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KALAS 2024 Conference
Symposium 12: Developing Cell-Gene Treatment (CGT) Human Cell Immunotherapy Using Humanized Animal Models

In Vivo and *In Vitro* Studies for Evaluation of Tumorigenicity of Cell Therapy Products

Yoji SATO, Ph.D.

Head, Division of Drugs

(Immediate Former Head, Division of Cell-Based Therapeutic Products)

National Institute of Health Sciences, Japan

DISCLAIMER

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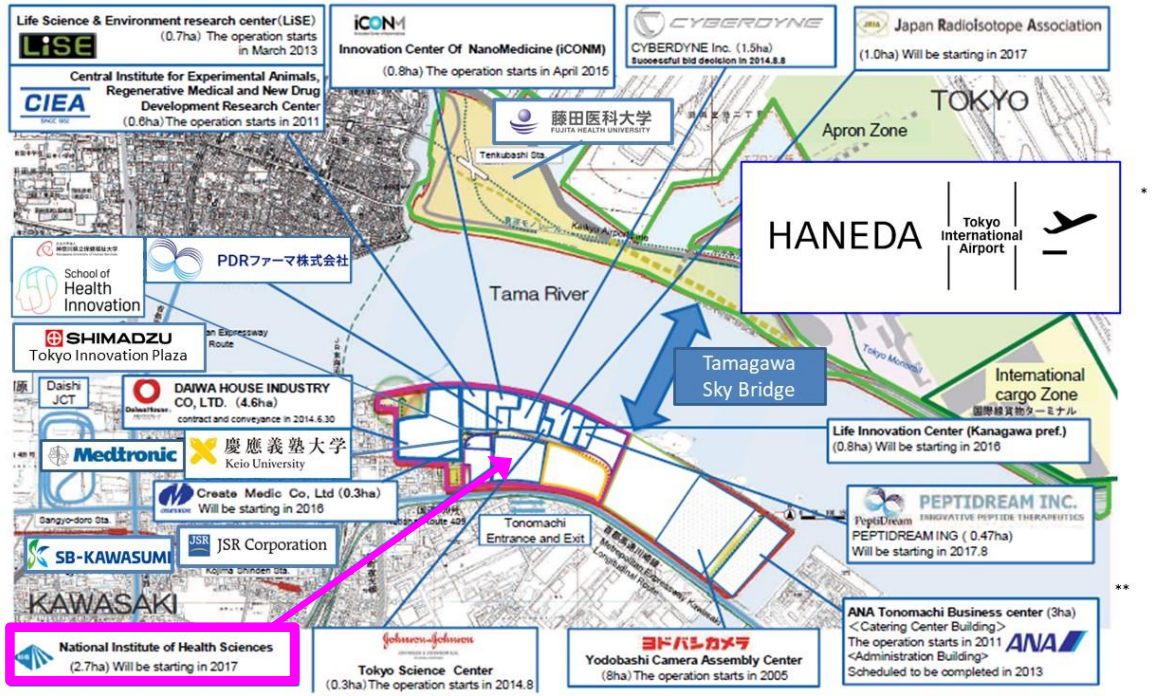
National Institute of Health Sciences

- **Established in 1874** as “Tokyo Pharmaceutical Testing Laboratory”
- Located just across the river from Haneda Airport (Tokyo International Airport)
- Serves as the Base of Research and Evaluation in the Field of **Regulatory Science**

This year marks its 150th anniversary.

Our institute sounds like US NIH, **but is actually more like** the research sections of of **US FDA or like NIFDS of Korean MFDS.**



* https://www.oag.com/hubfs/air-canada-787.jpg

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

“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.

https://www.fda.gov/science-research/science-and-research-special-topics/advancing-regulatory-science

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 and gene 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**.

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

Safety & eligibility of raw materials

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

Ensuring the quality of the final product

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

Prediction of safety & efficacy in the non-clinical phase

13. Design and interpretation of clinical trials
14. Efficacy and safety follow-up

Clinical Evaluation

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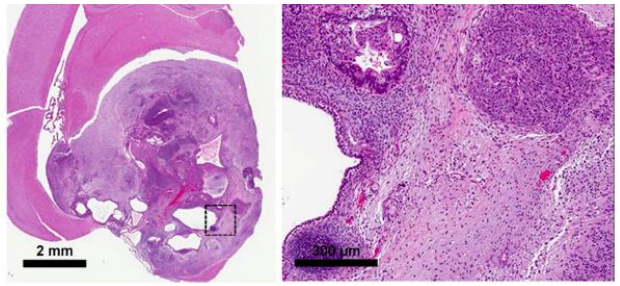
Clinical Evaluation

Tumorigenicity

… is one of the major concerns for cell therapy products, especially for pluripotent stem cell-derived 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.



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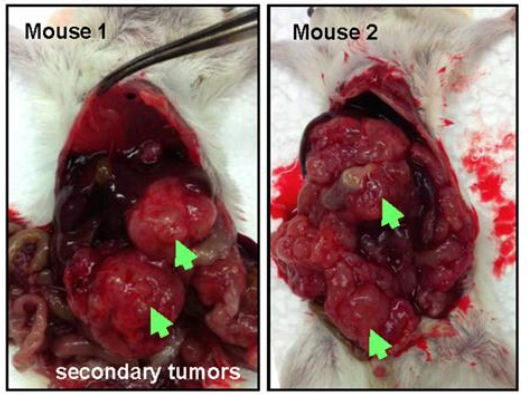
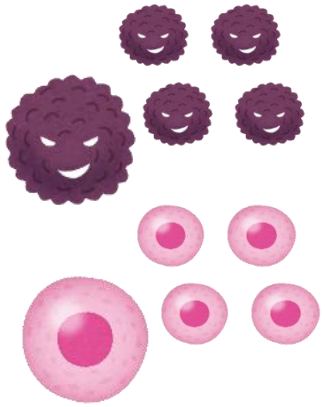


MOUSTAFA M et al. STEM CELLS TRANSLATIONALMEDICINE 2016;5:694–702

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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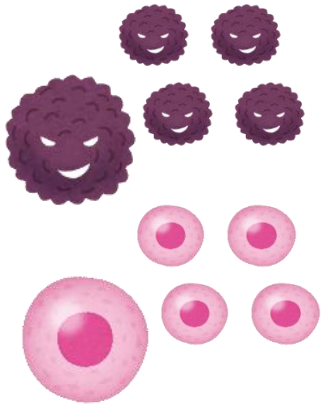

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- Quantitative evaluation is possible.
- Their impact could depend on the micro-environment of the engraftment site.

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Currently, we have no information that enables to predict their impact in specific cell types/products.

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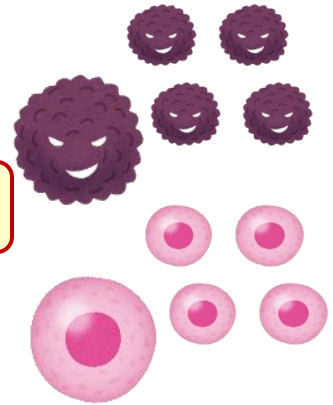

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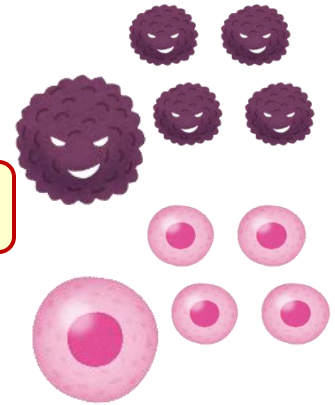

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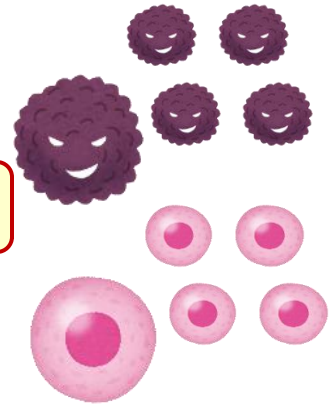

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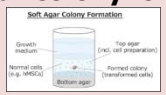
Development of Test Methods for Detection of Transformed Cells

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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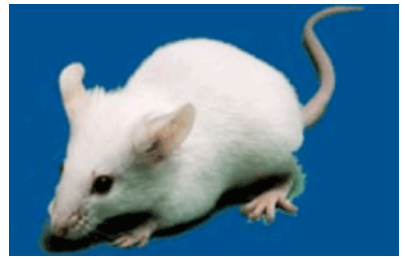

Development of Test Methods for Detection of Transformed Cells

Example 1

Tumorigenic Cellular Impurities
=Hazards of PSC-Derived Products

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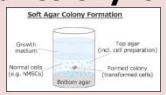
Assays/ Platform	Conventional soft agar colony formation 	Digital soft agar colony formation 	Cell growth analysis 
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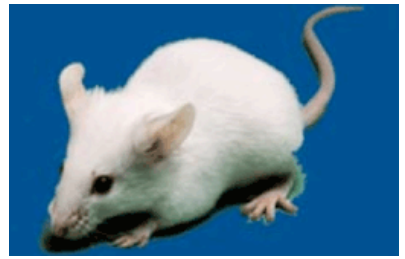
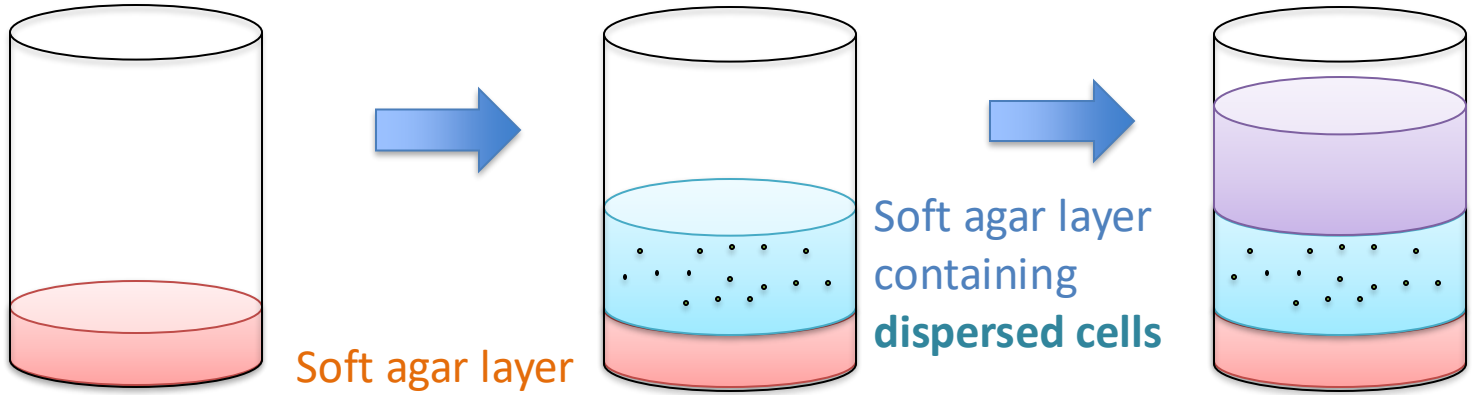


Conventional Soft Agar Colony Formation Assay

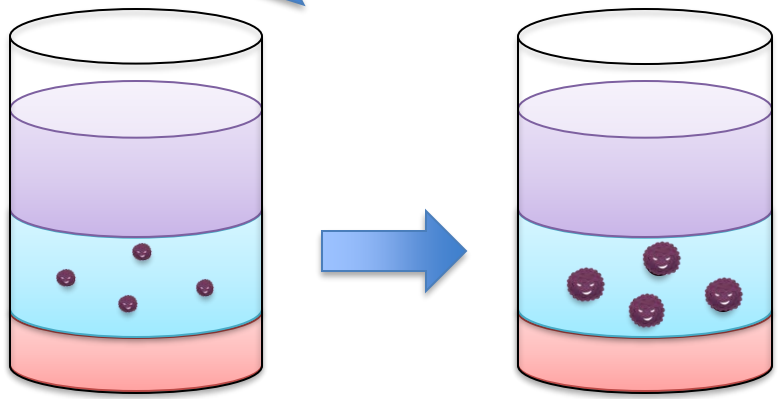
Purpose: Detection of scaffold-independent proliferation (= **malignant transformed cells**)



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



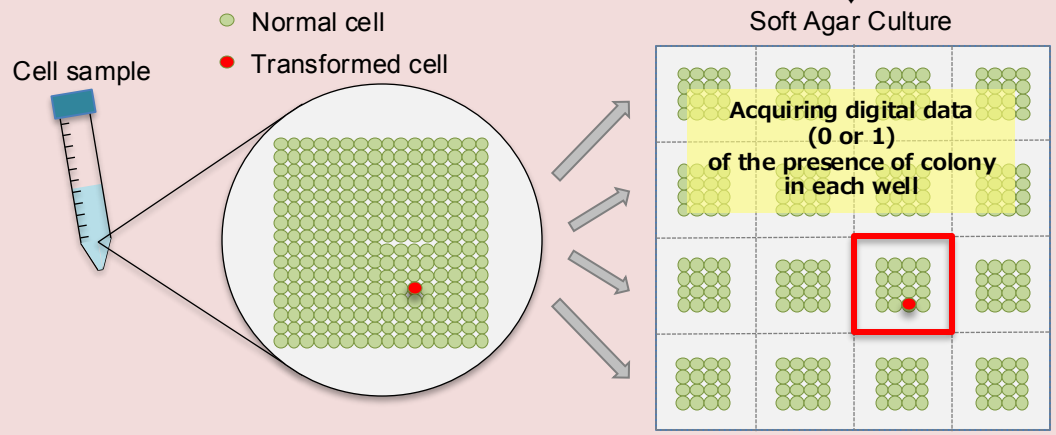
Malignant transformed cells
(= cancer cells) can grow without a scaffold,
resulting in colony formation.

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

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.

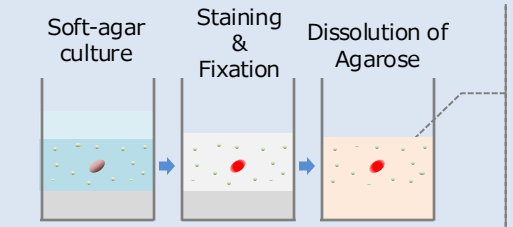
Low S/N ratio

↓

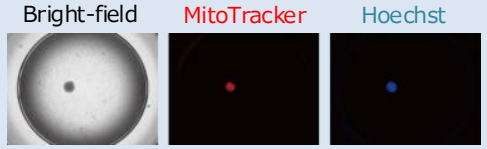
High S/N ratio

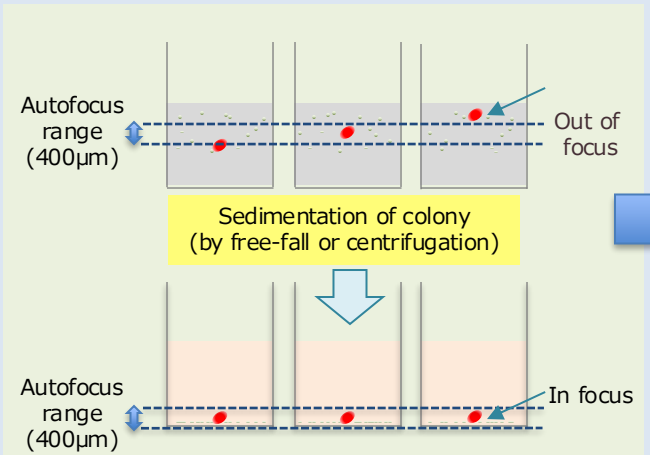
Procedures

Soft-agar culture & sample preparation

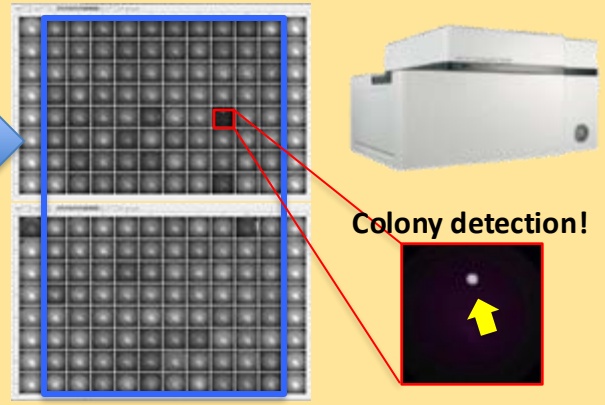


Post-sedimentation images (fluorescent images)



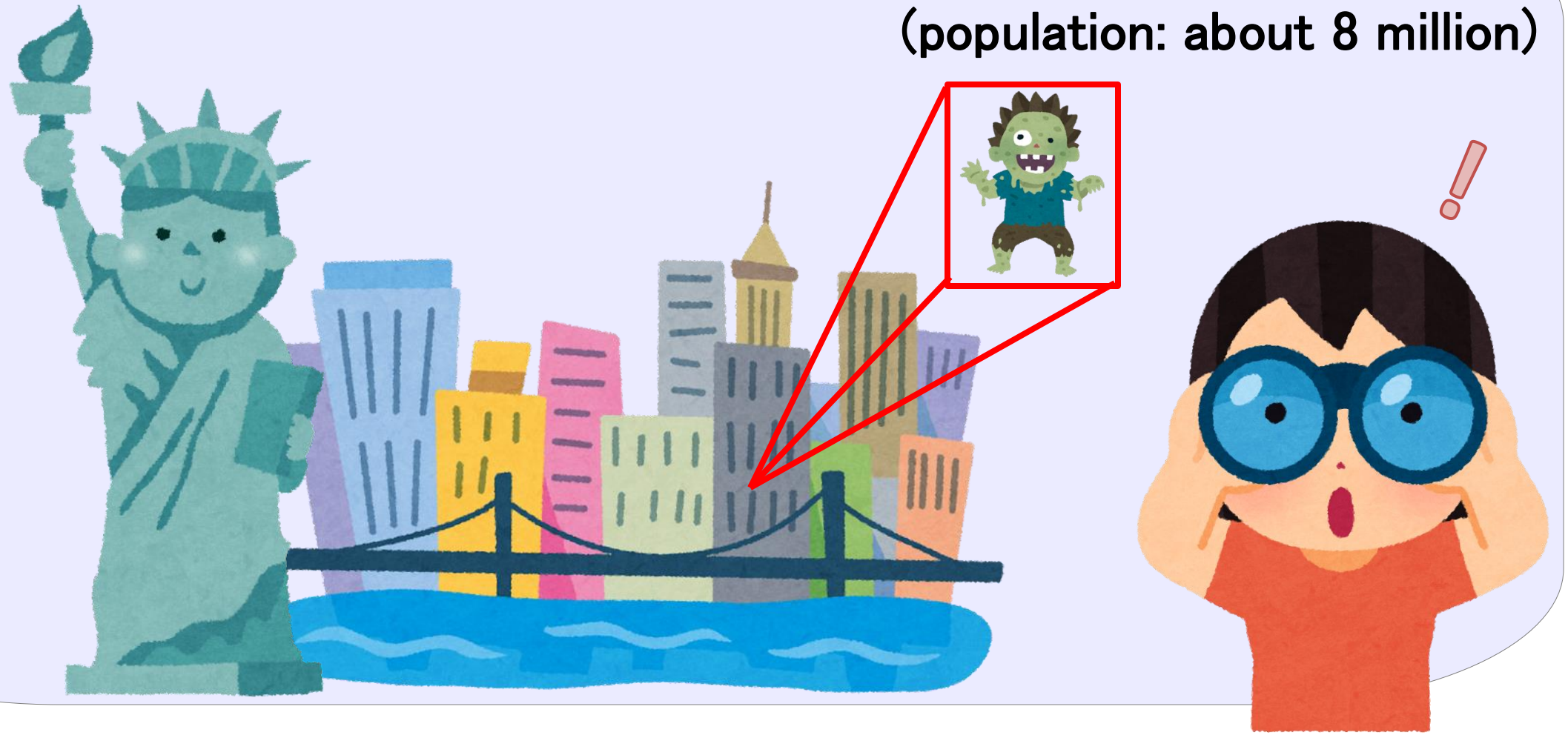


High-throughput screening of colony formation using an imaging cytometer



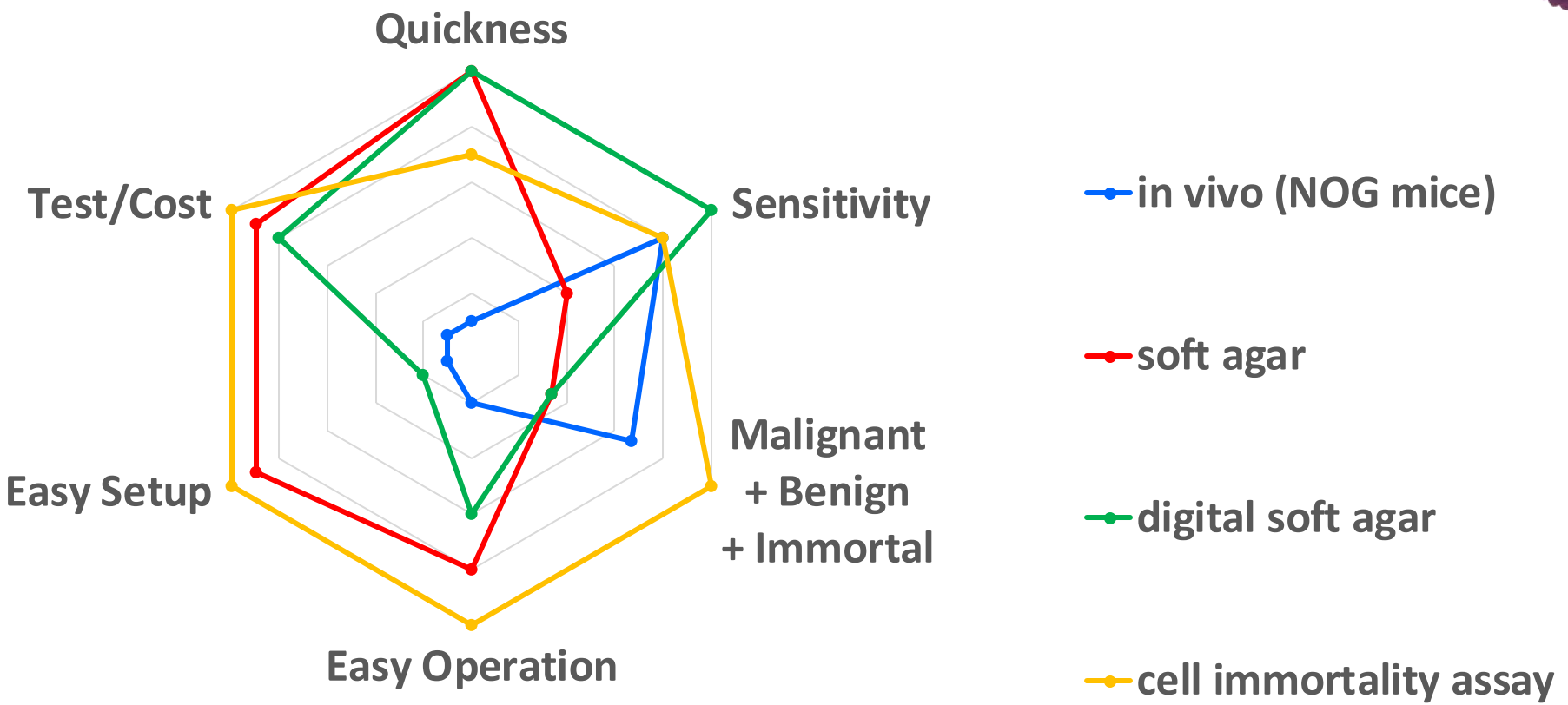
Digital Soft-Agar Colony Formation Assay has achieved the ability to detect cancer cells in normal cells at a ratio of 1 in 10 million

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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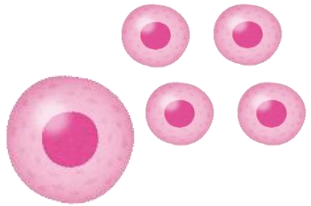
Development of Test Methods for Detection of Residual Undiffrentiated PSCs



Tumorigenic Cellular Impurities ↑
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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

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

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
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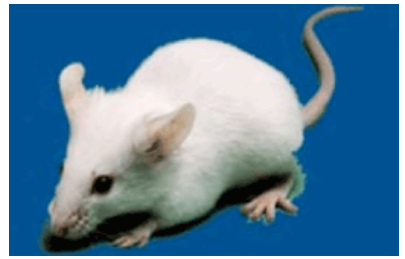

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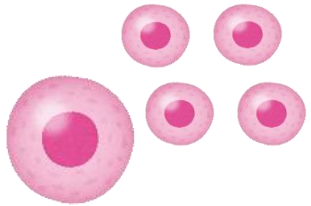

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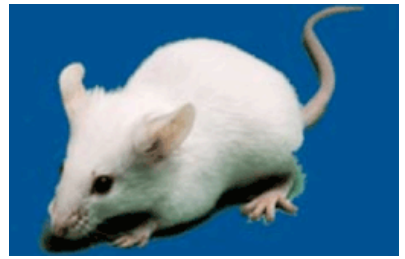
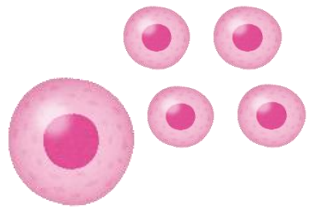


Highly-Efficient Culture (HEC) Assay

Example 2

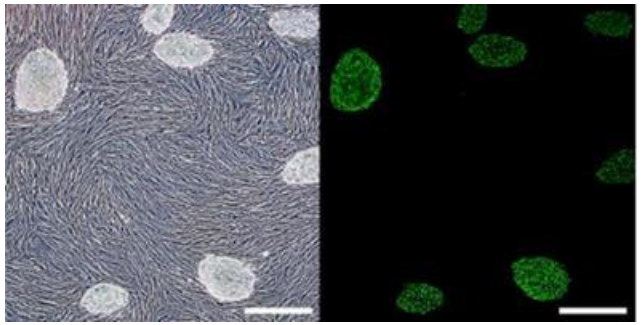
detects **residual undifferentiated pluripotent stem cells (PSCs)** in cell therapy products using highly efficient culture system which favors the growth of PSCs



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

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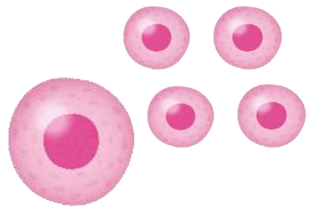

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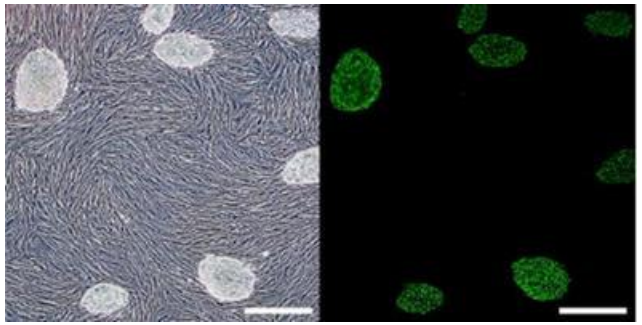

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Improvement of detection method for residual undifferentiated iPS cells (tumorigenic cells) in differentiated cells derived from human iPS cells

Cytotherapy 23 (2021) 176–183

Contents lists available at ScienceDirect

CYTOTHERAPY

journal homepage: www.isct-cytotherapy.org

International Society ISCT Cell & Gene Therapy®

ELSEVIER

FULL-LENGTH ARTICLE

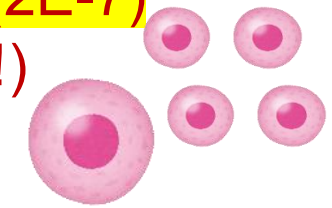
Regulatory Policies

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!!)



A B S T R A C T

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.

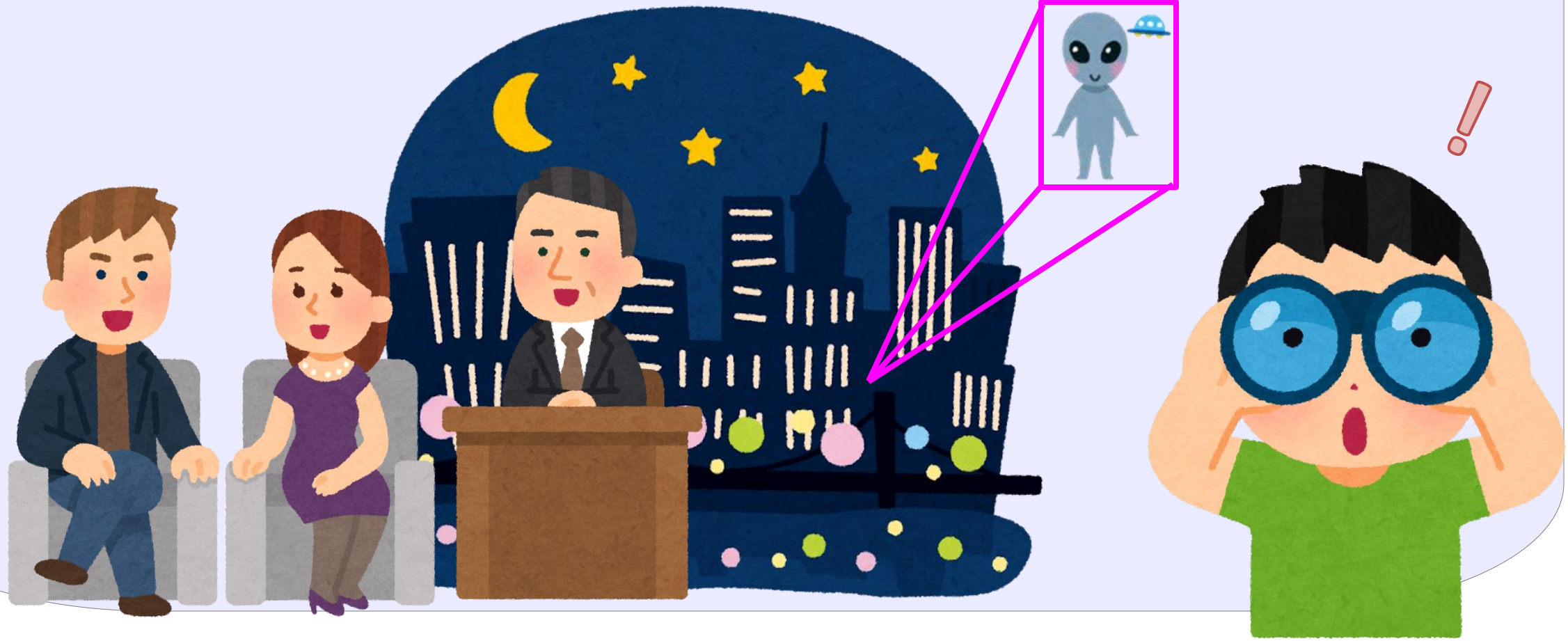
Methods: MEASURE first validated the detection sensitivity of HEC assay and then investigated the feasibility of magnetic-activated cell sorting (MACS) to improve sensitivity.

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.

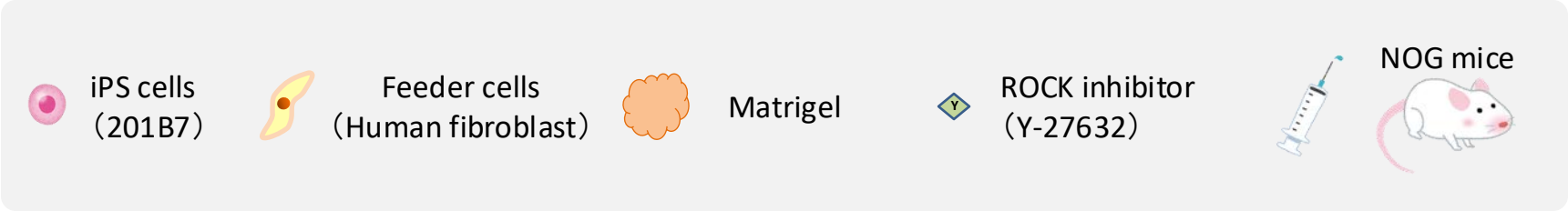
The improved Highly-Efficient Culture (HEC) Assay has achieved the ability to detect residual iPSCs in differentiated cells at a ratio of 1 in 5 million

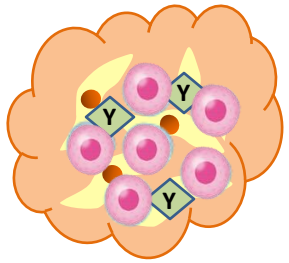
Comparable to the ability to find one alien in Los Angeles
(population: about 4 million)



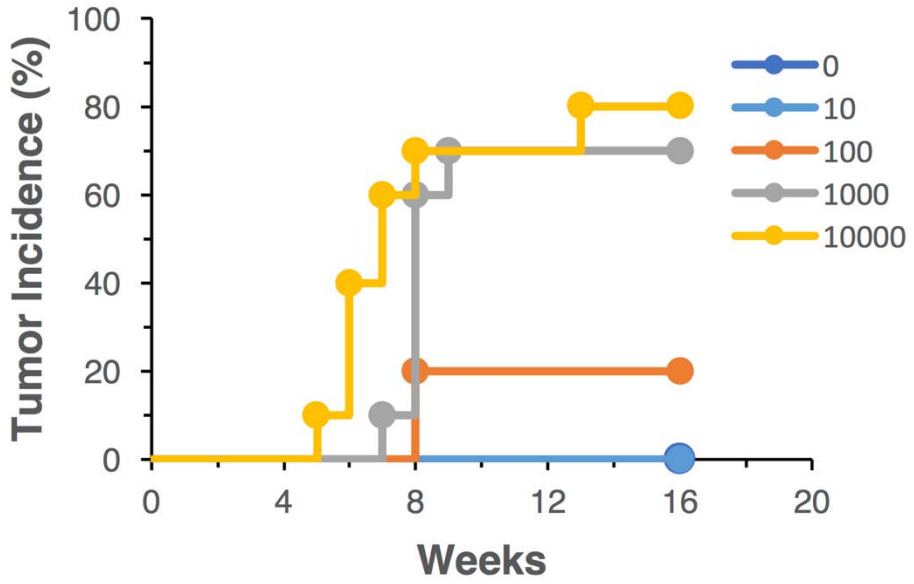
In vivo Tumorgenicity Test using NOG mice subcutaneously transplanted with iPSCs

Yasuda et al.,
PLoS One 2018





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.

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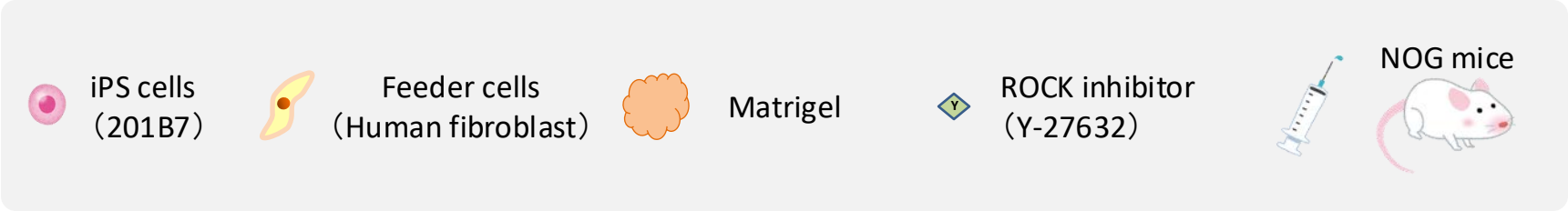

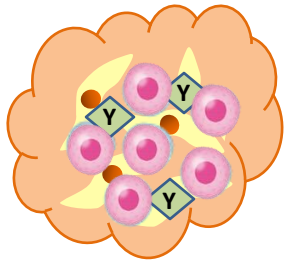

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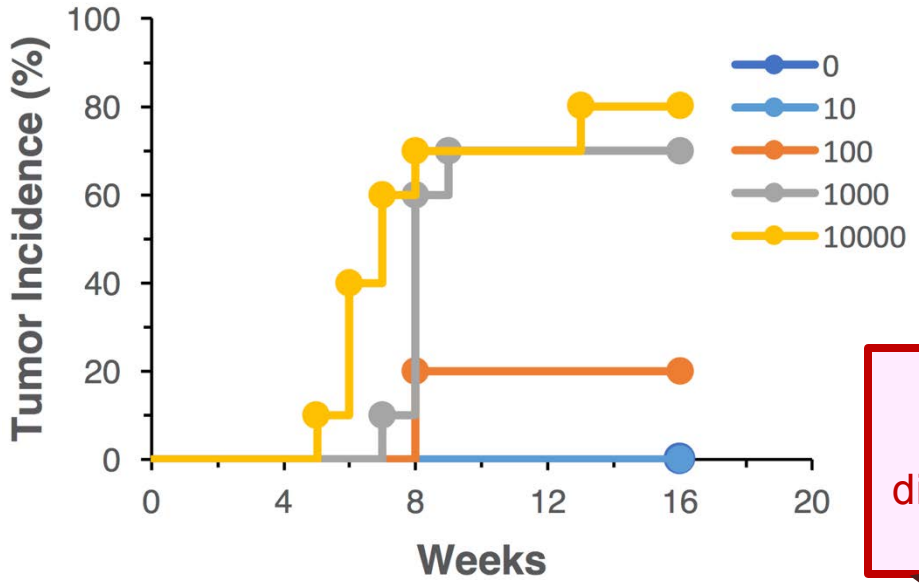

TPD_{50} = 631 cells

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

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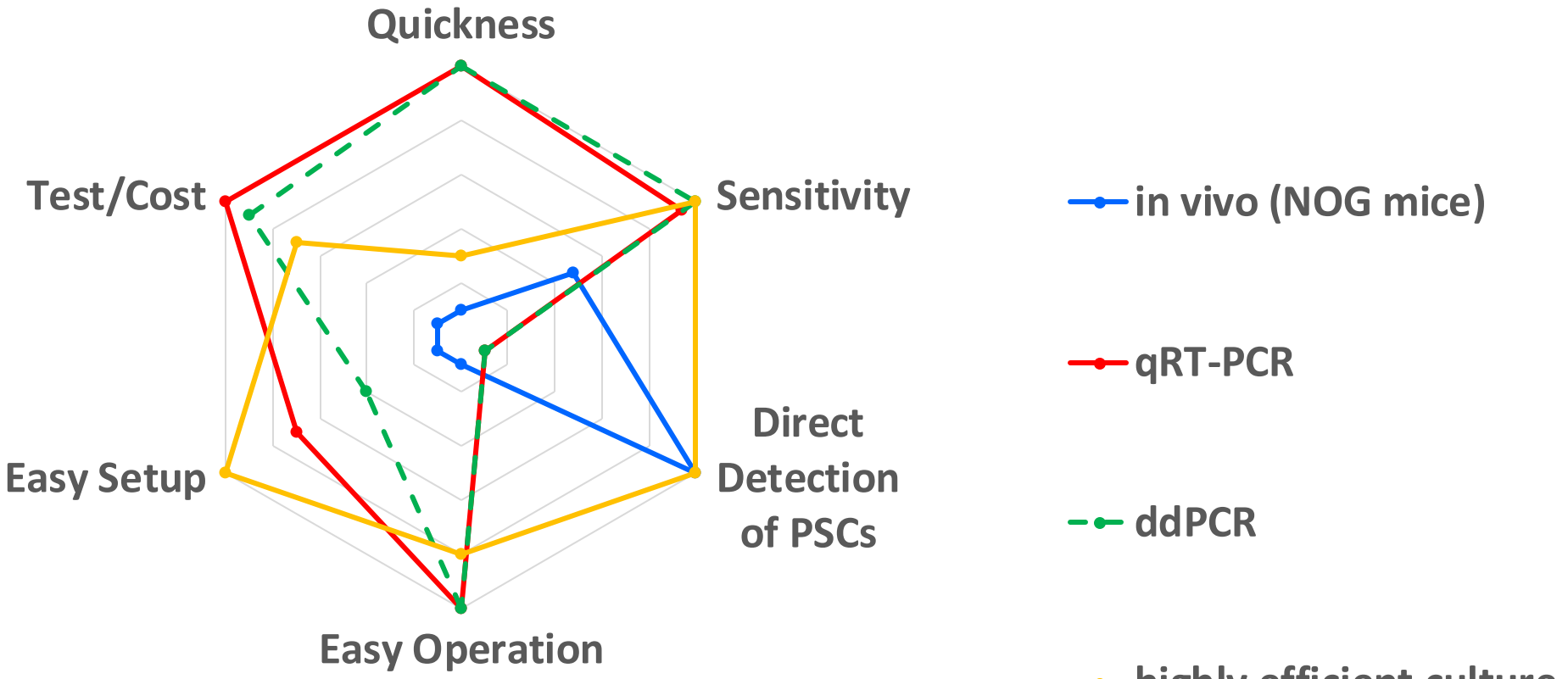
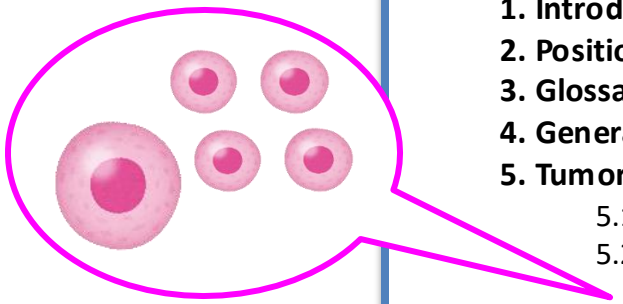
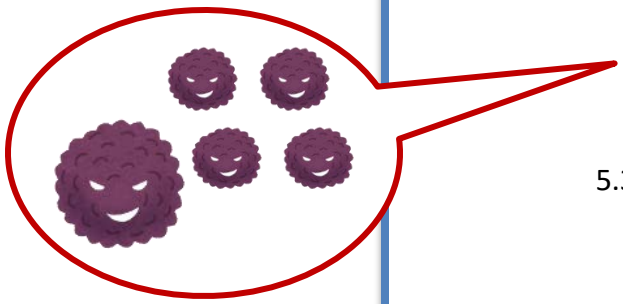
Qualitative Comparisons of Test Methods for Detection of Residual PSCs

(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)





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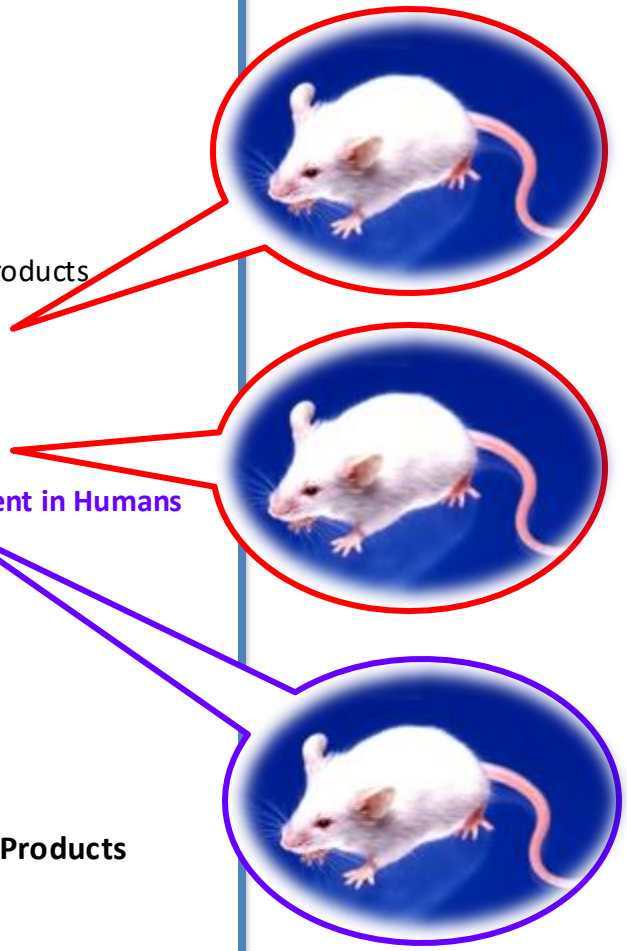
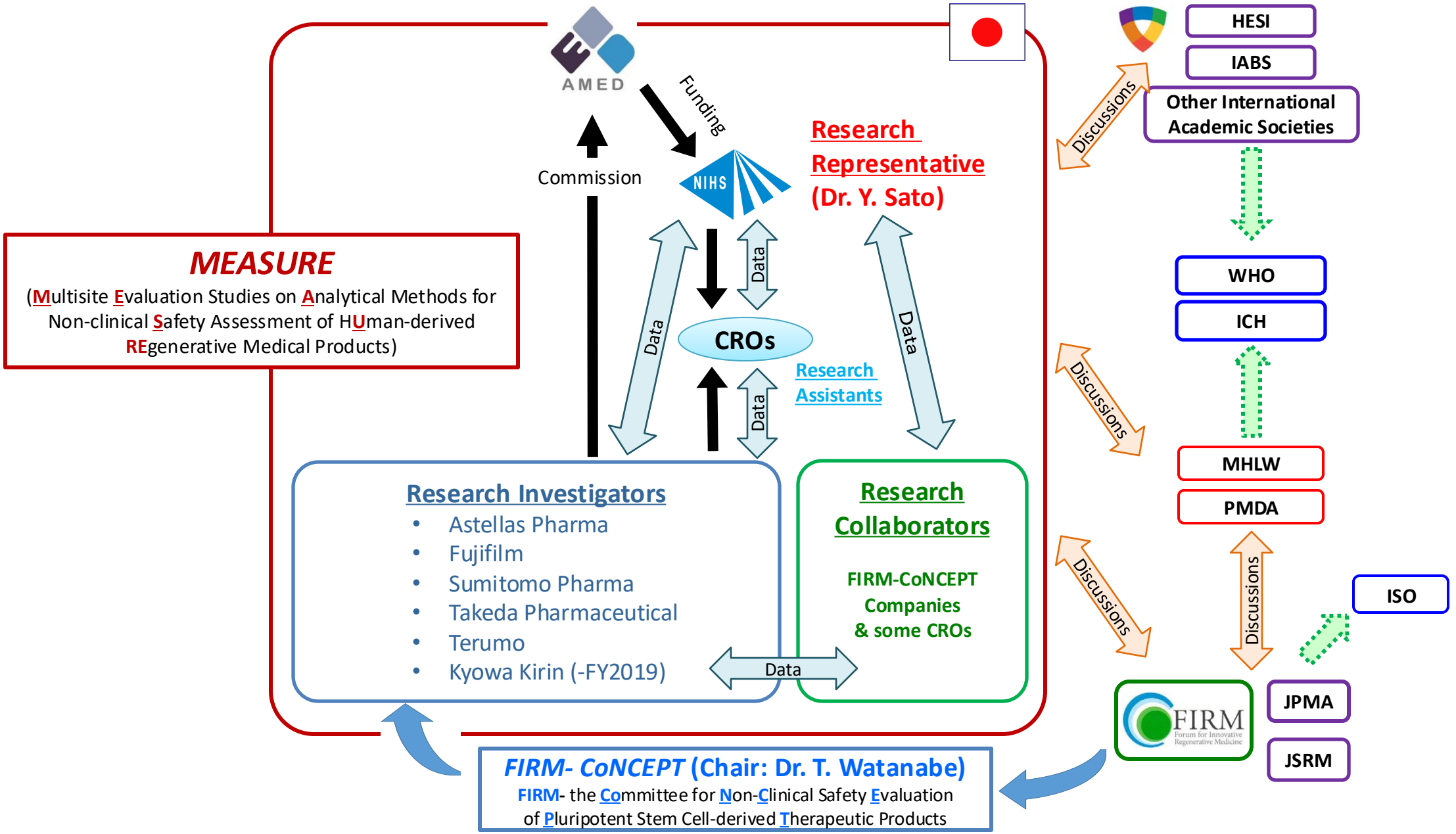


https://www.clea-japan.com/promotion/japanese/nextgenerationnog

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

MEASURE
(Multisite Evaluation Studies on Analytical Methods for Non-clinical Safety Assessment of HUman-derived REgenerative Medical Products)

AMED
Funding
Commission
NIHS
Data
Data
Data
CROs
Data

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

Research Collaborators
FIRM-CoNCEPT Companies & some CROs

MHLW
PMDA
Discussions
Discussions
ISO
FIRM Forum for Innovative Regenerative Medicine
JPMA
JSRM

***FIRM- CoNCEPT* (Chair: Dr. T. Watanabe)**
FIRM- the Committee for Non-Clinical Safety Evaluation of Pluripotent Stem Cell-derived Therapeutic Products

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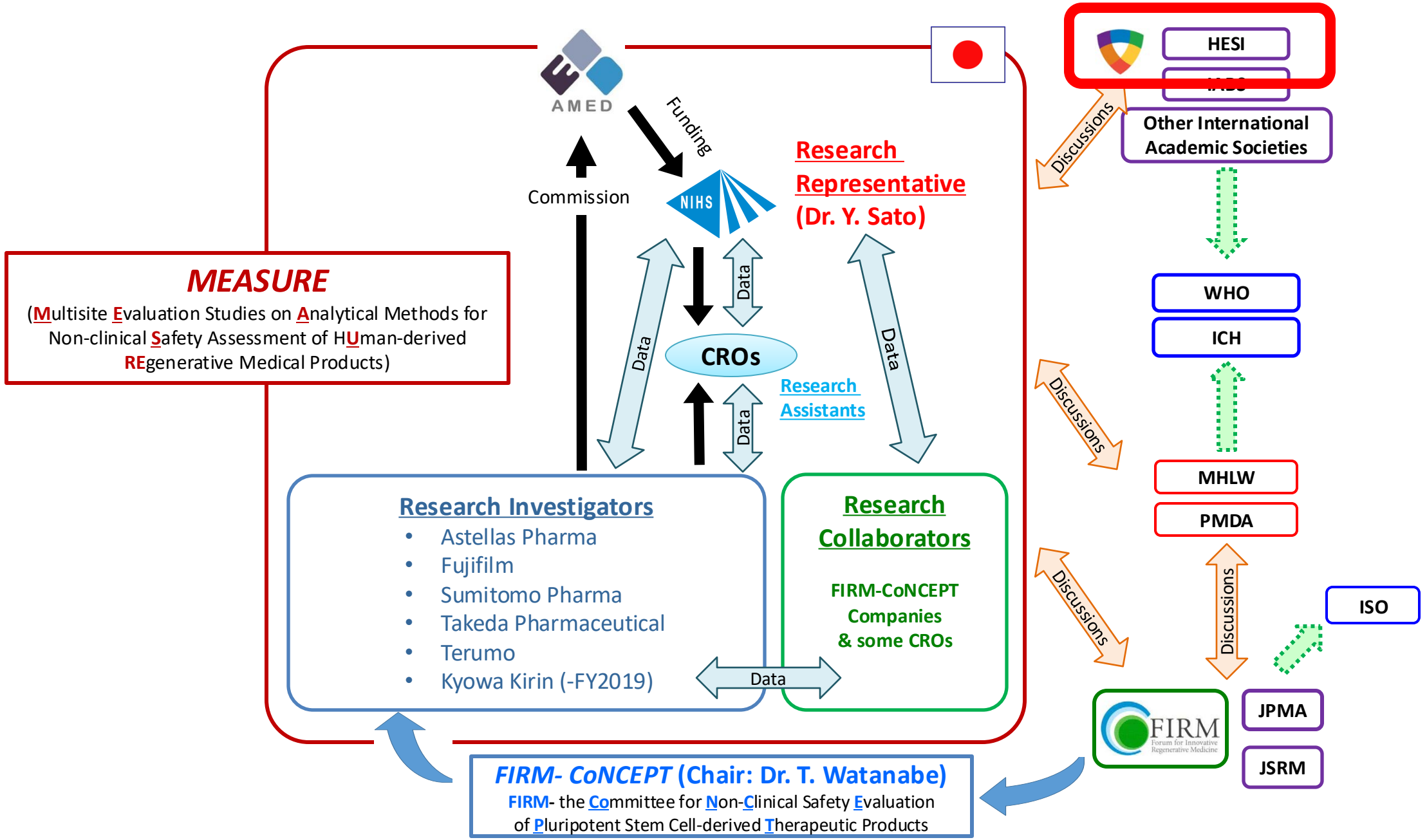

NGOs / Consortia:

CATAPULT Cell Therapy

eatris
European infrastructure for translational medicine

FIRM Forum for Innovative Regenerative Medicine

Universities/ Research Centers:

Universitäts Klinikum Freiburg

King's College London University of London

1884 Memorial Sloan Kettering Cancer Center

Newcastle University

The University Of Sheffield.

Stanford Cardiovascular Institute

UCL

Universiteit Leiden

Wageningen University & Research

The University of Sydney

HESI

CT-TRACS Members

(2022 data)

>100 Participants

>30 Organizations

Government & Regulatory bodies:

FDA

CBG MEB Medicines Evaluation Board

MHRA

NIH National Institutes of Health

NIHS

NIST National Institute of Standards and Technology

astellas Leading Light for Life

AstraZeneca

Athersys inc.

Bayer

Bristol Myers Squibb

Broken String Biosciences Unlocking future therapies

Cellular Dynamics international

Celsense

charles river

cytiva

MilliporeSigma

CRISPR Therapeutics

janssen

NOVARTIS

Roche

SANOFI

SONOMA BIOTHERAPEUTICS

Sumitomo Pharma

Takeda

TWINSTRAND BIOSCIENCES

VisiCELL MEDICAL

Courtesy of Dr. Lucilia Mouriès, HESI

- **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)

- Cell Tracking DB
- Education & Outreach
- Research *New in 2022!*

FIRM Forum for Innovative Regenerative Medicine

Collaboration with Japanese Consortium
FIRM-CoNCEPT & AMED MEASURE

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

- Multi-site studies for residual iPSCs
  - ddPCR
  - HEC assay
- Advanced sequencing for off target (CRISPR)
- IL-2 for CAR-T *New in 2022!*
- SACF/GILA (CRISPR) *New in 2022!*

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

Courtesy of Dr. Lucilia Mouriès, HESI

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Courtesy of Dr. Lucilia Mouriès, HESI

Position Paper of HESI CT-TRACS Tumorigenicity WG

Addressing Challenges & Needs

Cytotherapy. 2019;**21:**1095-1111

Cytotherapy, 2019; 21: 1095–1111

International Society ISCT Cell & Gene Therapy

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 MOURIÈS[11], M. SHARPE[7], W. SHINGLETON[12,†], B. SURMACZ-CORDLE[7], K. YAMAMOTO[13] & J.W. VAN DER LAAN[3,*]

[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

Multi-site Validation Studies by HESI CT-TRACS and MEASURE Consortium (FIRM-CoNCEPT & NIHS) on Test Methods for Tumorigenicity Assessment of Cell Therapy Products

- Yasuda S, Bando K, Henry MP, Libertini S, Watanabe T, Bando H, Chen C, Fujimori K, Harada K, Kuroda T, Lemmens M, Marginean D, Moss D, Mouriès LP, Nicholas N, Smart MJK, Terai O, Sato Y. Detection of residual pluripotent stem cells in cell therapy products utilizing **droplet digital PCR**: an international multisite evaluation study. ***Stem Cells Translational Medicine***. 2024 (in press)
- Watanabe T, Yasuda S, Kusakawa S, Kuroda T, Furukawa H, Futamura M, Shimizu S, Morishita A, Hata S, Koeda A, Komatsu K, Sato Y. Multisite studies for optimization of a **highly efficient culture assay** used for in vitro detection of residual undifferentiated human pluripotent stem cells intermingled in cell therapy products. ***Regenerative Therapy***. 2024 (in press)
- Bando K, Kusakawa S, Adachi H, Yamamoto M, Iwata M, Kitanaka A, Ogimura E, Osada T, Tamura M, Terai O, Watanabe T, Yoda T, Yotsumoto T, Zaizen K, Sato Y. Protocol improvement and multisite validation of a **digital soft agar colony formation assay** for tumorigenic transformed cells intermingled in cell therapy products. ***Cytotherapy***. 2024 (in press)
- Watanabe T, Yasuda S, Chen CL, Delsing L, Fellows M, Foldes G, Kusakawa S, Pereira Mouriès L, Sato Y. International evaluation study of an **HEC assay** for detection of residual human pluripotent stem cells in the products. ***Regenerative Medicine***. 2023;18:219-227.
- Hirai T, Kono K, Kusakawa S, Yasuda S, Sawada R, Morishita A, Hata S, Wakita A, Kageyama T, Takahashi R, Watanabe S, Shiraishi N, Sato Y. Evaluation of the reproducibility and positive controls of **cellular immortality test** for the detection of immortalized cellular impurities in human cell-processed therapeutic products. ***Regenerative Therapy***. 2022;21:540-546.
- Watanabe T, Yasuda S, Kusakawa S, Kuroda T, Futamura M, Ogawa M, Mochizuki H, Kikkawa E, Furukawa H, Nagaoka M, Sato Y. MEASURE: Multisite studies for validation and improvement of a **highly efficient culture (HEC) assay** for detection of undifferentiated human pluripotent stem cells intermingled in cell therapy products. ***Cytotherapy***. 2021;23:176-183
- Kamiyama Y, Naritomi Y, Moriya Y, Yamamoto S, Kitahashi T, Maekawa T, Yahata M, Hanada T, Uchiyama A, Noumaru A, Koga Y, Higuchi T, Ito M, Komatsu H, Miyoshi S, Kimura S, Umeda N, Fujita E, Tanaka N, Sugita T, Takayama S, Kurogi A, Yasuda S, Sato Y. **Biodistribution studies** for cell therapy products: Current status and issues. ***Regenerative Therapy***. 2021;18:202-216.
- Sato Y, Bando H, Di Piazza M, Gowing G, Herberts C, Jackman S, Leoni G, Libertini S, MacLachlan T, McBlane JW, Pereira Mouriès L, Sharpe M, Shingleton W, Surmacz-Cordle B, Yamamoto K, van der Laan JW. Tumorigenicity assessment of cell therapy products: The need for **global consensus and points to consider**. ***Cytotherapy***. 2019;21:1095-1111.

AGENDA

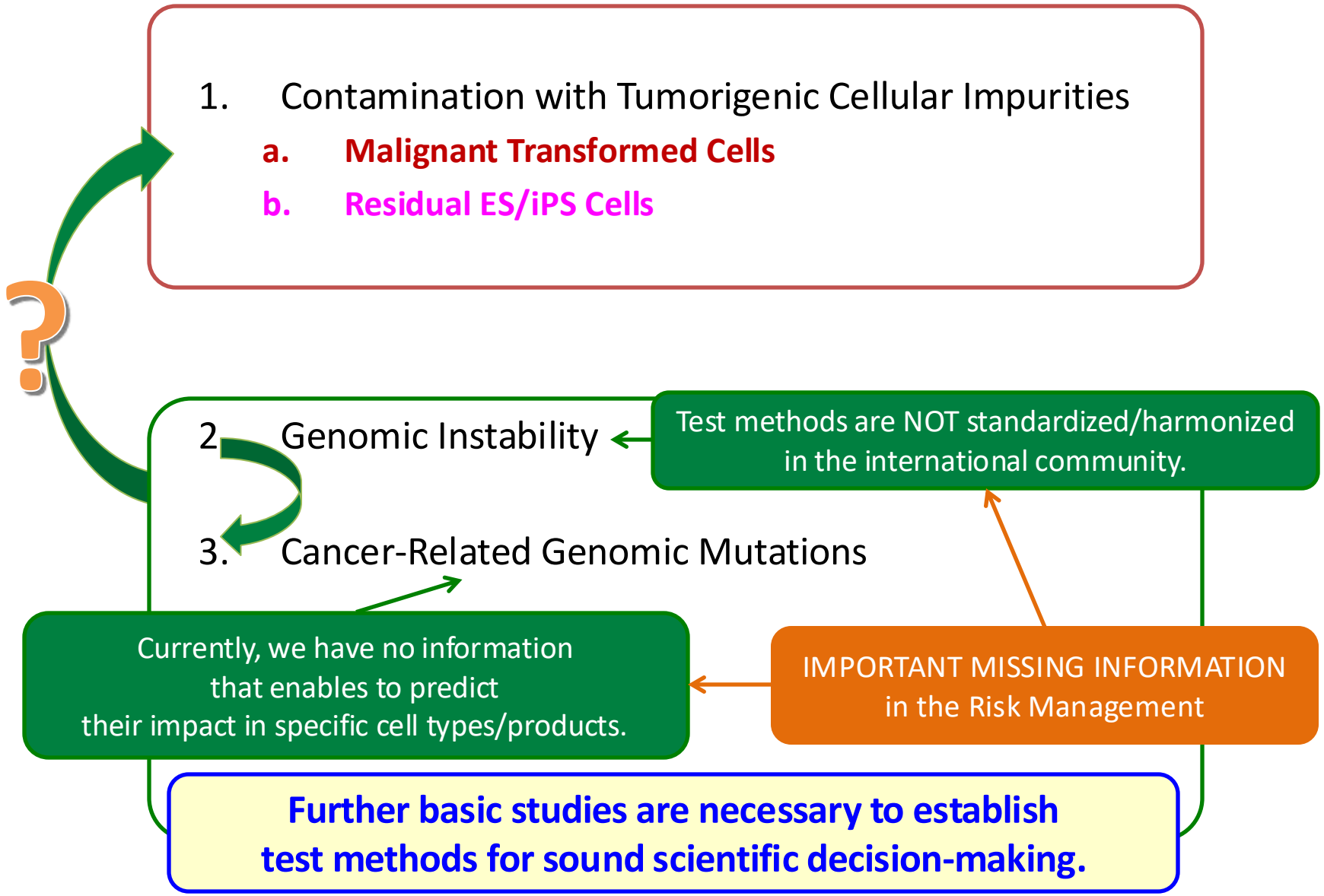
1. What is tumorigenicity? –The risk of tumorigenesis and its hazards–

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



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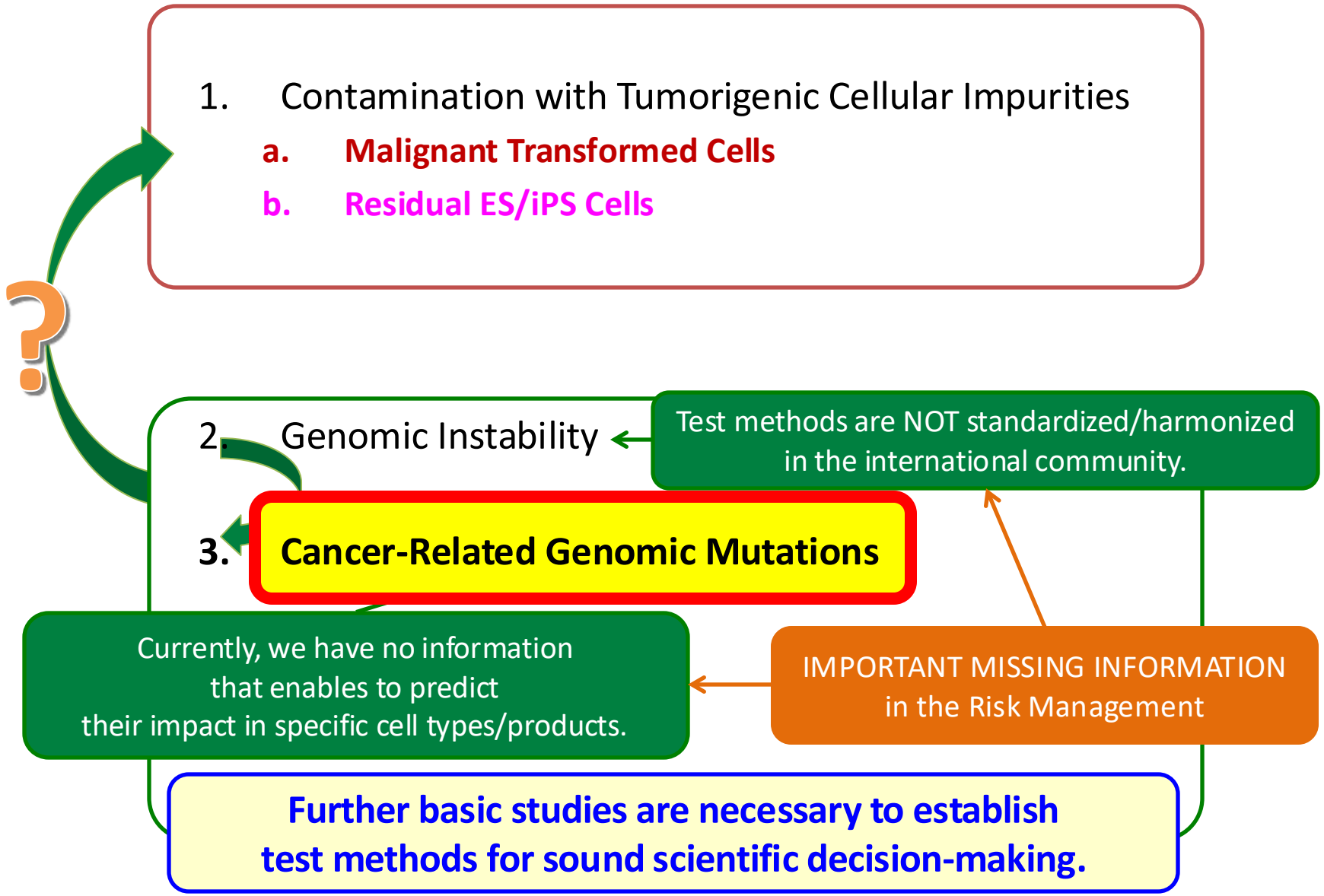

The human body is a mosaic of different genomes

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

Nature (NEWS on 06 June 2019)

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

RESEARCH ARTICLE

RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues

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

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

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

[3]Harvard Medical School, Boston, MA, USA.

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

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

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

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

*Corresponding author. Email: gadgetz@broadinstitute.org

– Hide authors and affiliations

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

The human body is a mosaic of different genomes

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

Nature (NEWS on 06 June 2019)

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

RESEARCH ARTICLE

RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues

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

[1]Broad Institute of MIT and Harvard, Cambridge, MA, USA.
[2]Center for Cancer Research, Massachusetts General Hospital, Boston, MA, USA.
[3]Harvard Medical School, Boston, MA, USA.
[4]Ocular Genomics Institute, Department of Ophthalmology, Massachusetts Eye and Ear, Boston, MA, USA.
[5]Biorepositories and Biospecimen Research Branch, Cancer Diagnosis Program, National Cancer Institute, Bethesda, MD, USA.
[6]Oncological Sciences, Icahn School of Medicine at Mount Sinai Hospital, New York, NY, USA.
[7]Department of Pathology, Massachusetts General Hospital, Boston, MA, USA.
*Corresponding author. Email: gadgetz@broadinstitute.org
Hide authors and affiliations

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…means "we currently have no way"

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Cristian Tomasetti, Johns Hopkins Medicine

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

MEXT

MHLW PMDA

AMED

Cancer-related SNVs (HiSeq 2500 / NovaSeq 6000)

Data feed back

Differentiation resistance

PCR for factors

WGS

reprogramming

primary cell

② Discuss required QC

④ Share data

NIHS Y.Sato

G-band SNV/Indel, CNV Methylation

PI:FBRI Kawamata

CiRA N.Takasu

iPSC supplier

① Share the data

Draft of Safety Guideline

Cell from donor

iPSC

Diff. cell

③ Share the data

Differentiation

In vivo tumorigenicity test

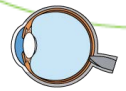
CNVs (KaryoStat)

FBRI S.Kawamata

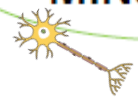
Riken CBD M.Takahashi

Keio Univ M.Nakamura

CM



RPE



NSC

QC test

QC test + clinical protocol

Courtesy of Dr. Kawamata with some modifications

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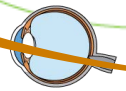

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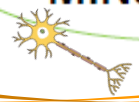

NSC

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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 / Expectancy		SNV(-) Normal	SNV(+) Abnormal	Discriminative ratio	Overall predictability
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 / Expectancy		CNV(-) Normal	CNV(+) Abnormal	Discriminative ratio	Overall predictability
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	

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

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

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

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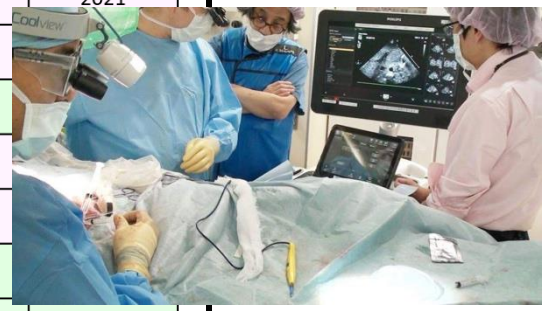
https://nd.natureasia.com/figure/4438/56992/phone/1

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

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



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

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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I would like to express my sincere appreciation to:

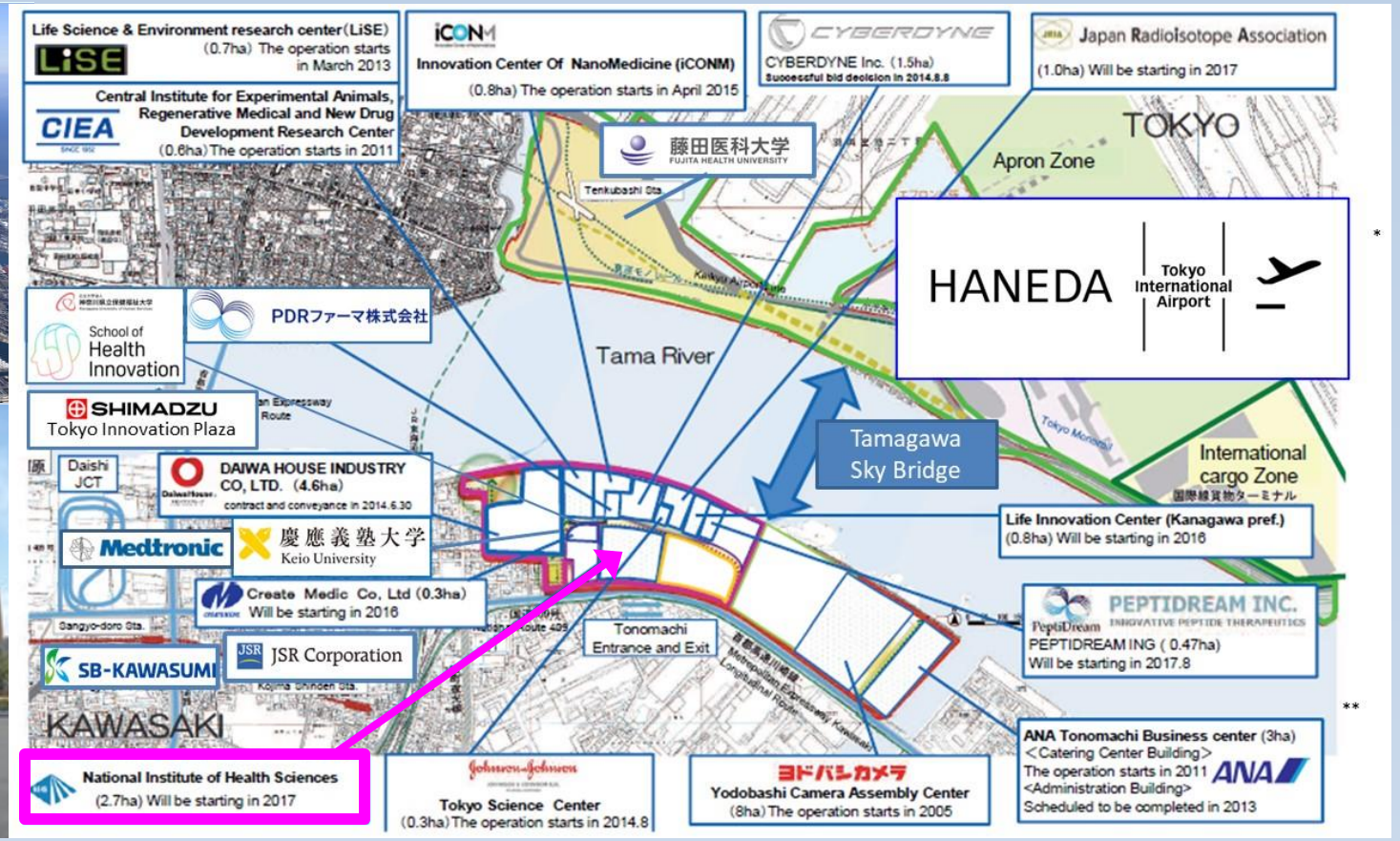
- The member companies of the Committee for Non-Clinical Safety Evaluation of Pluripotent Stem Cells-derived Therapeutic Products, the Forum for Innovative Regenerative Medicine (FIRM-CoNCEPT)
- The member companies of the Japan Association of Contract Laboratories for Safety Evaluation (JACL) and the other Japanese companies that participated in MEASURE 1 or MEASURE 2 Projects
- Global public and private sector organizations that are participating or participated in HESI CT-TRACS joint research
- Our collaborators in the AMED Research Project for Regulatory Harmonization and Evaluation of Medical Products
- Our collaborators (Dr. Shin Kawamata, etc.) in the AMED Research Project for Practical Application of Regenerative Medicine
- The Secretariat of the Forum for Innovation in Regenerative Medicine (FIRM)
- AMED Regulatory Science Division and Regenerative Medicine R&D Division
- PMDA Regenerative Medicine Products Review Division
- The Medical Device Review and Management Division, Ministry of Health, Labour and Welfare (MHLW),

and

- All of my excellent and hard-working colleagues at the Division of Cell-Based Therapeutic Products, National Institute of Health Sciences

Thank you for your attention!

Yoji SATO, Ph.D.
Head, Division of Drugs
National Institute of Health Sciences
3-25-26 Tonomachi, Kawasaki Ward, Kawasaki City 210-9501, Japan
E-mail: yoji@nihs.go.jp



* https://www.oag.com/hubfs/air-canada-787.jpg
** http://www.city.kawasaki.jp/en/page/0000038680.html