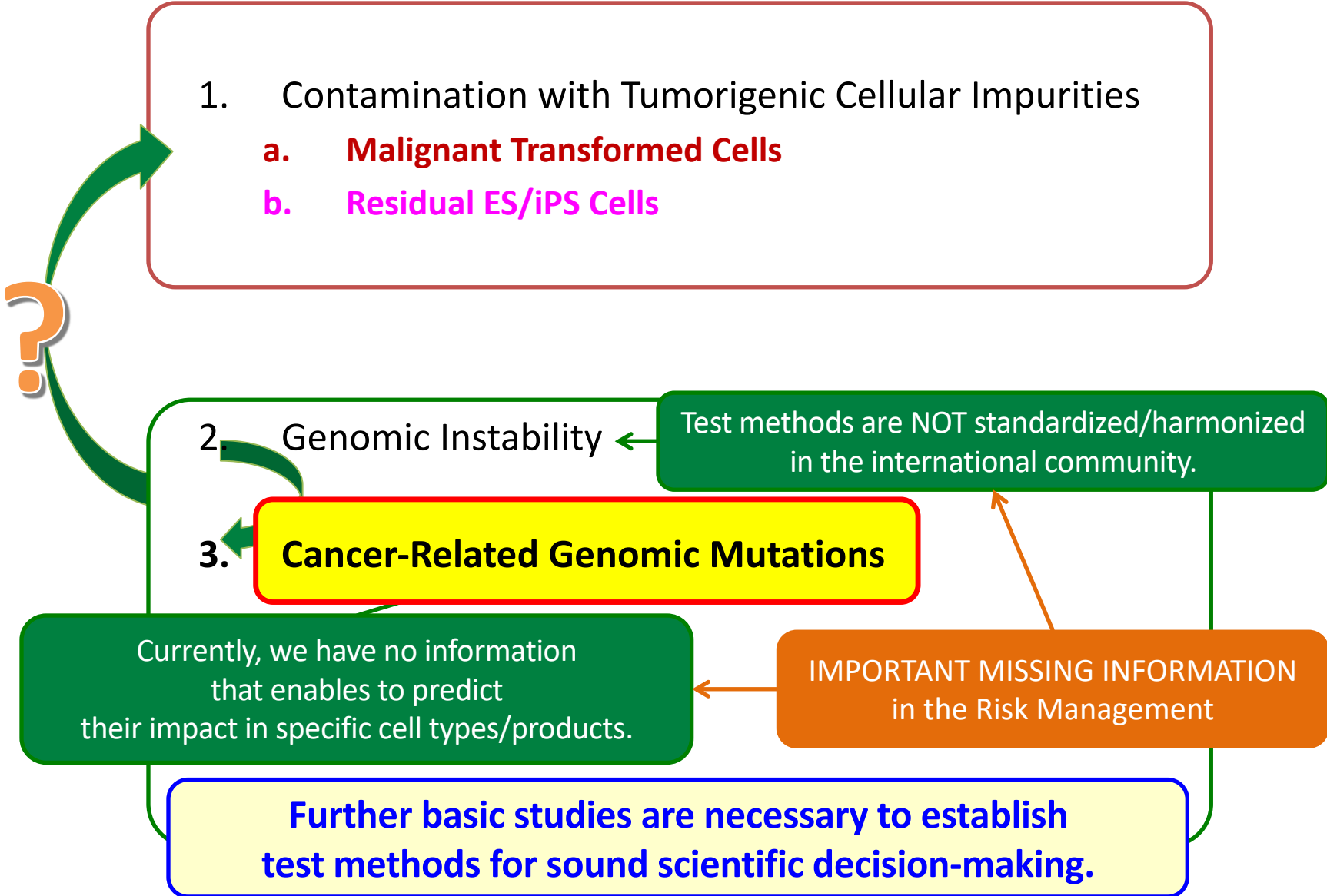
AGENDA

1. **Regulatory science on emerging S&Q issues for hiPSC-derived products**

2. **Development and validation of test methods for tumorigenicity assessment of hiPSC-derived products**

3. **Study on the correlation between genomic variations in hiPSC-derived products and abnormal tissue formation**

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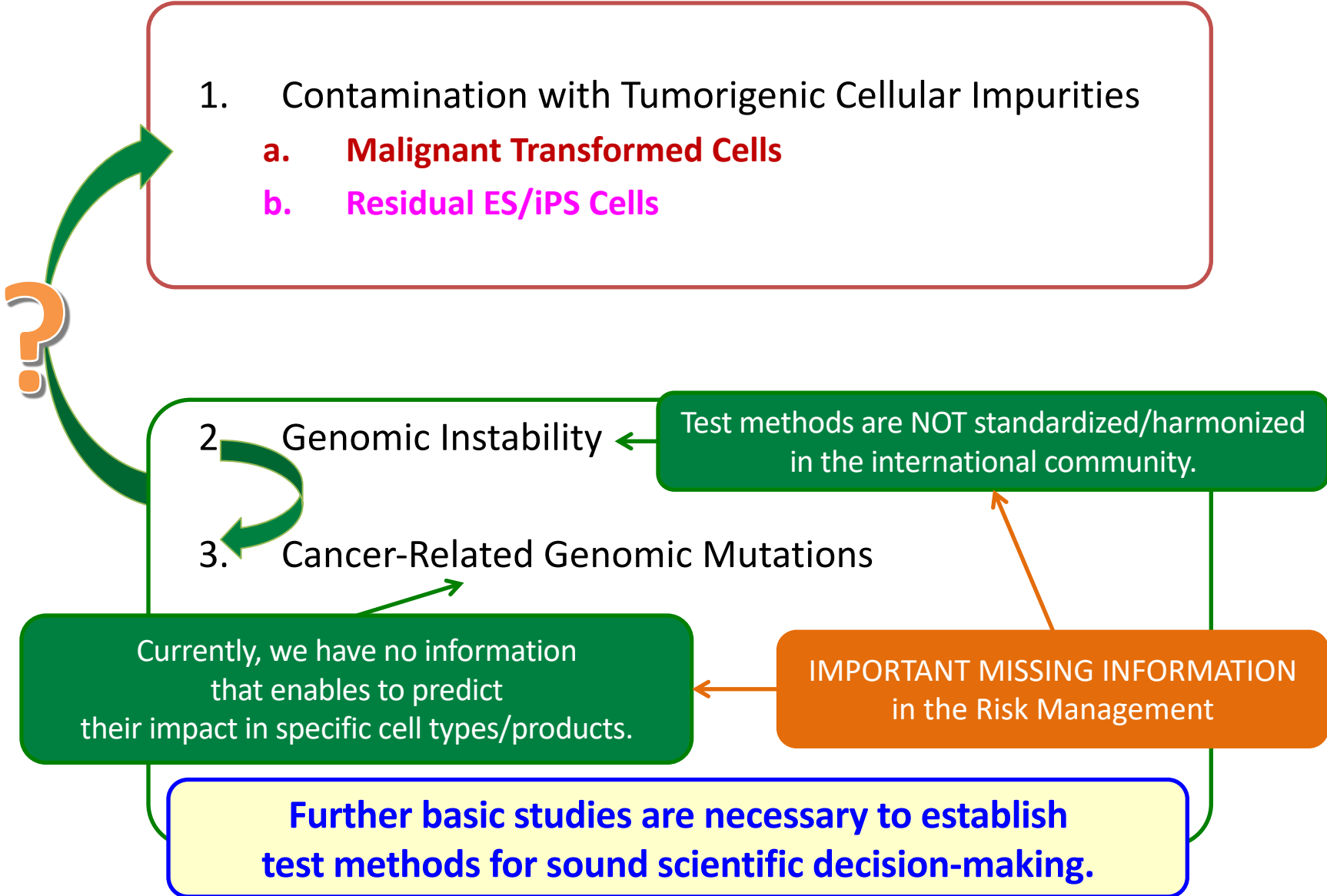
Potential Hazards for the Tumorigenicity Risk of Pluripotent Stem Cell-Derived Therapeutic Products

The human body is a mosaic of different genomes
Survey finds that 'n ormal' human tissues are riddled with mutations.

Nature (NEWS on 06 June 2019)

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

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

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Cristian Tomasetti, Johns Hopkins Medicine

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…means "we currently have no way"

"Researchers now need to find ways to sort out

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"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]*
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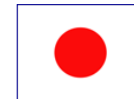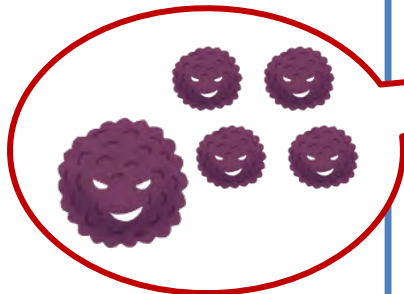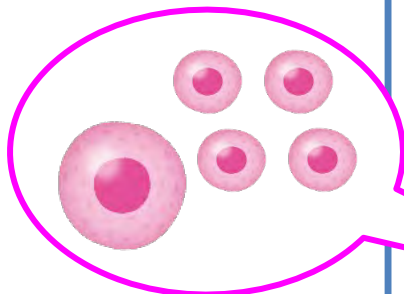
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7. General Considerations for Genomic Stability

"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

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

C. Explanatory variable: CNV (- : CNV ≤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%			
Overall predictivity			86%			
Likelihood ratio for abnormal outcome		0.0	4.5	Correlation ratio η: 0.75		

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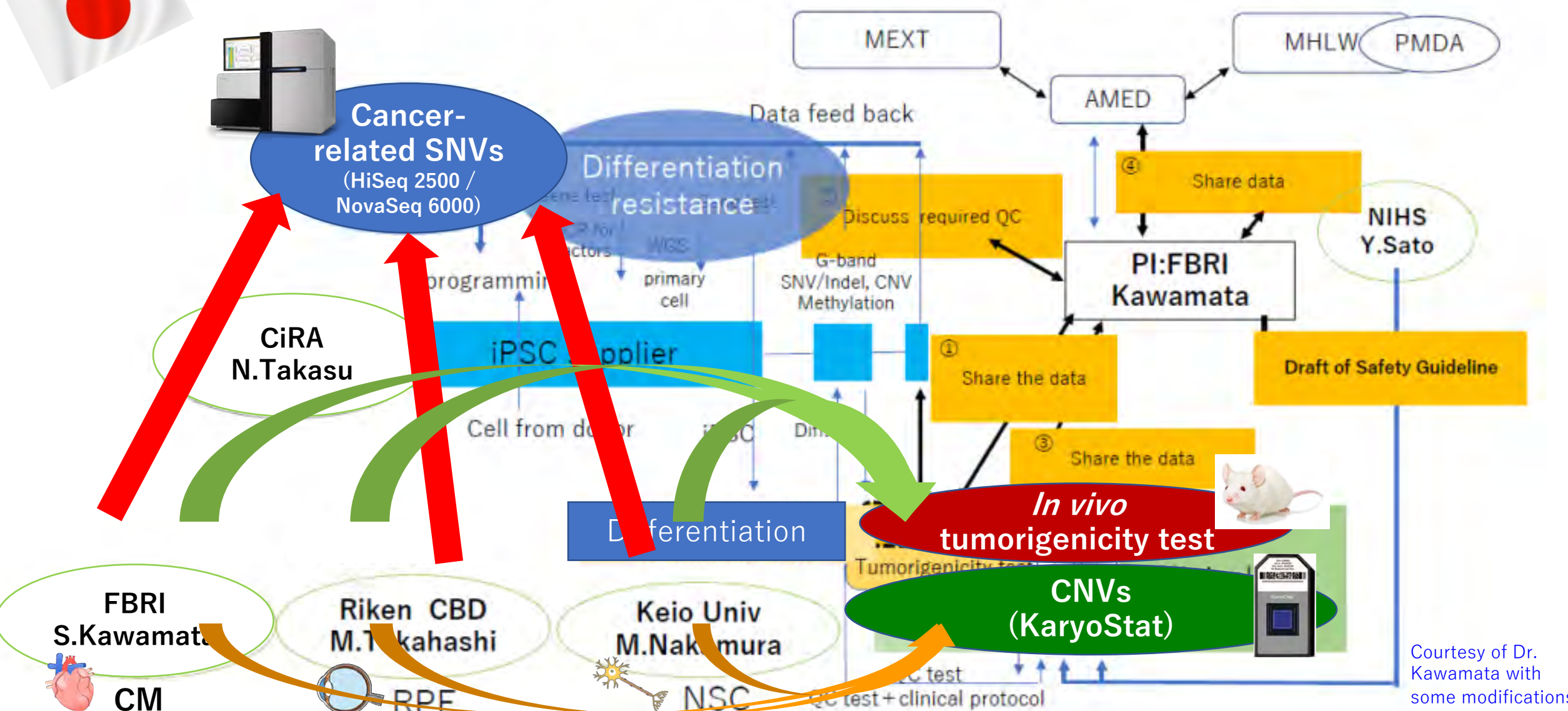
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16H12	non-CMs	SNV(+)	CNV(-)	Normal
15M38	RPEs	SNV(-)	CNV(+)	Abnormal
15M38	non-CMs	SNV(-)	CNV(+)	Abnormal
1210B2	NSCs	SNV(+)	CNV(-)	Normal
Ff-WJ	NSCs	SNV(-)	CNV(-)	Normal
Ff-I01	RPEs	SNV(-)	CNV(+)	Abnormal
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> CNVs may help predict abnormal tissue formation, including tumorigenesis, after product implantation.

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

Explanatory variable		CNV(-)	CNV(+)	Discriminative ratio		Overall predictability
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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	*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
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

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paminergic 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	
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Cartilage	Allogeneic iPSCs	Knee articular cartilage injury	Kyoto Univ.	Non-commercial clinical research under the RM Safety Act	2020	
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Cardiomyocytes	Allogeneic iPSCs	Ischemic Cardiomyopathy	Heartseed, Novo Nordisk	Clinical trial under the PMD Act	2021	2023

https://japan-forward.com/osaka-university-team-does-worlds-first-successful-ips-cell-derived-corneal-transplant/

https://nd.natureasia.com/figure/4438/56992/phone/1

https://english.kyodonews.net/news/2020/01/47a1ba1f19f1-japan-researchers-conduct-worlds-1st-transplant-of-ips-heart-muscles.html

https://www.sankei.com/article/20200521-B5I5HI55EBI6XMQ5AVIKYLXQVY/photo/UDRYD4AHVFJPDHGFB54X2ZSB2Q/

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

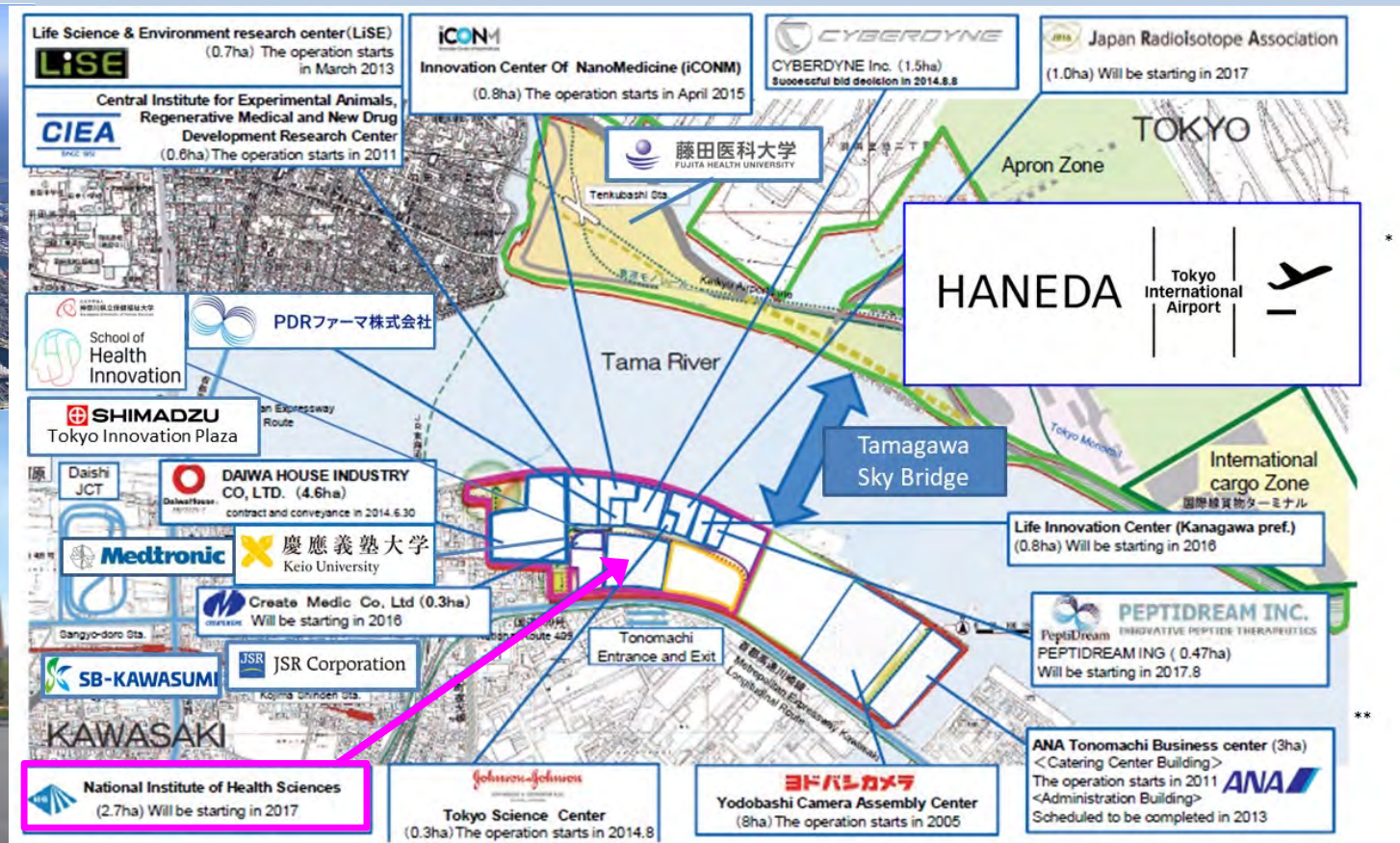# ACKNOWLEDGMENTS

Thank you for your attention!

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