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2024 iPSC Research, Clinical Application, and Regulatory Considerations

Regulatory Considerations in Japan for Ensuring the Quality and Safety of iPSC-derived Products

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DISCLAIMER

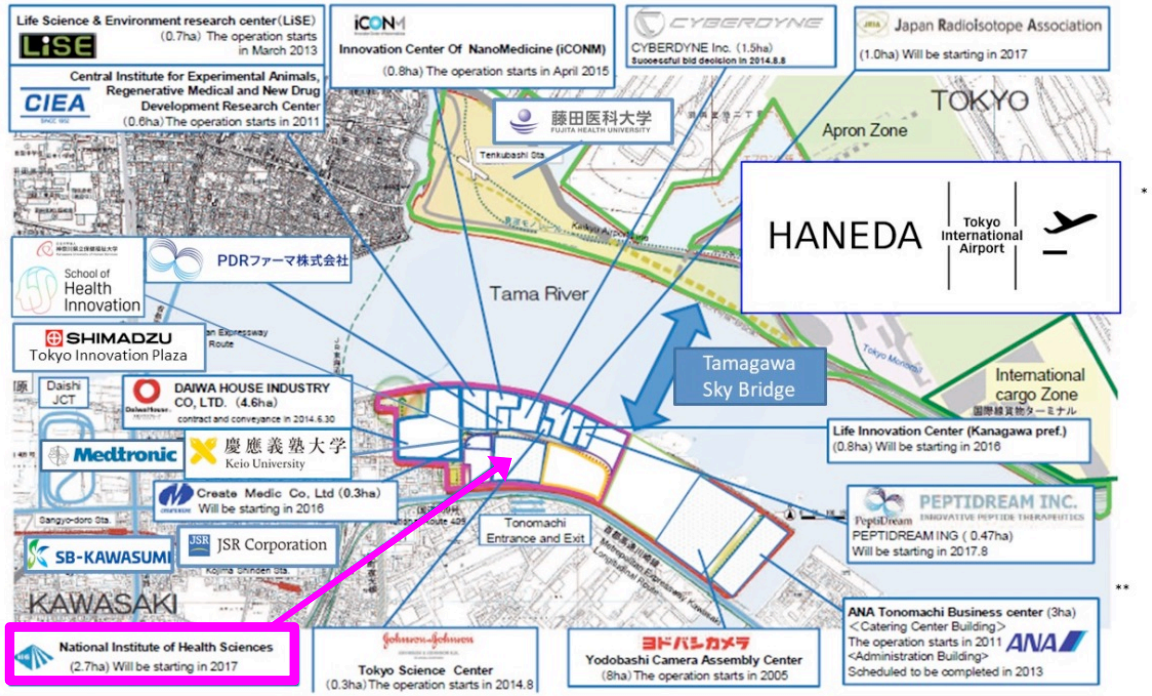
The views and opinions expressed in this presentation are those of the presenter and do not necessarily represent the official policy or position of the Japanese National Institute of Health Sciences or the Japanese Ministry of Health, Labour & Welfare. Also, the presenter has no COI to disclose in connection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

National Institute of Health Sciences

This year marks its 150th anniversary.

- **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**

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



* 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**.

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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AGENDA (1)

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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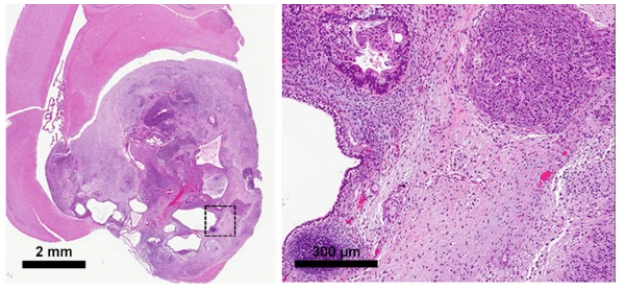
Tumorigenicity

… 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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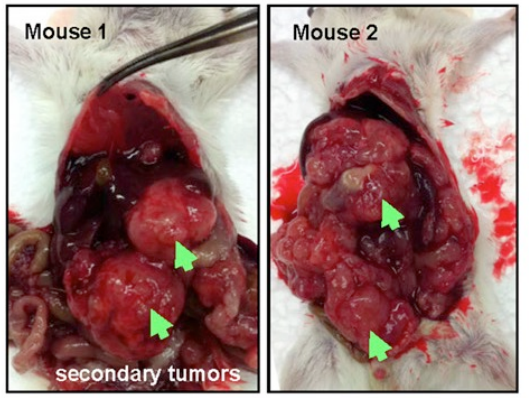
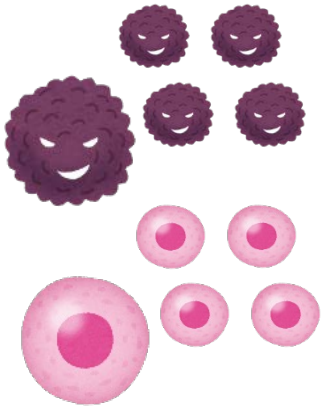


MOUSTAFA M et al. STEM CELLS TRANSLATIONALMEDICINE 2016;5:694–702

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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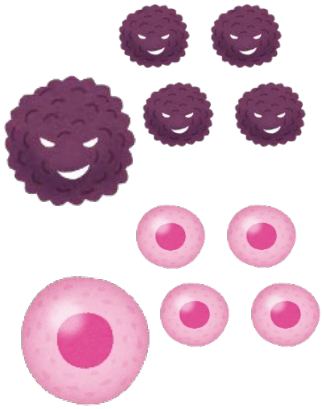

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in vitro (or in vivo) tests

- 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

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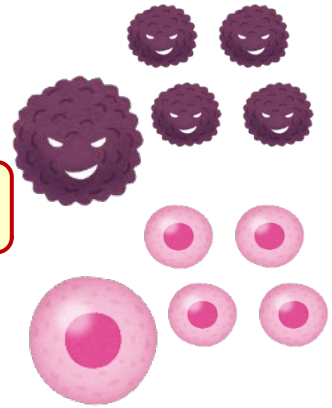

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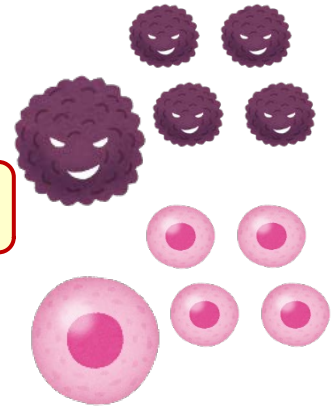

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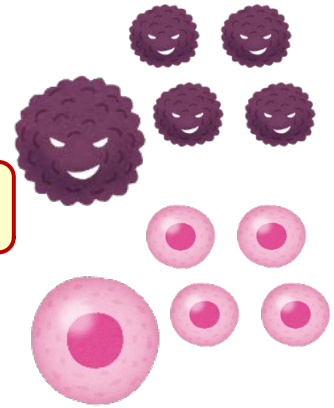

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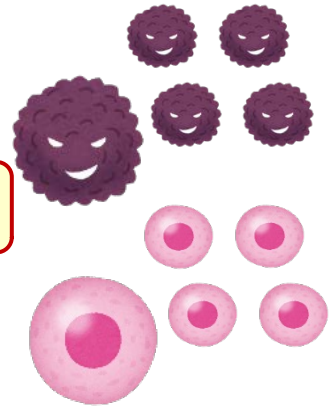

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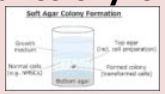
Development of Test Methods for Detection of Transformed Cells

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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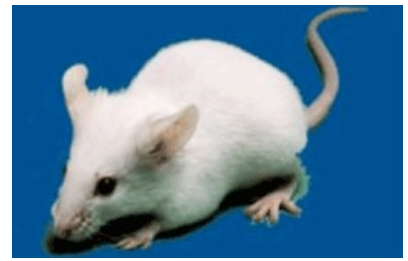

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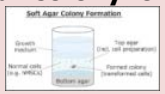
Example 1

In Vitro Assays

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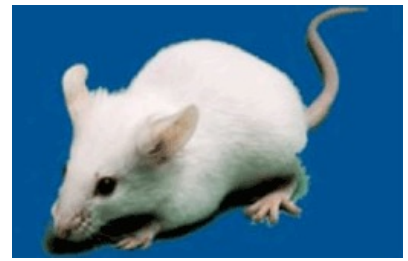
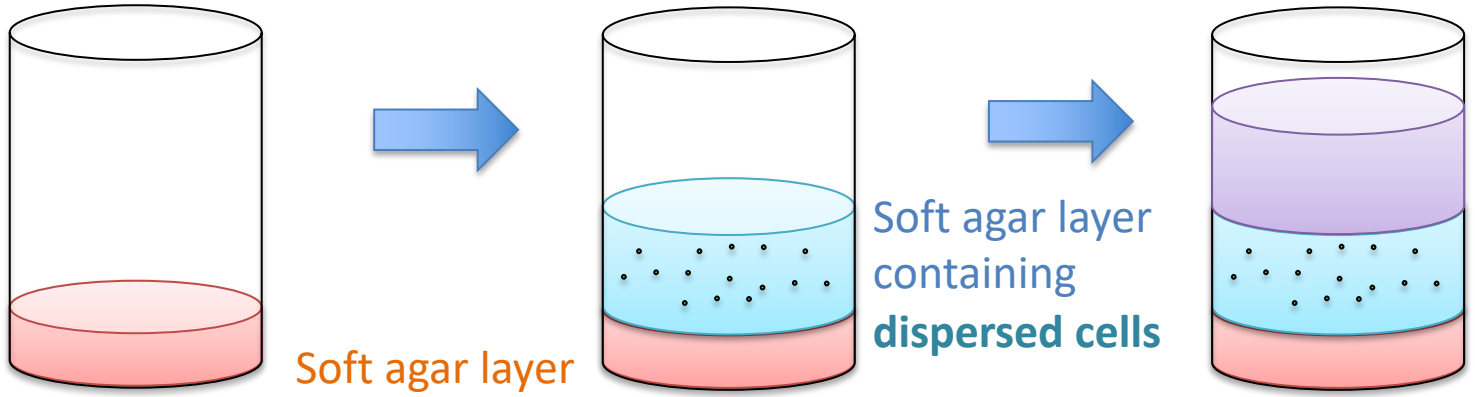


Conventional Soft Agar Colony Formation Assay

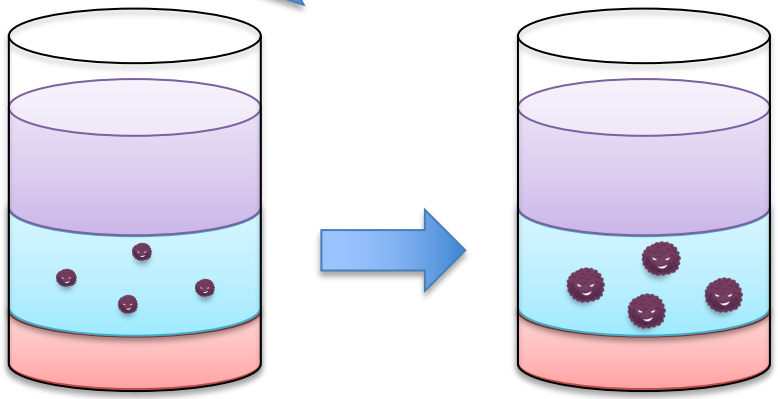
Purpose: Detection of scaffold-independent proliferation (= **malignant transformed cells**)



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



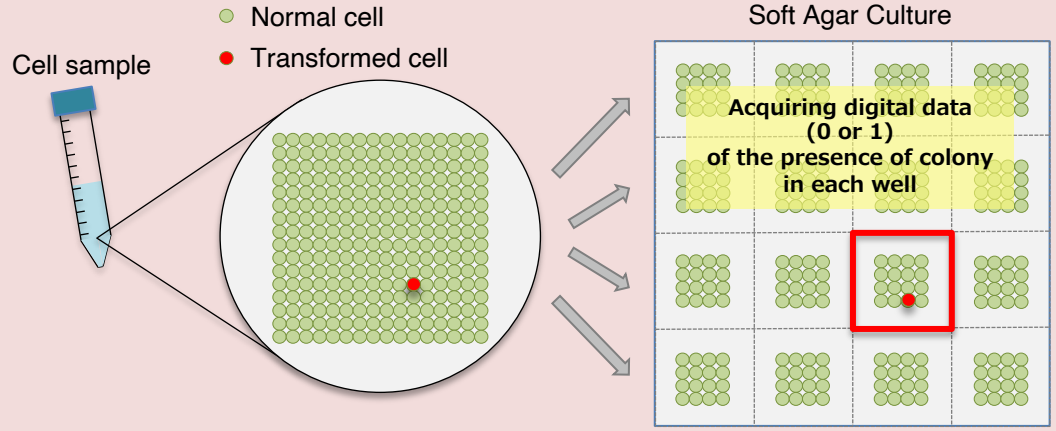
Malignant transformed cells
(= cancer cells) can grow without a scaffold,
resulting in colony formation.

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.

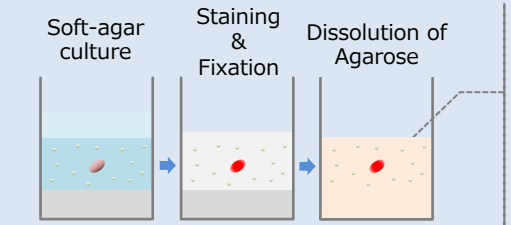
Low S/N ratio

↓

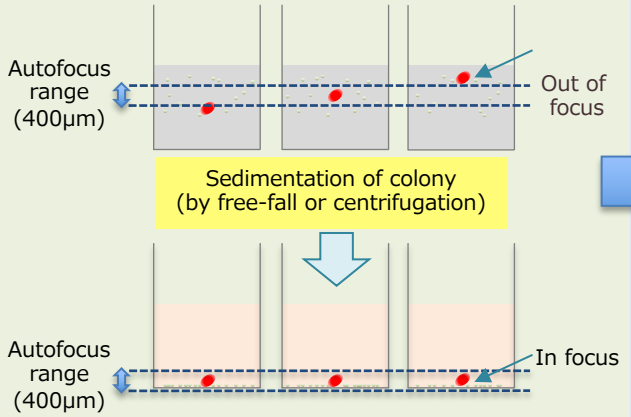
High S/N ratio

Procedures

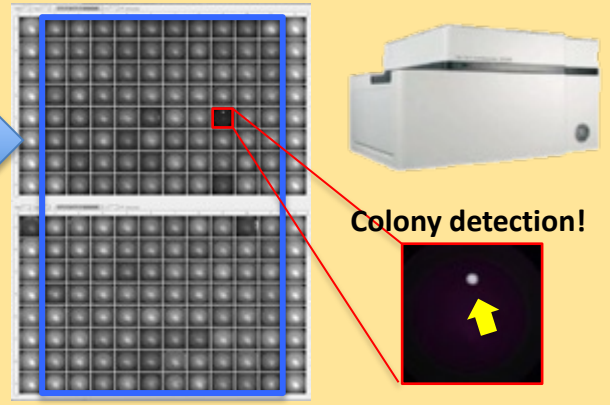
Soft-agar culture & sample preparation



Post-sedimentation images
(fluorescent images)

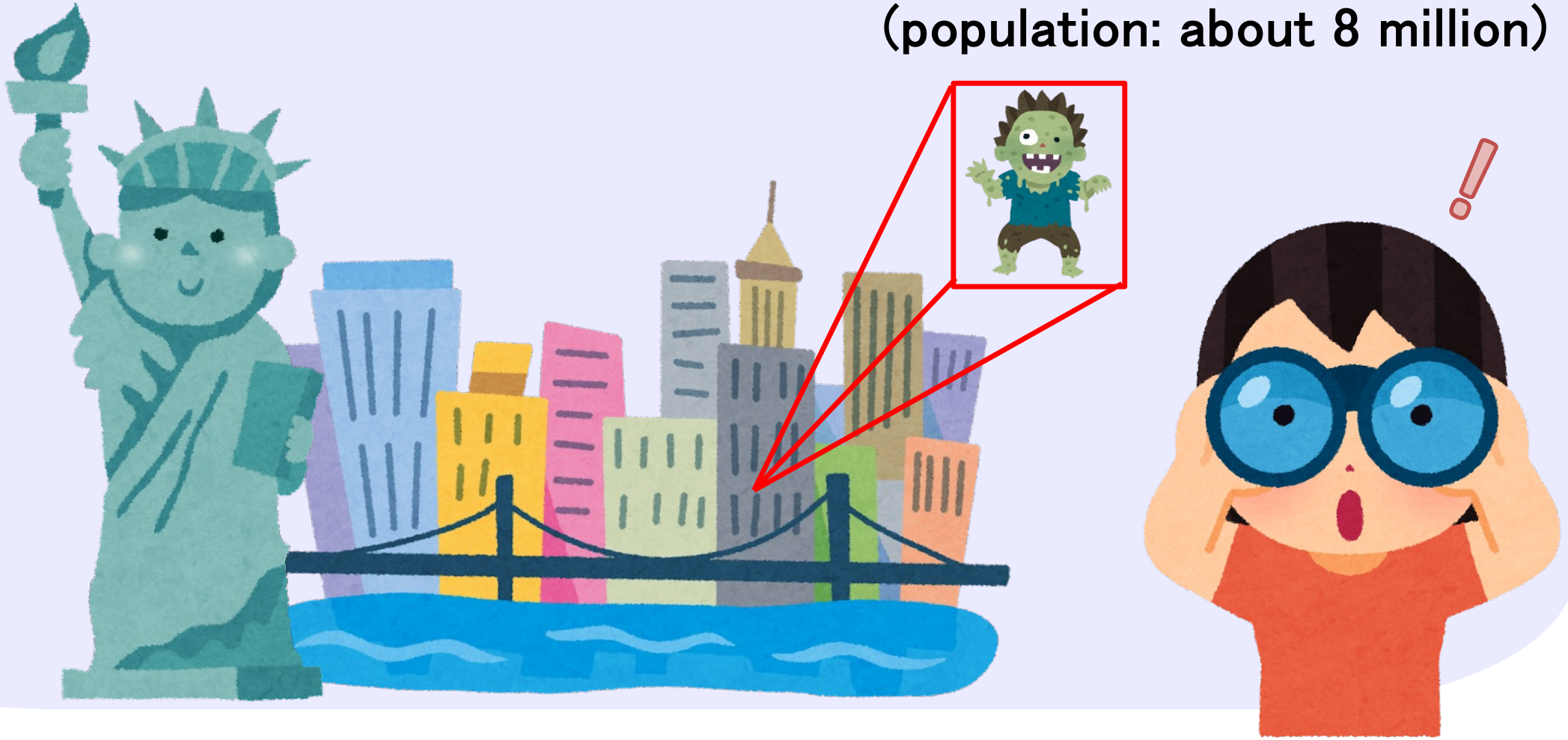


High-throughput screening of colony formation using an imaging cytometer



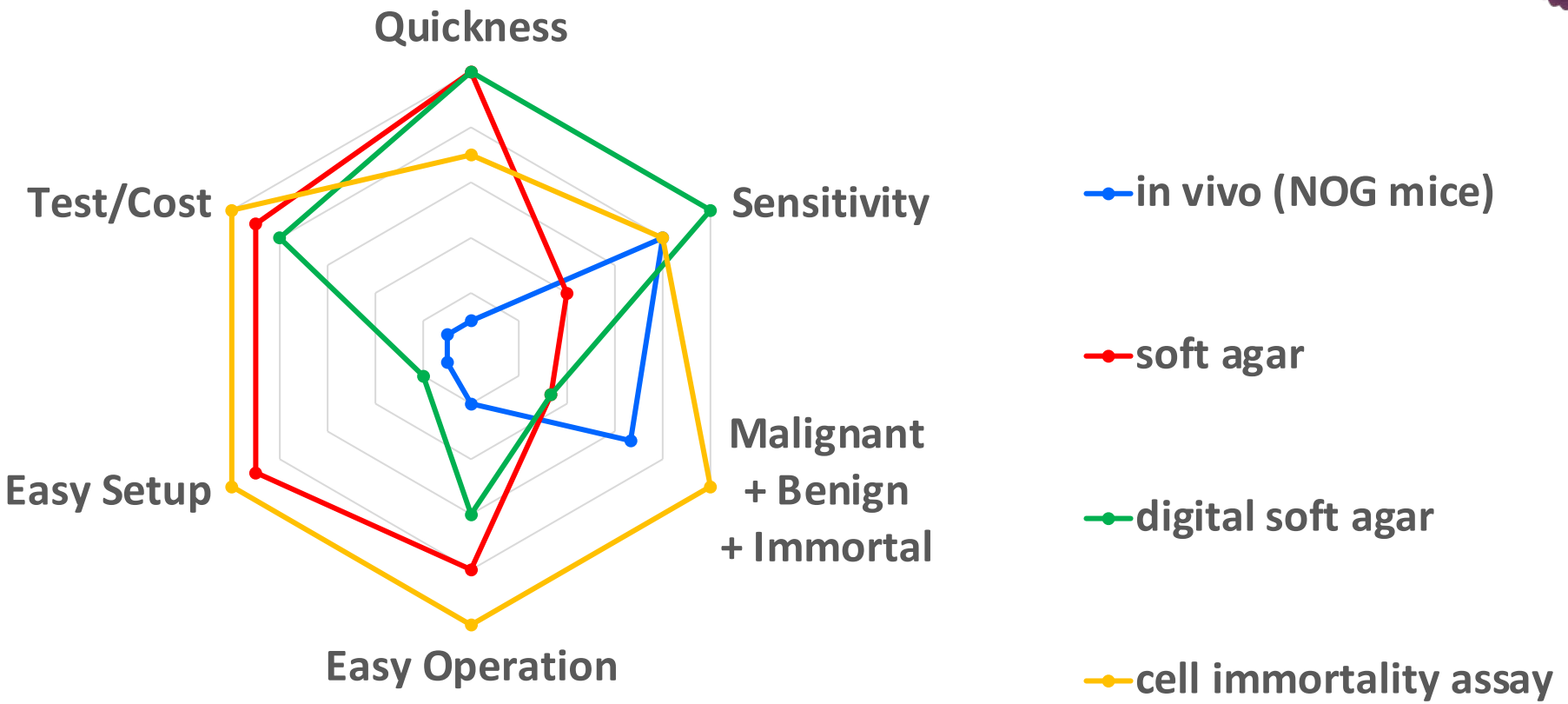
Digital Soft-Agar Colony Formation Assay has achieved the ability to detect cancer cells in normal cells at a ratio of 1 in 10 million

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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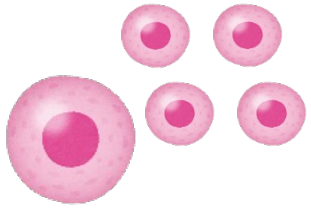
Development of Test Methods for Detection of Residual Undiffrentiated PSCs



Tumorigenic Cellular Impurities ↑
=Hazards of PSC-Derived Products

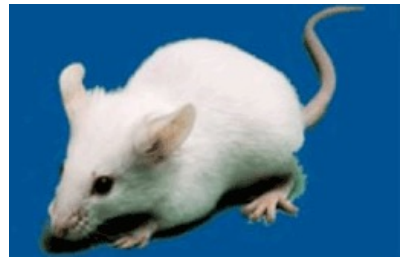
In Vitro Assays

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

*: 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
Reference	Kanemura et al., Sci Rep. 2013; Kawamata et al., J Clin Med. 2015



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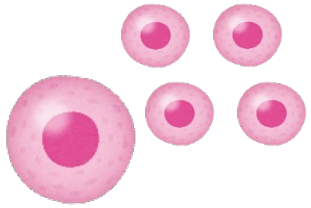


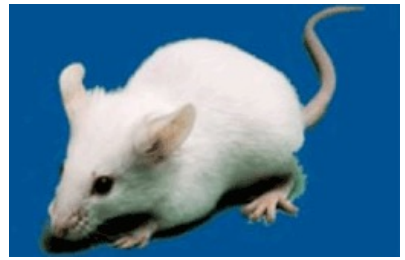
In Vitro Assays

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

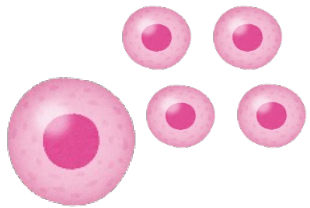
Assays/Platform	Tumorigenicity Test
Animals	NOG mice
Route	Subcutaneous transplantation
Positive control	iPS cells
Duration	17-30 weeks
Pros	Direct evaluation in micro environment (expected clinical use site)
Cons	High cost, Long time, Especial facility, Low through put, Histopathological evaluation 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
Reference	Kanemura et al., Sci Rep. 2013; Kawamata et al., J Clin Med. 2015



Highly-Efficient Culture (HEC) Assay

Example 2

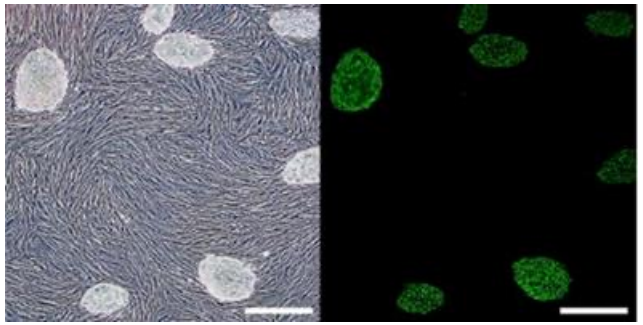
detects **residual undifferentiated pluripotent stem cells (PSCs)** in cell therapy products using highly efficient culture system which favors the growth of PSCs



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.



Tano et al., PLoS ONE. 2014

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

Highly-Efficient Culture (HEC) Assay

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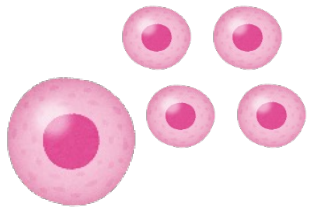

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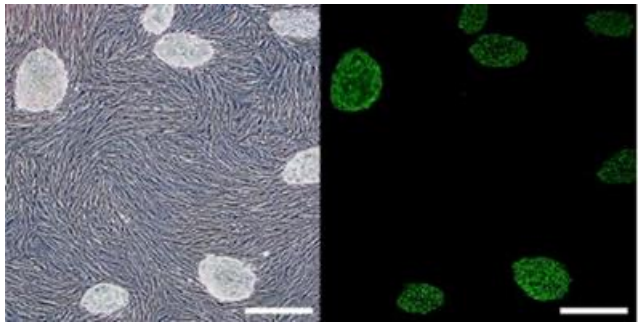

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Improvement of detection method for residual undifferentiated iPS cells (tumorigenic cells) in differentiated cells derived from human iPS cells

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

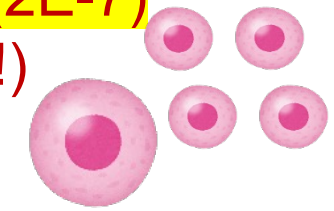
Regulatory Policies

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.

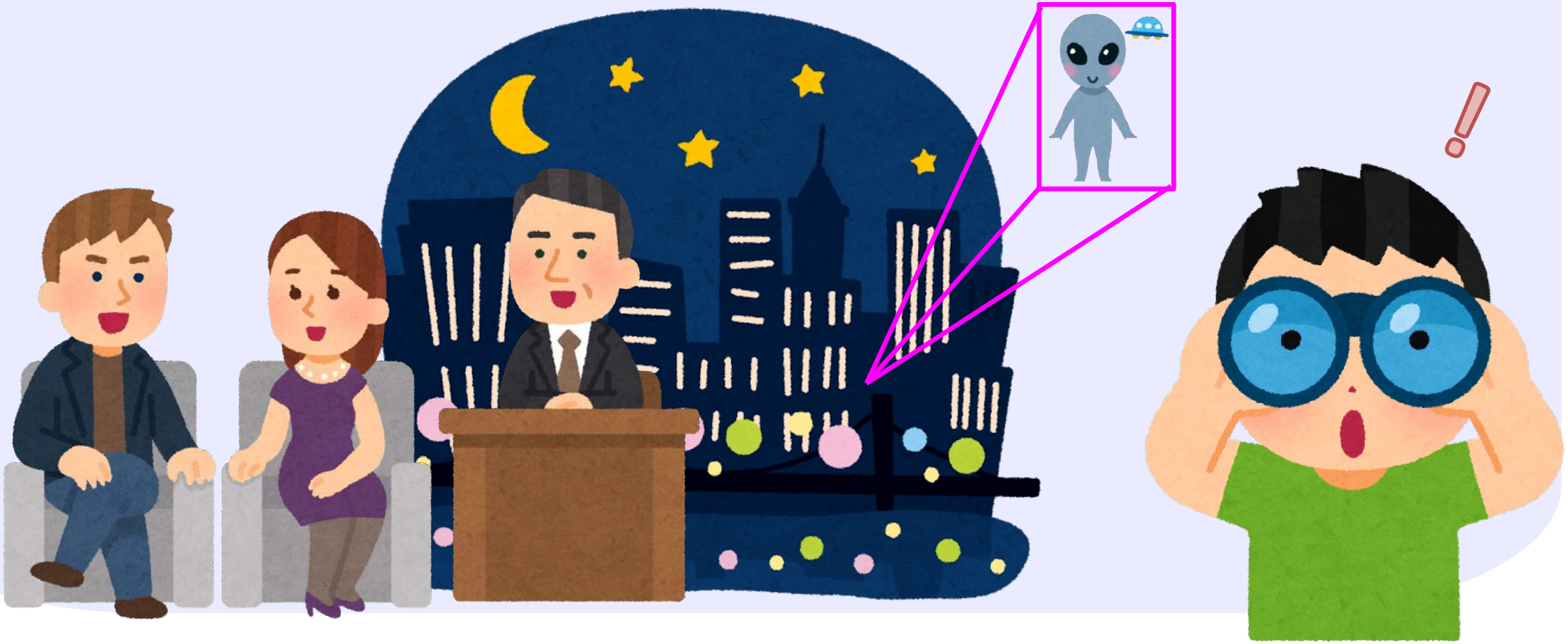
Methods: MEASURE first validated the detection sensitivity of HEC assay and then investigated the feasibility of magnetic-activated cell sorting (MACS) to improve sensitivity.

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.

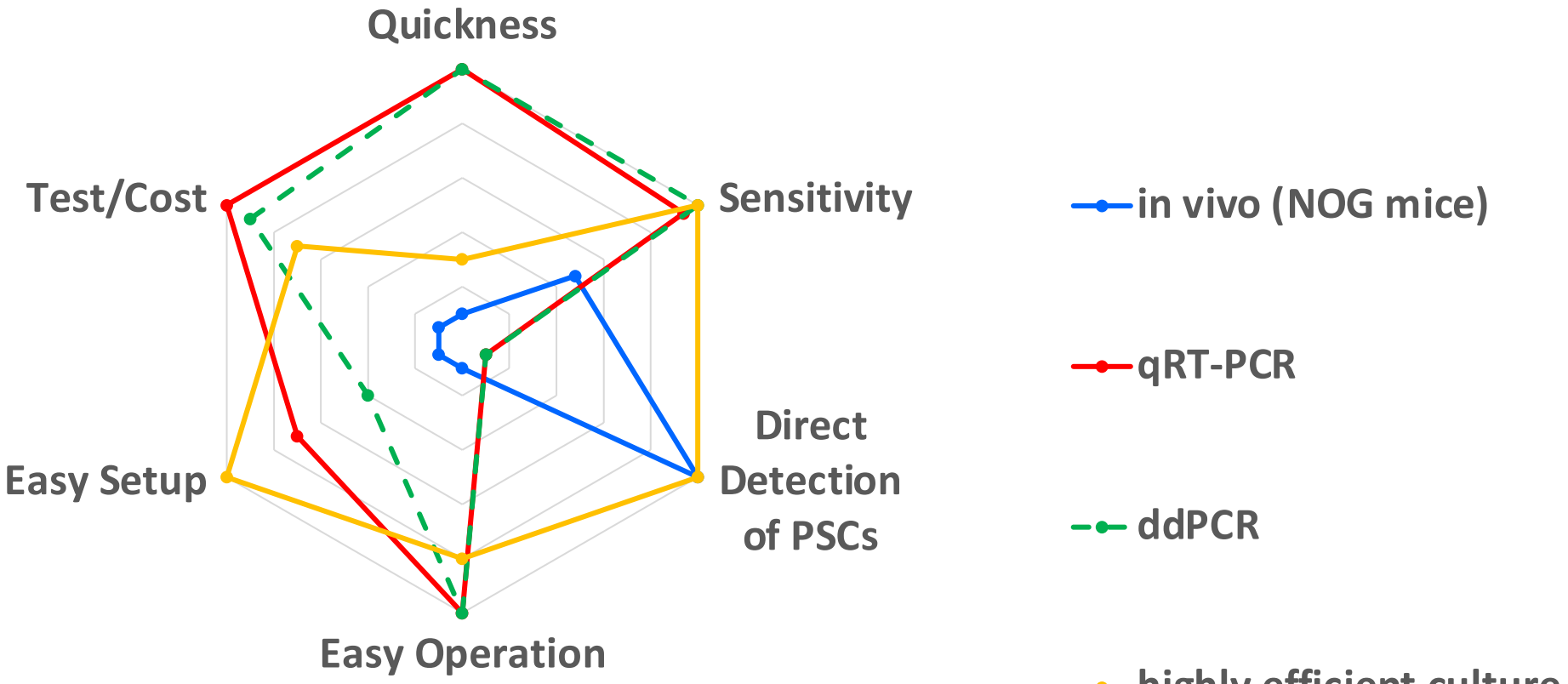
The improved Highly-Efficient Culture (HEC) Assay has achieved the ability to detect residual iPSCs in differentiated cells at a ratio of 1 in 5 million

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



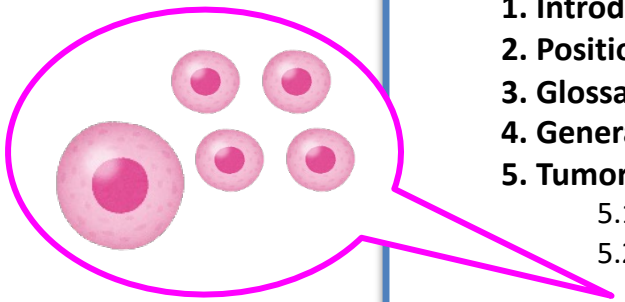
Qualitative Comparisons of Test Methods for Detection of Residual PSCs

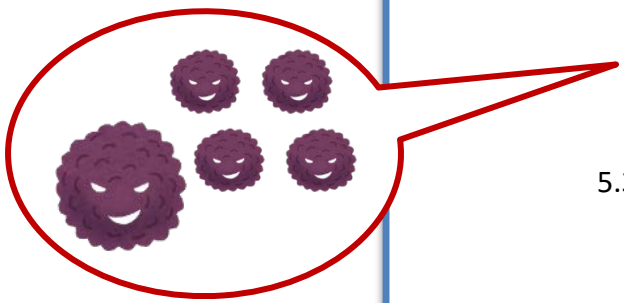
(based on our validation studies and past literature)



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

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

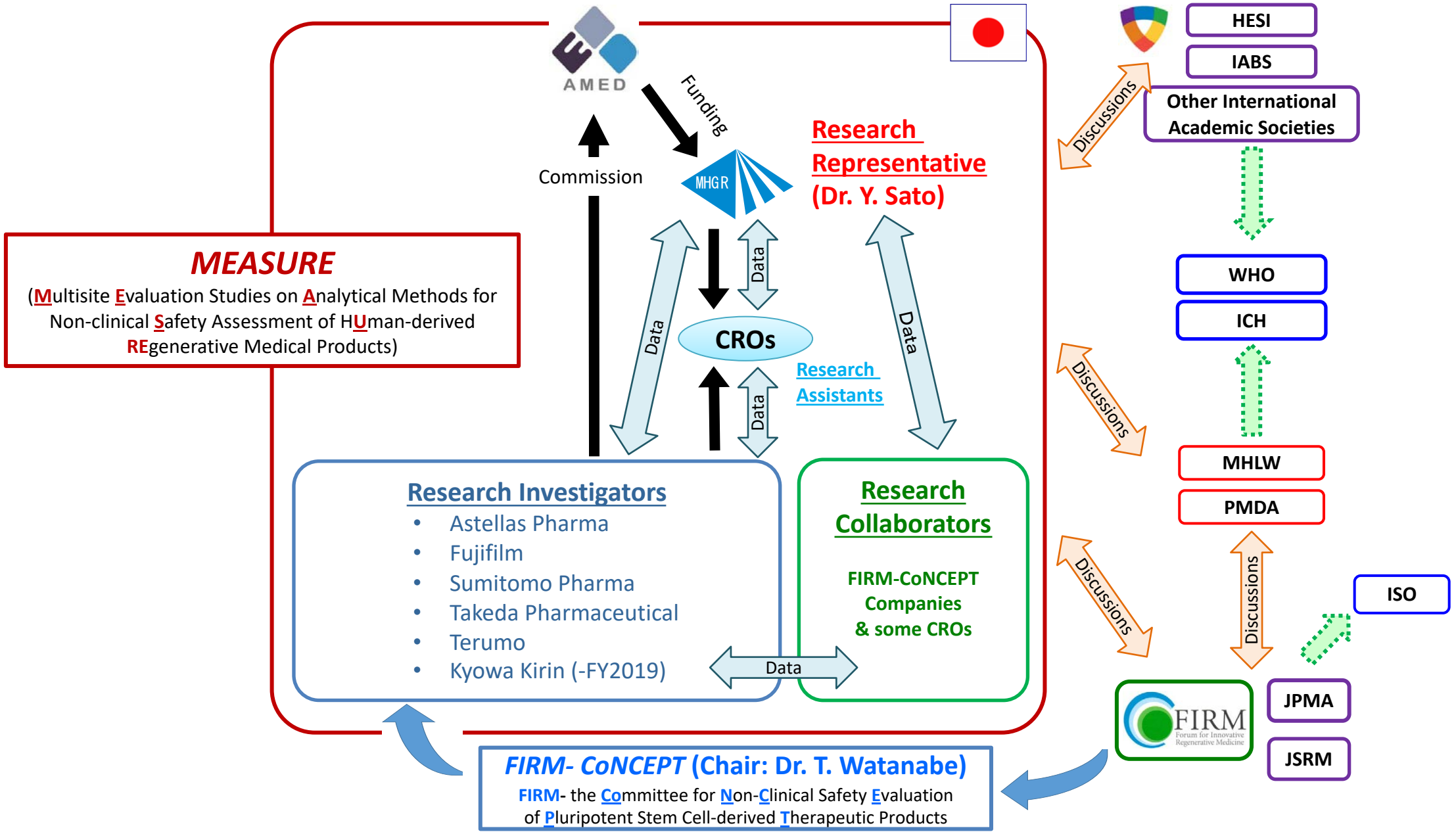




Contents

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

MHGR

Data

Data

Data

CROs

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

Data

Research Collaborators

FIRM-CoNCEPT Companies & some CROs

MHLW

PMDA

Discussions

Discussions

ISO

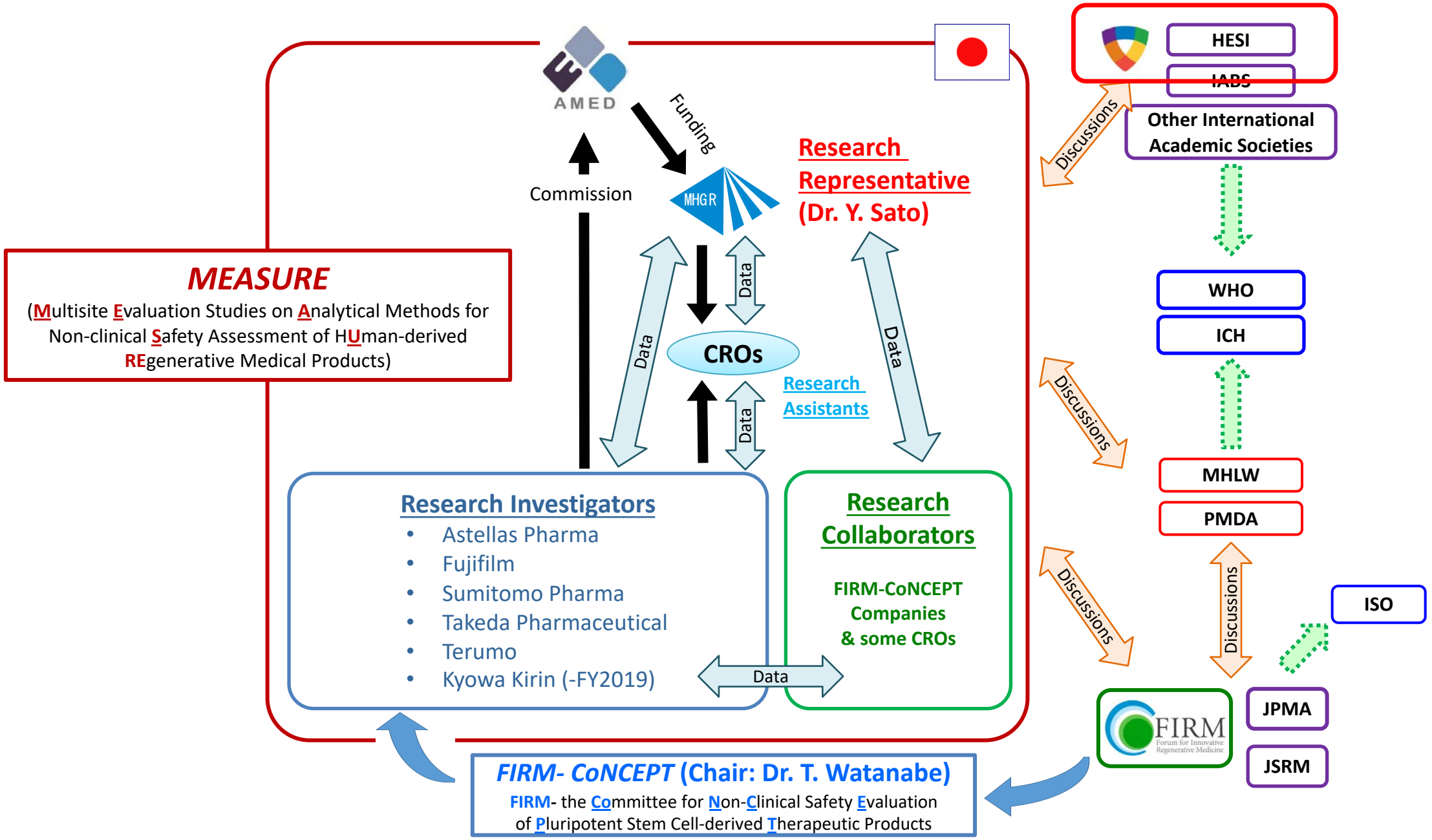
FIRM Forum for Innovative Regenerative Medicine

JPMA

JSRM

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

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



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

Memorial Sloan Kettering Cancer Center

Newcastle University

The University Of Sheffield.

Stanford Cardiovascular Institute

UCL

Universiteit Leiden

Wageningen University & Research

The University of Sydney

CT-TRACS Members

(2022 data)

>100 Participants

>30 Organizations

Government & Regulatory bodies:

FDA

cBG MEB Medicines Evaluation Board

MHRA

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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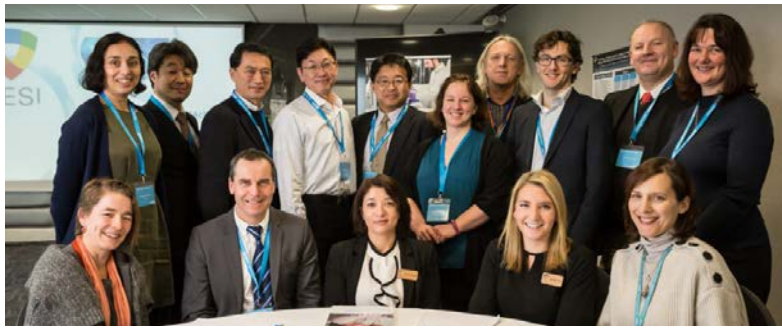
Courtesy of Dr. Lucilia Mouriès, HESI

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[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

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:szae058. doi: 10.1093/stcltm/szae058. Online ahead of print.

- 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***. 2024:26:315-323.

- 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;26:769-777.

- 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.

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- 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)

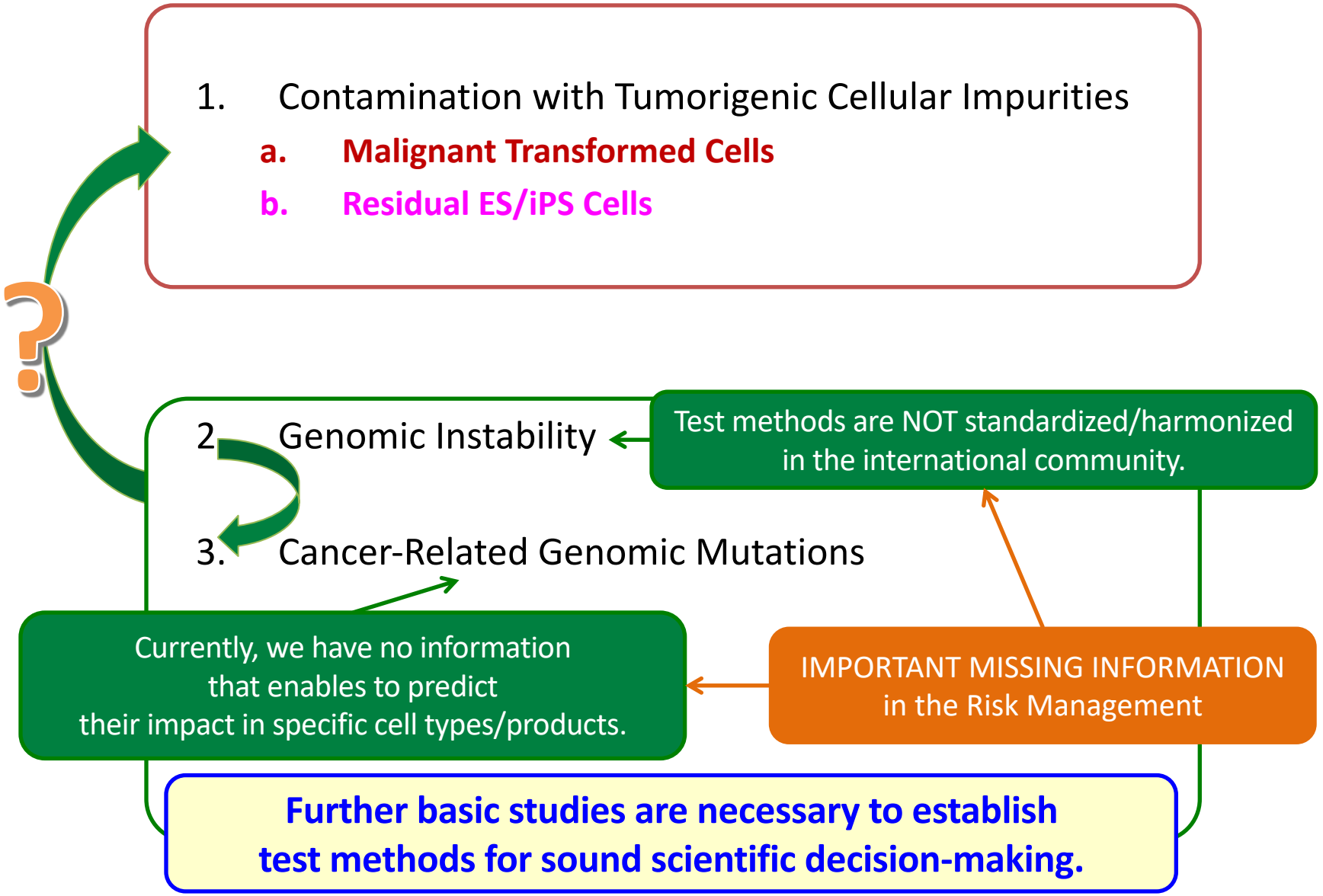
1. What is tumorigenicity? –The risk of tumorigenesis and its hazards–

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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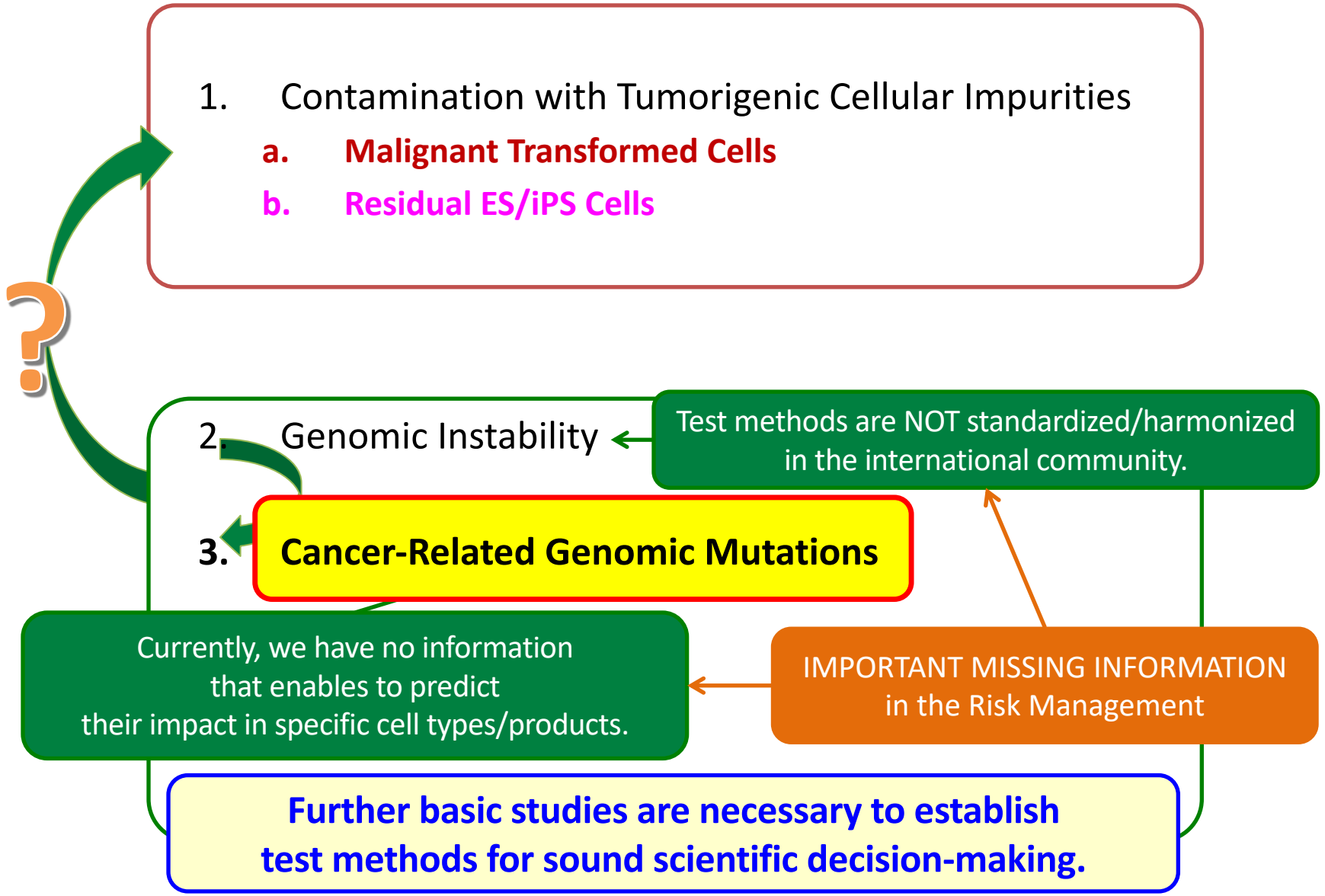

The human body is a mosaic of different genomes

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

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Science 07 Jun 2019:
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Somatic mosaicism in normal tissues

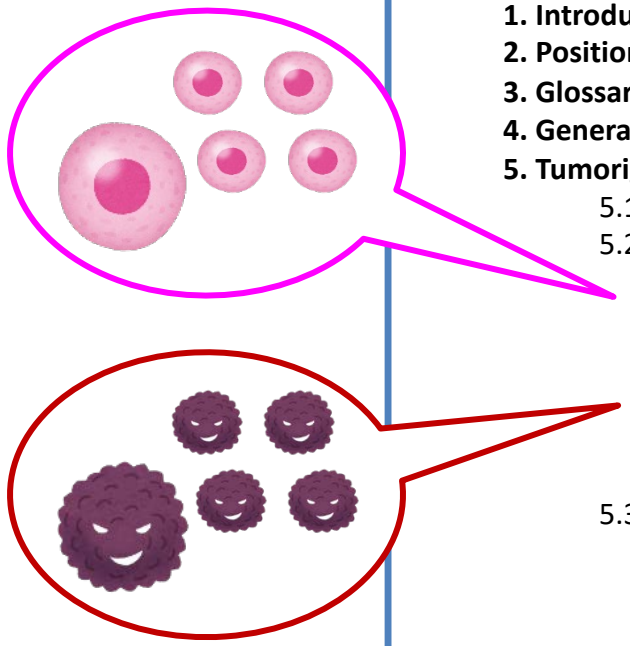
Somatic cells can accumulate mutations over the course of an individual's lifetime. This generates cells that differ genetically at specific loci within the genome. To explore how this genetic diversity in individuals contributes to disease, Yizhak *et al.* developed a method to detect mutations from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

…means "we currently have no way"

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

Cristian Tomasetti, Johns Hopkins Medicine

“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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Annex of Notification No. 0627-1 Issued on June 27, 2019, MHLW

7. General Considerations for Genomic Stability

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

….

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

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

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

Cancer-related SNVs
(HiSeq 2500 / NovaSeq 6000)

MEXT

MHLW PMDA

AMED

Data feed back

Differentiation resistance

PCR for factors

WGS

reprogramming

primary cell

G-band
SNV/Indel, CNV
Methylation

② Discuss required QC

④ Share data

NIHS
Y.Sato

PI:FBRI
Kawamata

CiRA
N.Takasu

iPSC supplier

Cell from donor

iPSC

Diff. cell

① Share the data

Draft of Safety Guideline

③ Share the data

In vivo
tumorigenicity test

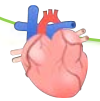
Differentiation

CNVs
(KaryoStat)

QC test

QC test＋clinical protocol

FBRI
S.Kawamata



CM

Riken CBD
M.Takahashi

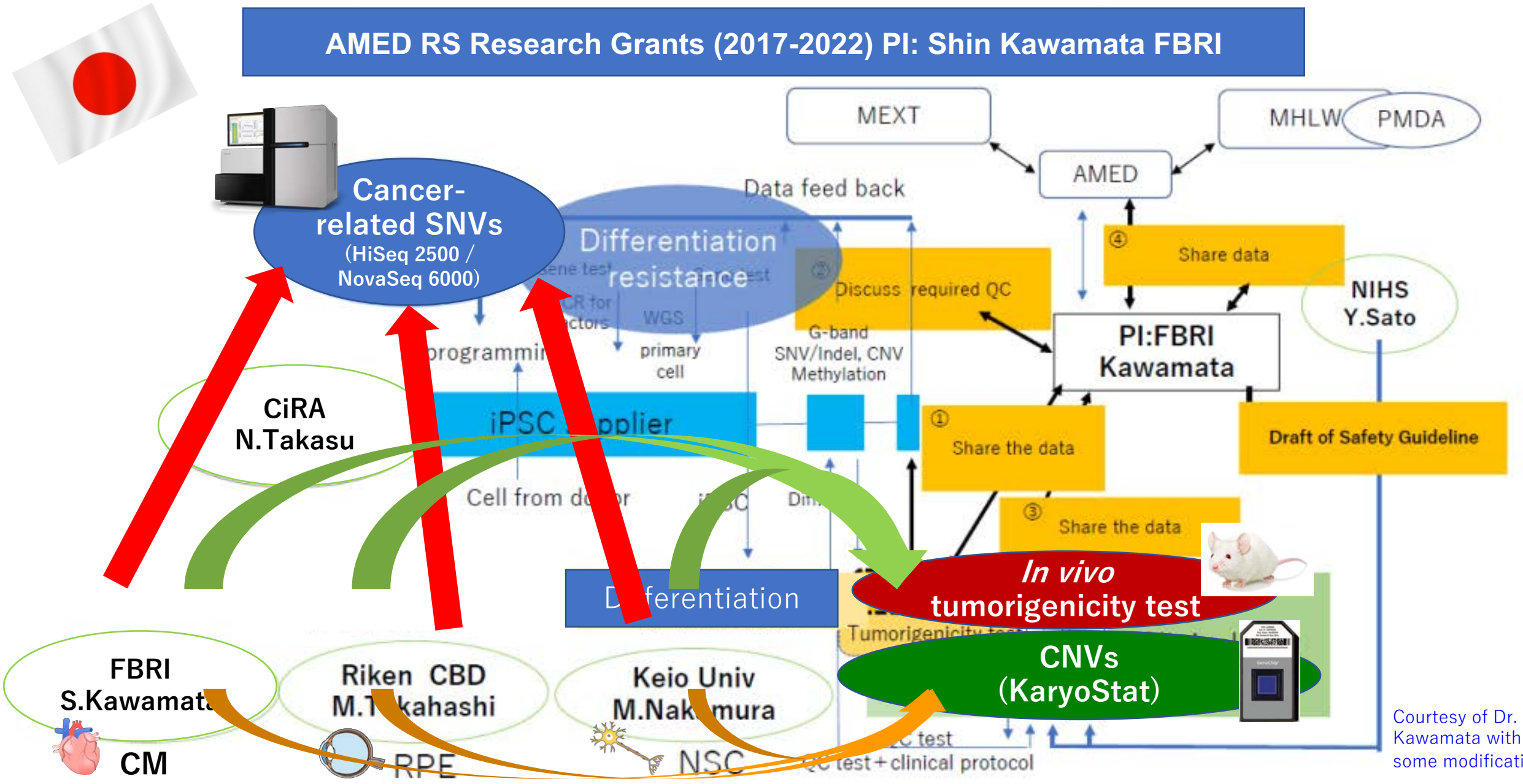
RPE

Keio Univ
M.Nakamura

NSC

Courtesy of Dr. Kawamata with some modifications

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



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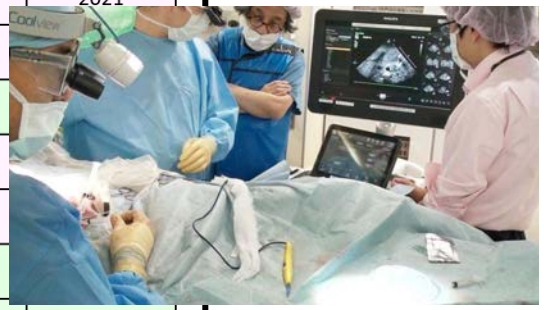
https://japan-forward.com/osaka-university-team-does-worlds-first-successful-ips-cell-derived-corneal-transplant/

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/

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.

Conclusions 1

- One of the safety and quality risks of iPS cell-derived products is their tumorigenicity. However, methods for evaluating the tumorigenicity of cell therapy products (CTP), including iPS cell-derived products, are not sufficiently developed/characterized.
- We have developed a number of *in vitro* and *in vivo* methods for evaluating tumorigenicity.
- By drafting and publishing guidelines on the performance and limitations of these tests, we have contributed to the facilitation of clinical applications of iPS cell-derived products in Japan.
- Validation of these test methods is currently underway with domestic and global stakeholders, which would contribute to the standardization and regulatory harmonization of the test methods in the future.

- iPS細胞由来製品の安全性・品質上のリスクとして、製品の造腫瘍性がある。しかし、 iPS細胞由来製品を含む細胞治療製品（CTP）の造腫瘍性評価法は十分には整備されていません。
- 我々は、数多くの *in vitro*又は*in vivo*の造腫瘍性評価法を開発してきました。
- これらの試験の性能と限界についてのガイドラインを執筆・発出することで、我々は日本におけるiPS細胞由来製品の促進に貢献してきました。
- 現在、国内外のステークホルダーと共同でこれらの試験法のバリデーションが進められており、将来の試験法の標準化・規制調和に貢献することが期待されています。

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

AGENDA (2)

1. **What is Comparability? – An Essential Requirement for Quality when Changing the Manufacturing Process of Cell Therapy Products –**

2. **CQA Mining – A New Approach for Stem Cell Pharmacotaxonomy –**

3. **MHLW Guideline for Comparability of Human Cell-Processed Products Subject to Changes in Their Manufacturing Process**

Comparability

An essential requirement for quality when changing the manufacturing process of cell therapy products

Essential requirement for changes in the manufacturing process of biological products, including CTPs
細胞加工製品を含むバイオ医薬品等の製造工程の変更時の必須要件

- The changes in the manufacturing process should **not adversely affect the product safety and efficacy**.
  - It is reasonable and effective to judge the pros and cons of changing the manufacturing method by **evaluating changes in the quality attributes of the product before and after the change**.
  - The need for confirmation in non-clinical and clinical trials is also determined by the content of the quality attribute evaluation.

- 製法変更によって少なくとも**製品の安全性と有効性に有害な影響を及ぼす変化がない**こと
  - 製法変更の是非は、変更前後の製品の**品質特性の変化を評価**することにより判断することが合理的かつ効果的。
  - 非臨床試験・臨床試験による確認尾必要性も、品質特性の評価の内容次第で判断。

“Comparable”
「同等・同質」 → ICH Q5E

- A conclusion that products **have highly similar quality attributes before and after manufacturing process changes and that no adverse impact on the safety or efficacy**, including immunogenicity, of the drug product occurred. This conclusion can be based on an analysis of product quality attributes. In some cases, nonclinical or clinical data might contribute to the conclusion.

- **製造工程変更前後の製品が品質特性において高い類似性を有し、製剤の免疫原性を含む安全性、あるいは有効性に有害な影響が生じていないこと**をいう。これは、製品の品質特性の分析に基づき判断できることが多いが、非臨床試験や臨床試験のデータを勘案する必要がある場合もある。

Basic Approach for Assessing Comparability Before and After Manufacturing Process Change (= ICH Q5E)

製法変更前後での同等性･同質性の評価の基本的考え方

1. Attempt to assess and assure the comparability, based on the analysis results of **quality attributes** of the product before and after the process change.

2. When the quality attributes of the product before and after the manufacturing process change appear to be changed, and the comparability cannot be fully explained, due to reasons such as the relationship between the quality attributes and safety/efficacy not being fully understood, consider the comparability assessment with the results of non-clinical or clinical trials.

1. 製法変更前後の製品の**品質特性**の分析結果で評価･保証することを試みる。

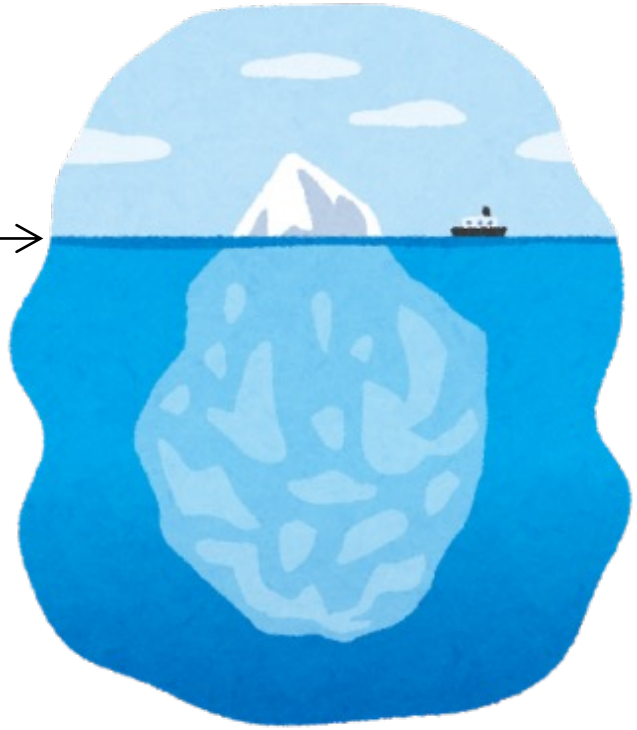
2. 製造工程変更前後の製品の品質特性に変化が認められ、また、品質特性と安全性及び有効性との関係が十分に解明されていないなどの理由により、同等性が十分に説明できない場合には、非臨床試験あるいは臨床試験の成績を組み合わせて評価する。

Cell Therapy Products are Complex
細胞加工製品は複雑

Limited Characterization Window
(Recognizable Quality Attributes)

Limit of Knowledge

Hidden/Unrecognizable
(but Potentially Critical)
Quality Attributes



Efficacy

Safety

・・・which creates UNCERTAINTY in the comparability assessment
(観察可能な)品質特性データのみで同等性を評価・保証することは難しいと予想される

Challenges in exploring and evaluating CQAs
CQAを探索・評価する際の課題

Test methods for viral safety, sterility, and tumorigenicity

- **Safety-related CQAs (characteristics and quantity of hazards)**

  Can you detect hazards and hazardous impurities that may have proliferative potential?

  Do you understand the sensitivity of your assays?

  = How can you avoid false negatives (and false positives)?

- **Efficacy-related CQAs**

  How do you identify attributes linked to cellular functions that

  … It's very difficult for products with unclear mechani

ウイルス安全性や無菌性
造腫瘍性の評価方法

- **安全性関連のCQA（ハザードの質と量）**

  増殖能を示すハザード・有害不純物を漏れなく検出できているか？測定法の感度を理解しているか？

  =偽陰性（＆偽陽性）の回避

- **有効性関連のCQA**

  有効性を裏付ける細胞機能とリンクした細胞特性をいかに同定する（掘り当てる）か？

  ・・・　作用機序が明確でない製品の場合は、とても難しい

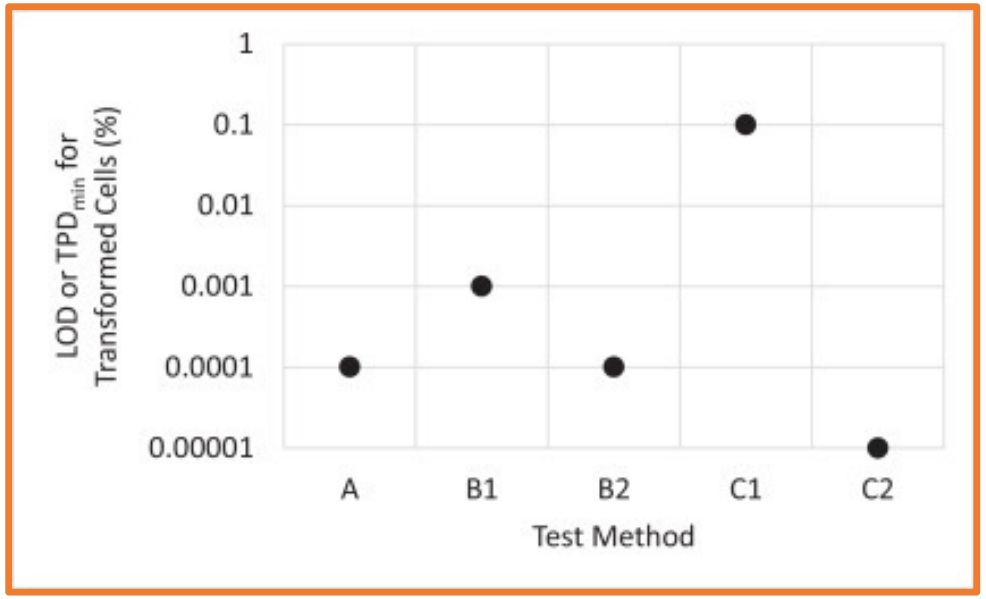
EXAMPLE

Sato Y, *et al., Cytotherapy.* 2019;21:1095-1111.

Limits of Detection (LODs) or Minimal Tumor Producing Doses (TPD_{min}) of Tumorigenic Cell Detection Tests

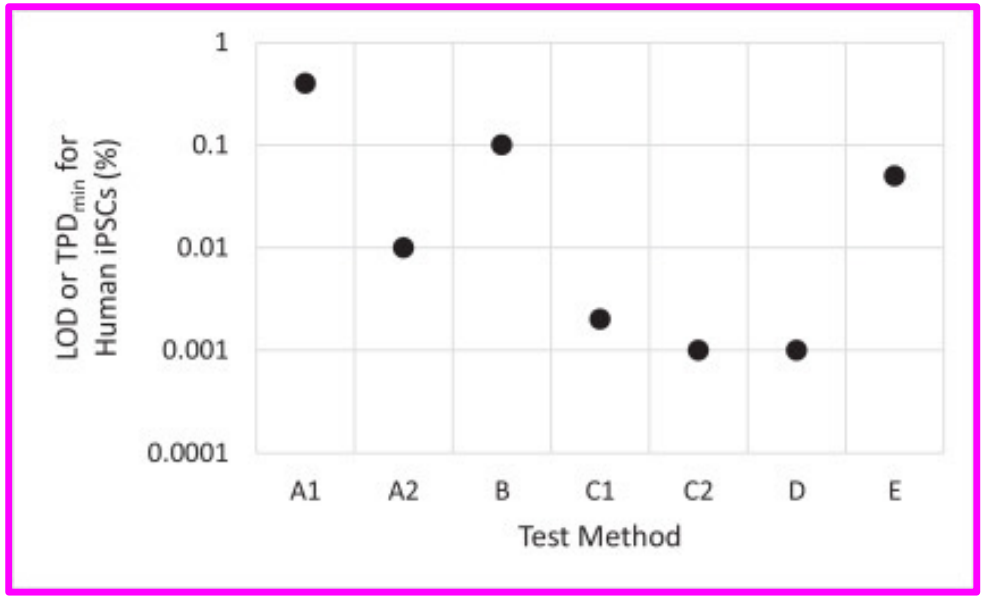
造腫瘍性細胞検出試験の検出限界(LODs)または最小腫瘍生成線量(TPDmin)

Transformed Cells in Normal Cells



A) TPD_{min} of ***in vivo*** **tumorigenicity test** (**HeLa cells/hMSC**, s.c. into NOG mice)
B1) LOD of **cell immortalization assay** (**immortalized hMSCs/hMSCs**)
B2) LOD of **cell immortalization assay** (**HeLa cells/hMSCs**)
C1) LOD of **conventional soft agar colony formation assay** (**HeLa cells/hMSCs**)
C2) LOD of **digital soft agar colony formation assay** (**HeLa cells/hMSCs**)

hiPSCs in Normal Cells



A1) TPD_{min} of ***in vivo*** **tumorigenicity test** (**hiPSCs/hRPE cells**, s.c. into NOG mice)
A2) TPD_{min} of ***in vivo*** **tumorigenicity test** (**hiPSCs/hNDF**, s.c. into NOG mice)
B) LOD of **flow cytometry** (**hiPSCs/hRPE cells**)
C1) LOD of **conventional qRT-PCR** (**hiPSCs/hRPE cells**)
C2) LOD of **droplet digital RT-PCR** (**hiPSCs/human cardiomyocytes**)
D) LOD of **highly efficient culture assay** (**hiPSCs/hMSCs**)
E) LOD of **GlycoStem-HP method** (**hiPSCs/HEK293 cells**).

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  How do you identify attributes linked to cellular functions that support efficacy?

  … It's very difficult for products with unclear mechanisms of action.

- **安全性関連のCQA（ハザードの質と量）**

  増殖能を示すハザード・有害不純物を漏れなく検出できているか？測定法の感度を理解しているか？

  =偽陰性（＆偽陽性）の回避

- **有効性関連のCQA**

  有効性を裏付ける細胞機能とリンクした細胞特性をいかに同定する（掘り当てる）か？

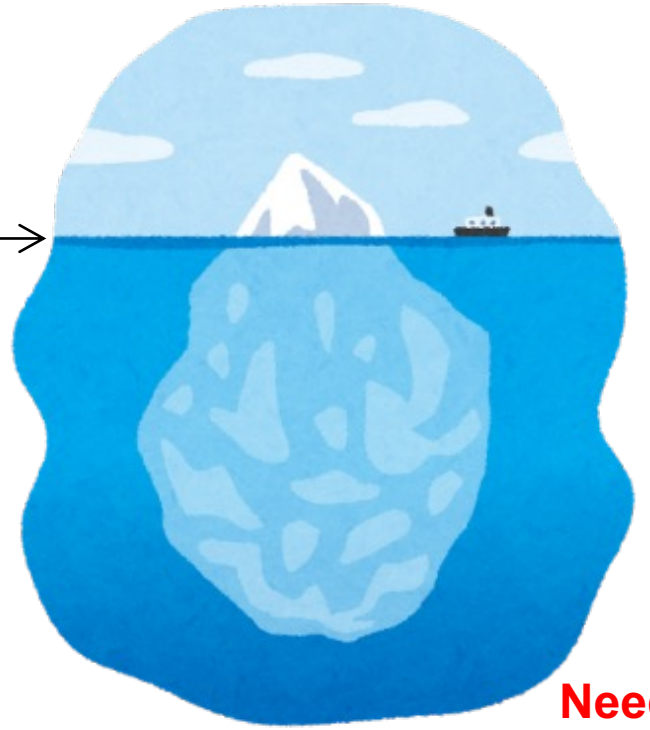
  ・・・　作用機序が明確でない製品の場合は、とても難しい

Cell Therapy Products are Complex
細胞加工製品は複雑

Limited Characterization Window
(Recognizable Quality Attributes)

Limit of Knowledge

Hidden/Unrecognizable
(but Potentially Critical)
Quality Attributes



Efficacy

The mode of action (MOA) is unclear in many cases.

Need for understanding MOA and CQAs related to the efficacy or *in vitro* potency.

Need for a tool for uncovering hidden CQAs

AGENDA (2)

1. **What is Comparability? – An Essential Requirement for Quality when Changing the Manufacturing Process of Cell Therapy Products –**

2. **CQA Mining – A New Approach for Stem Cell Pharmacotaxonomy –**

3. **MHLW Guideline for Comparability of Human Cell-Processed Products Subject to Changes in Their Manufacturing Process**

CQA Mining

A New Approach for Stem Cell Pharmacotaxonomy

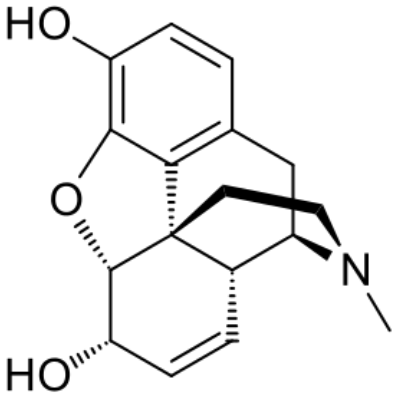
The Starting Point for Pharmacology

EXAMPLE : Isolation of Morphine

Opium, arguably the oldest herbal drug in human history, has always been in the spotlight throughout history as the only medicine that relieves pain and induces sleep.

In 1806, a German pharmacist Friedrich Sertürner（フリードリヒ・ゼルチュルナー） succeeded in the isolation of **morphine** from opium.

= The starting point for their pharmacology (and toxicology)



https://ja.wikipedia.org/wiki/%E3%82%A2%E3%83%98%E3%83%B3

https://en.wikipedia.org/wiki/Friedrich_Sert%C3%BCrner

At what stage is “regenerative medicine” as an academic field now?
学問としての「再生医学」は今どの段階にあるのか？

Crude Pharmaceutics → → Separation Science/Analytical Chemistry → → Modern Pharmacology

composed of diverse chemicals

= Cell Therapy Products → → → → → → → ？？？ → → → → → → → Science on their MOA

composed of complex and diverse cells

Science to understand the heterogeneity of cells and cell populations

「生薬」から「分離科学／分析化学」を経て「近代薬学」「薬理学」）が成立

多様な化学物質の集合体

=「細胞加工製品」から　「？？？」を経て　「再生医学」「細胞加工製品の薬理」を理解

複雑・多様な細胞の集合体

細胞・細胞集団の不均質性を理解するための科学

Need for technology to understand heterogeneity
不均質性を理解するための技術が必要

For example, **even when there are a total of 1 million cells, only 10,000 of them may be effective**.

“Visualization” of such heterogeneity and **characterization of those 10,000 cells** would **make identifying CQAs related to efficacy easier.**

例えば、**総細胞数が100万個**あっても、**そのうち有効性を発揮するのは1万個**しかないという場合もありうる。

このような**不均質性を「見える化」**することで、**その1万個の細胞がどのような特性を持つのか**を明らかにすれば、 **有効性に関連するCQA（重要品質特性）を発見しやすくなる（・・・と期待できる）**

EXAMPLE

Stem Cells Translational Medicine, 2023, **12**, 379–390
https://doi.org/10.1093/stcltm/szad029
Advance access publication 2 June 2023
Original Research

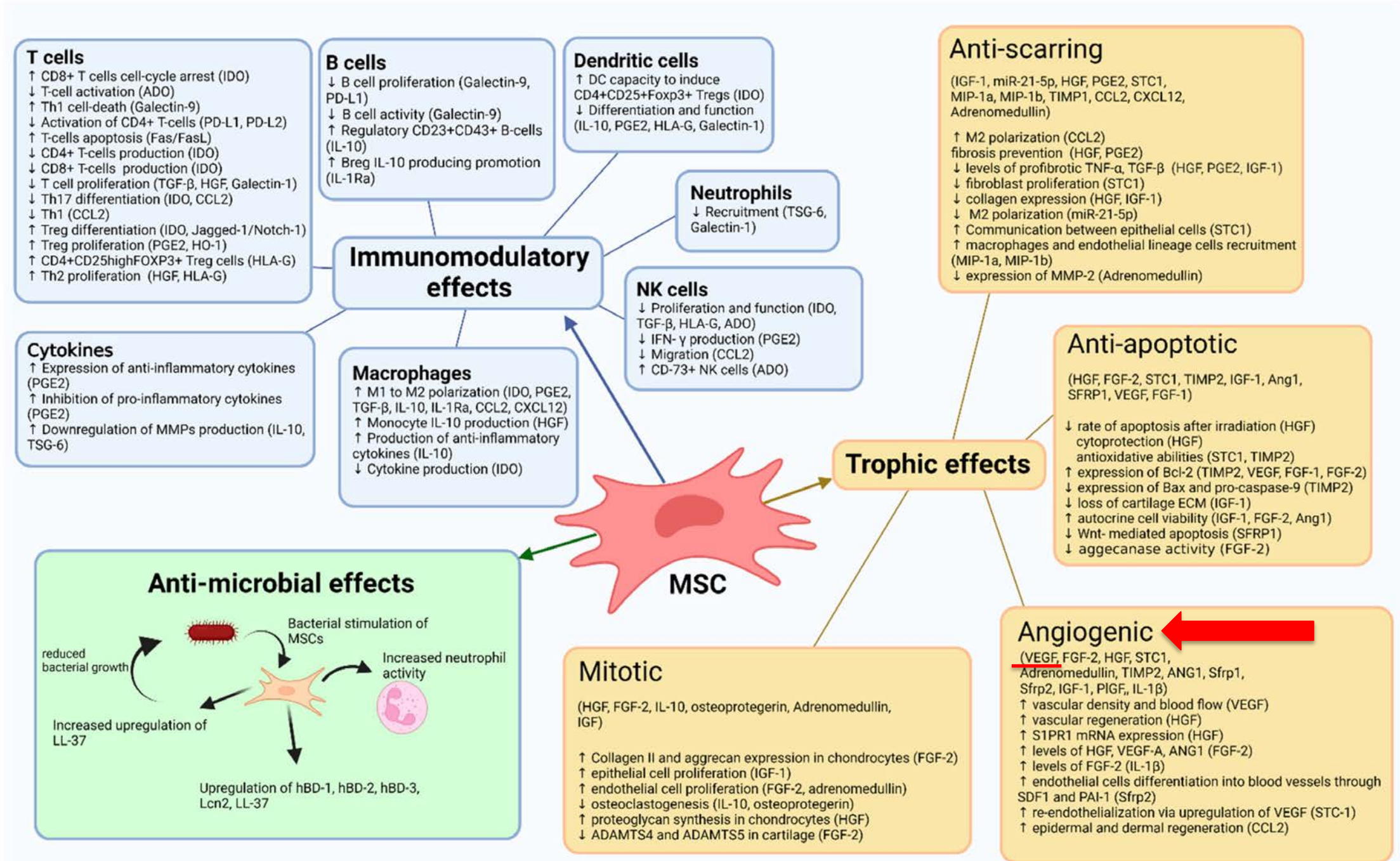
OXFORD

Single-Cell RNA-Seq Reveals *LRRC75A*-Expressing Cell Population Involved in VEGF Secretion of Multipotent Mesenchymal Stromal/Stem Cells Under Ischemia

Takumi Miura[1,2,‡], Tsukasa Kouno[3,‡], Megumi Takano[1], Takuya Kuroda[1], Yumiko Yamamoto[3], Shinji Kusakawa[1], Masaki Suimye Morioka[3], Tohru Sugawara[2,4], Takamasa Hirai[1], Satoshi Yasuda[1], Rumi Sawada[1], Satoko Matsuyama[1,5], Hideya Kawaji[3,6], Takeya Kasukawa[3], Masayoshi Itoh[3], Akifumi Matsuyama[5], Jay W. Shin[3,7], Akihiro Umezawa[2], Jun Kawai[3,8], Yoji Sato[*,1,8,9]

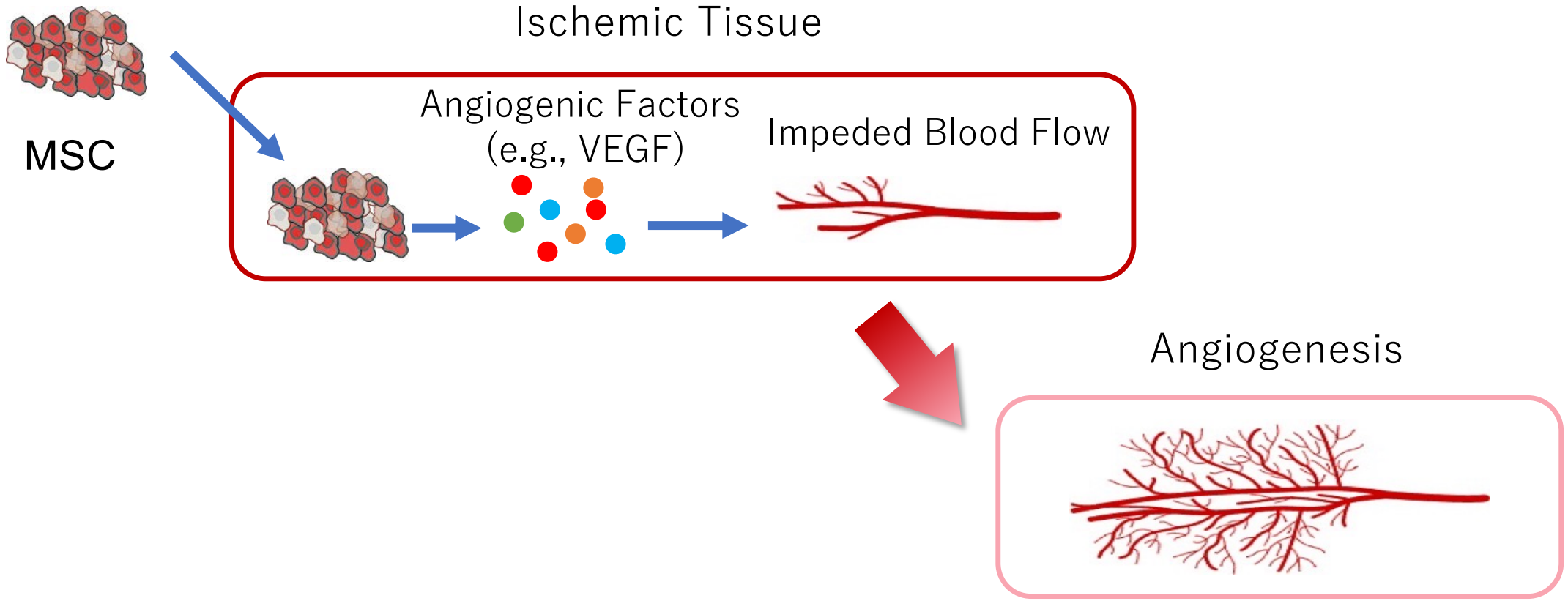
[1]Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, Kanagawa, Japan
[2]Center for Regenerative Medicine, National Center for Child Health and Development, Tokyo, Japan
[3]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[4]Biopharmaceutical and Regenerative Sciences, Graduate School of Medical Life Science, Yokohama City University, Yokohama, Japan
[5]Center for Reverse TR, Osaka Habikino Medical Center, Osaka Prefectural Hospital Organization, Osaka, Japan
[6]Research Center for Genome & Medical Sciences, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
[7]Genomic Institute of Singapore, Agency for Science, Technology and Research, Singapore
[8]Life Science Technology Project, Kanagawa Institute of Industrial Science and Technology, Kawasaki, Japan
[9]Department of Cellular and Gene Therapy Products, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

*Corresponding author: Yoji Sato, PhD, Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki Ward, Kawasaki City, Kanagawa 210-9501, Japan. Email: yoji@nihs.go.jp
‡Contributed equally.

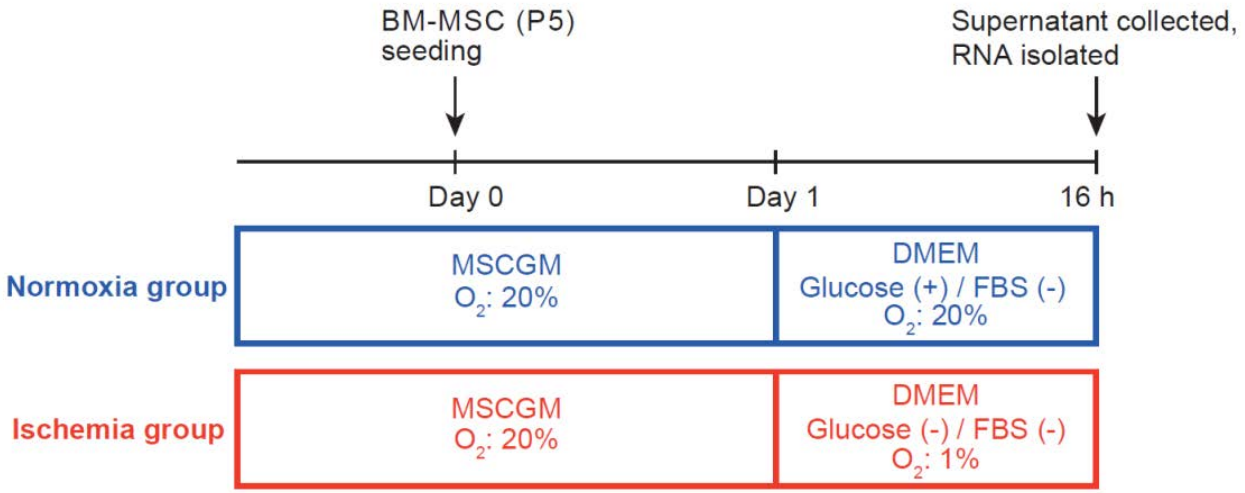


https://www.mdpi.com/genes/genes-13-00949/article_deploy/html/images/genes-13-00949-g001.png

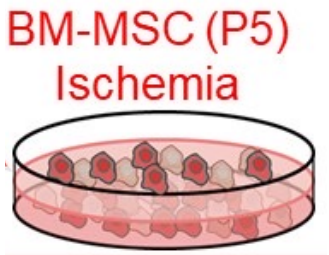
Design of an experimental condition mimicking the environment of the engraftment site

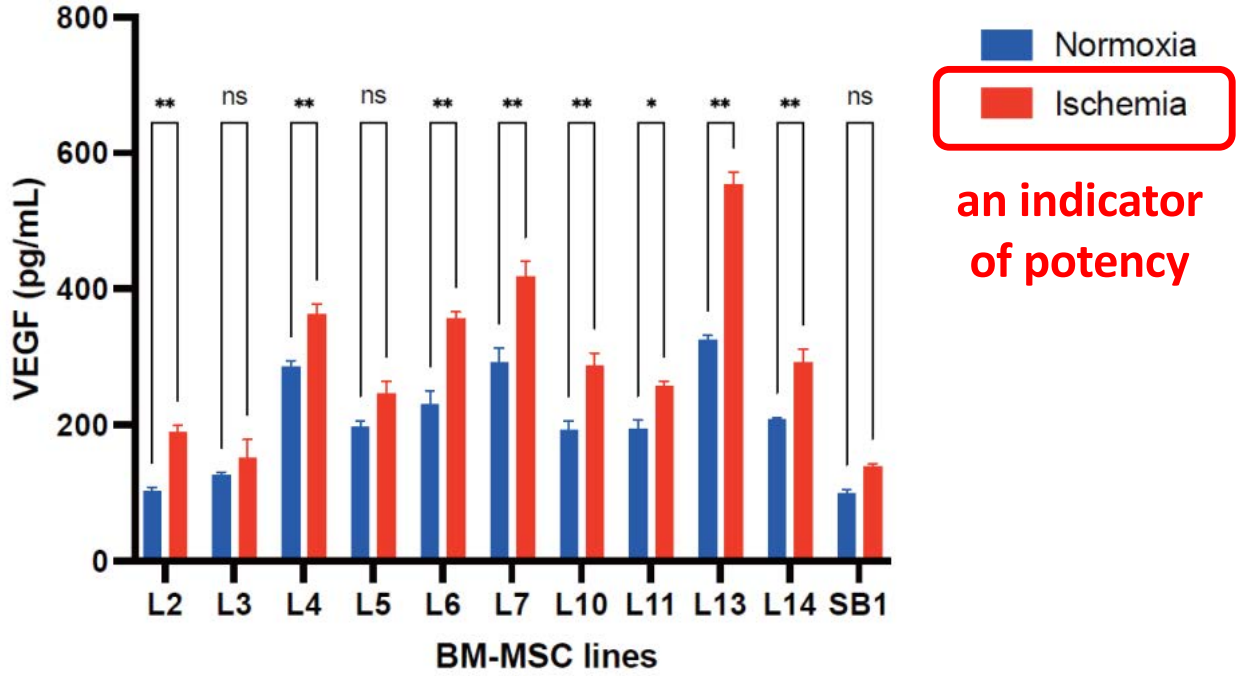


Design of an experimental condition mimicking the environment of the engraftment site



BM-MSCs under ischemia





an indicator of potency

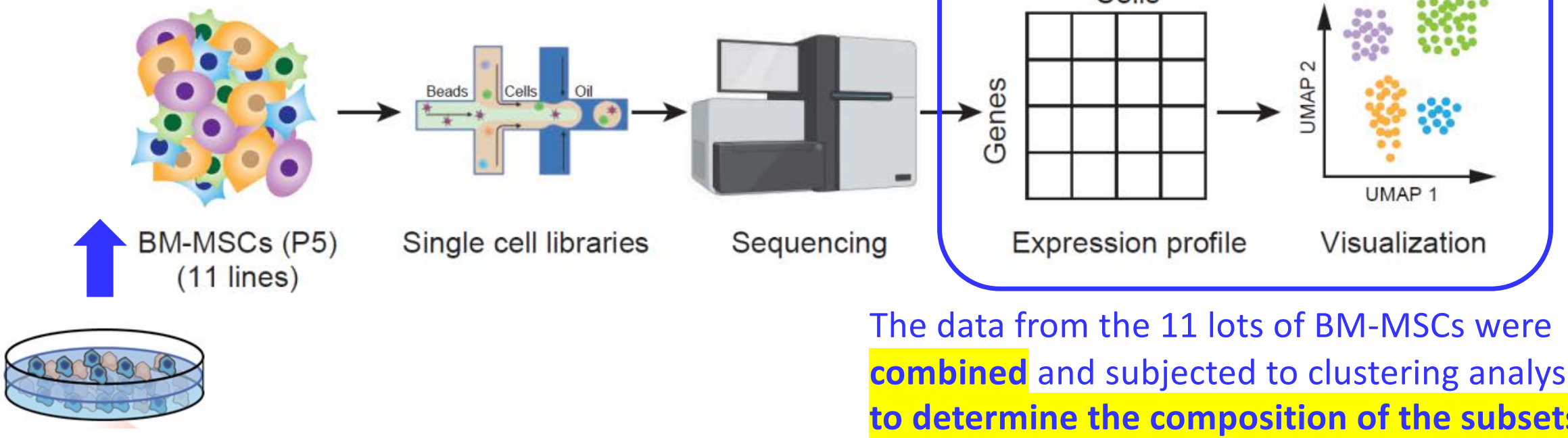
VEGF secretion varies widely between the lines.

Miura T *et al., Stem Cells Transl Med.* 2023;**12**:379-390.

Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

Single-Cell Transcriptome Experiments

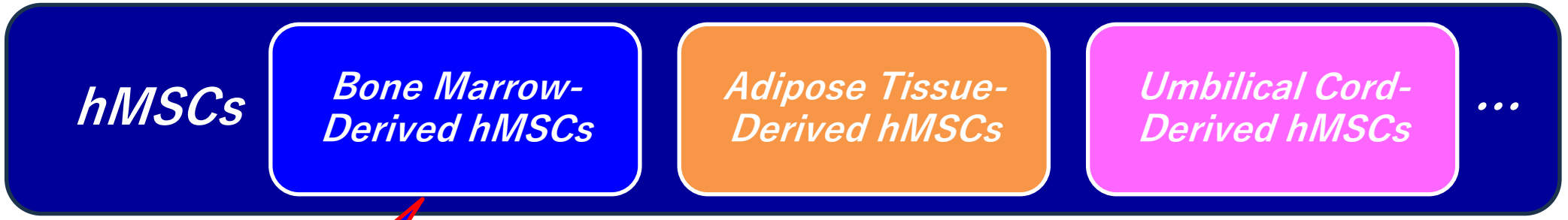
BM-MSCs under normoxia



The data from the 11 lots of BM-MSCs were **combined** and subjected to clustering analysis **to determine the composition of the subsets of "average BM-MSCs" (BM-MSCs as a population).**

Science on hMSCs

The Populations of hMSCs
(the Ideas in Plato's Philosophy)



Specific Samples
(Our Study)

11 Lines of Bone Marrow-Derived hMSCs

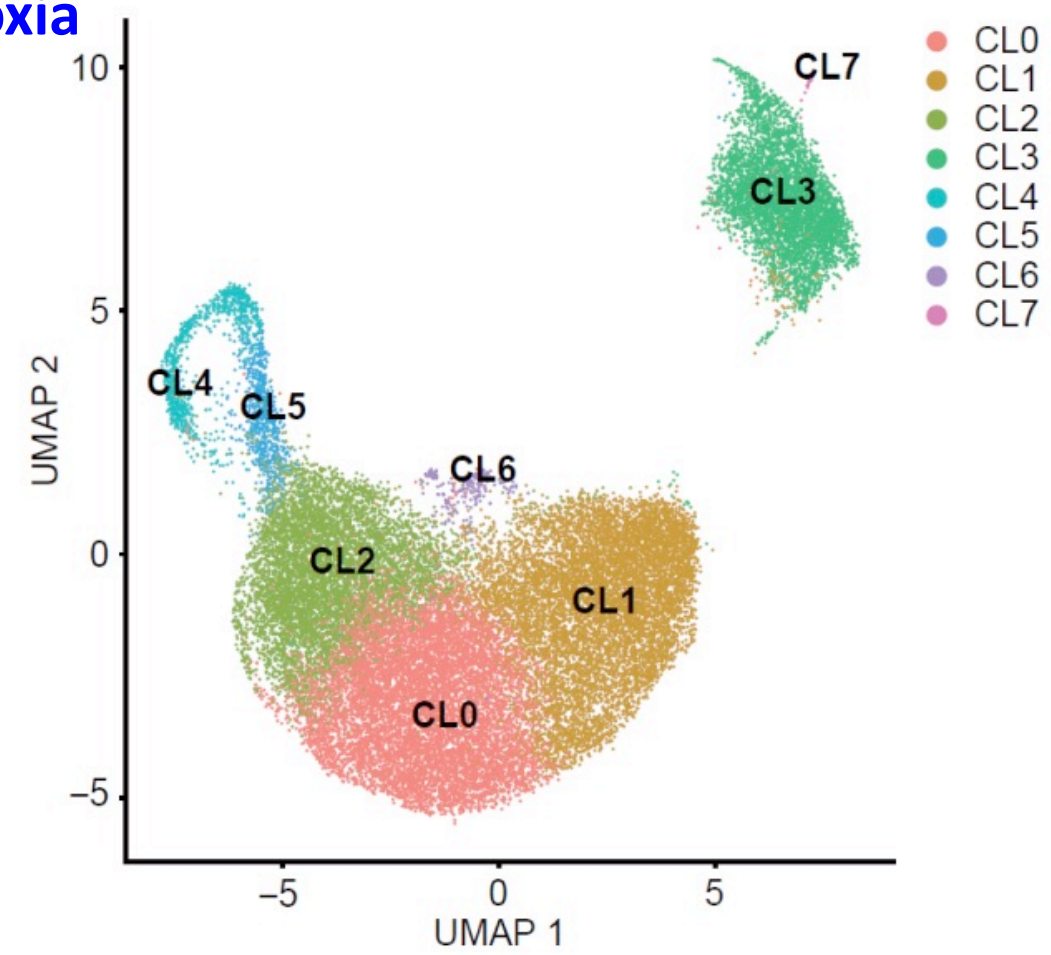
To estimate the heterogeneity of BM-MSCs in the population, all data from approximately equal numbers of cells derived from each line were clustered together.

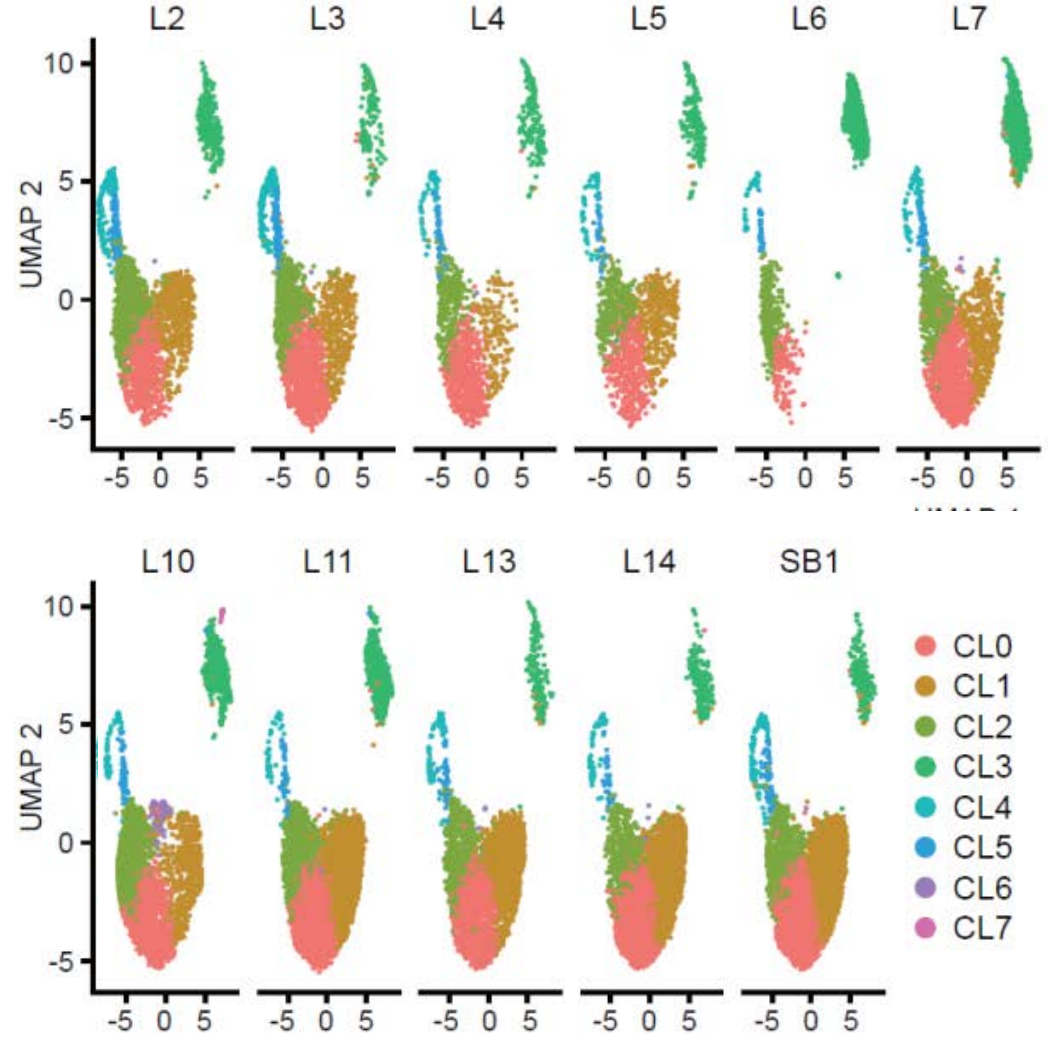
母集団中のBM-MSCの不均一性を推定するため、各ライン由来のほぼ同数の細胞のデータを全部併せてクラスタリング

Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

Single-Cell Transcriptome Experiments

BM-MSCs (P5)
Normoxia

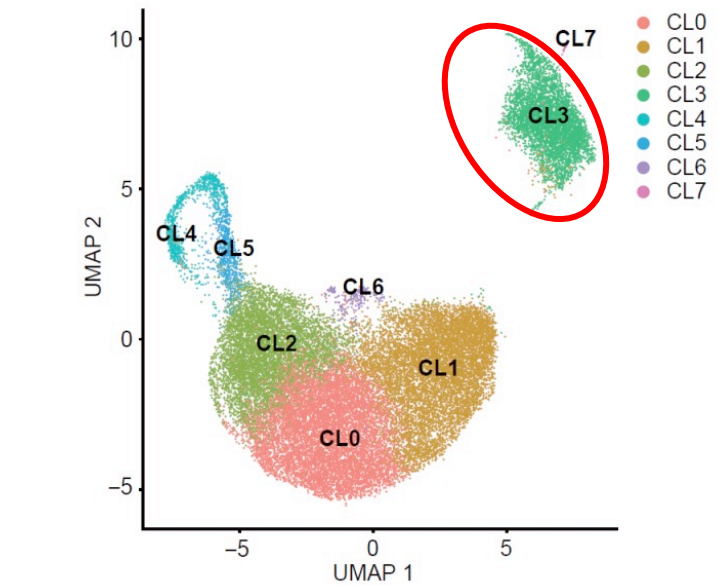




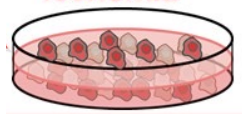
Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

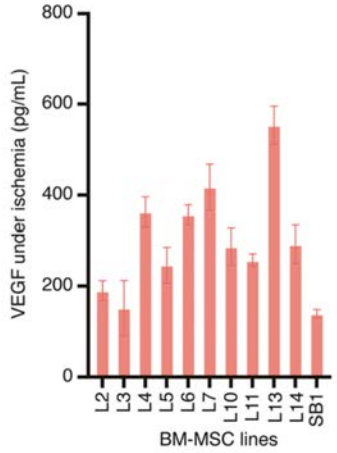
Single-Cell Transcriptome Experiments

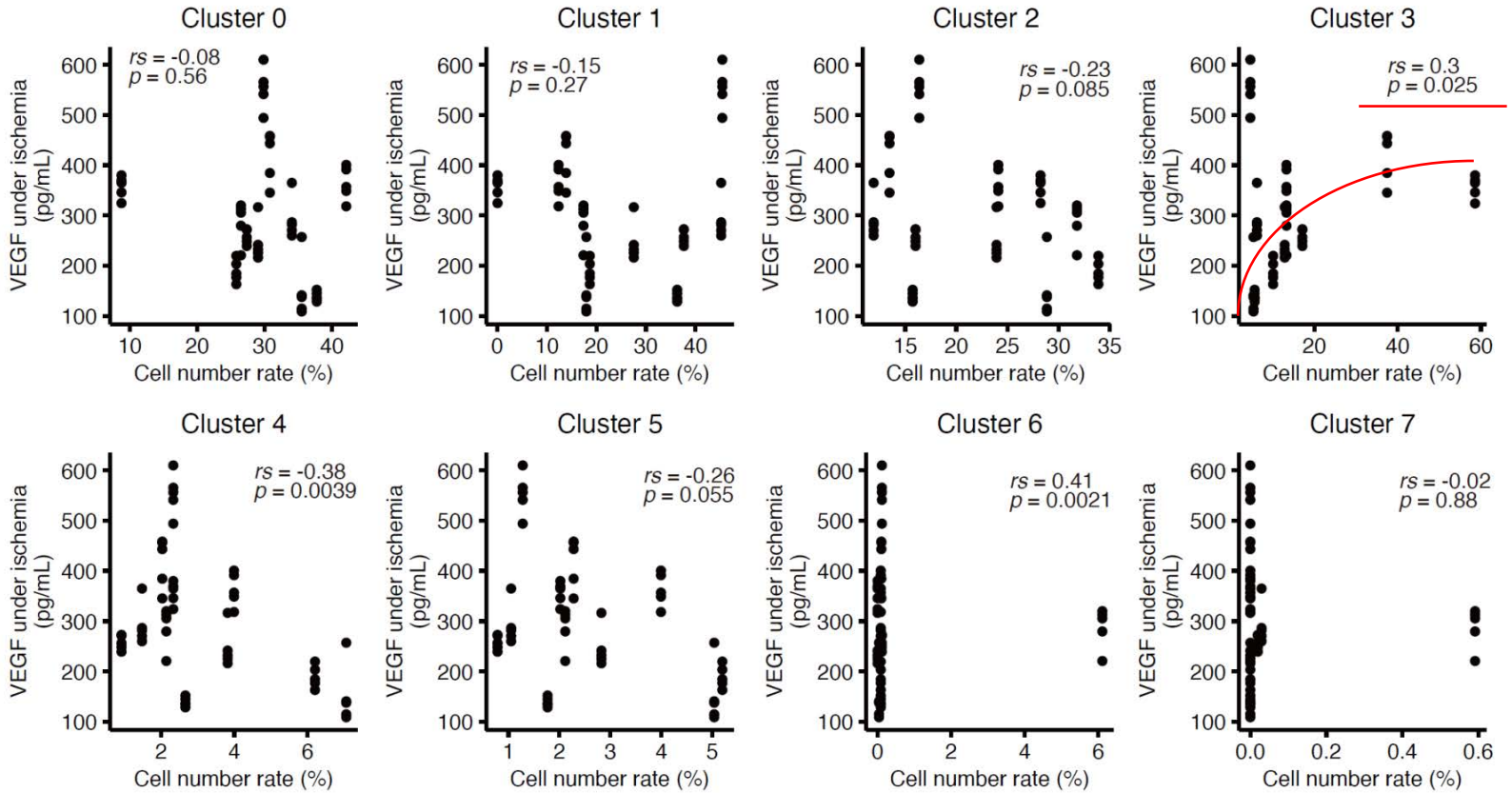
BM-MSCs (P5)
Normoxia



BM-MSC (P5)
Ischemia



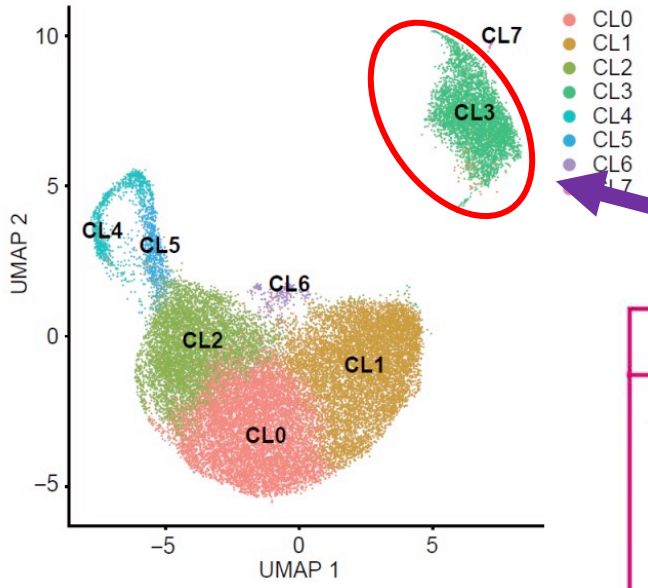




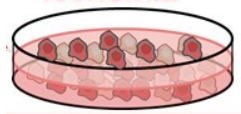
Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

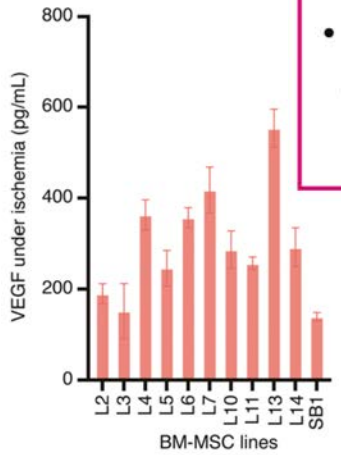
Functional involvement of LRRC75A

BM-MSCs (P5)
Normoxia



BM-MSC (P5)
Ischemia





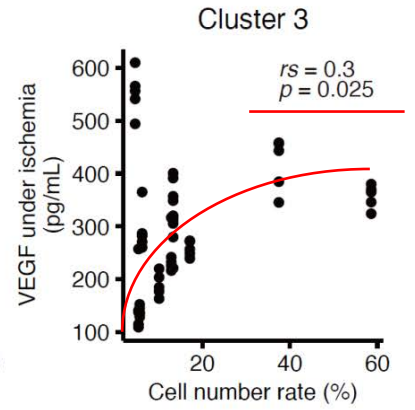
Top 20 upregulared genes of CL3

Gene name	Ave $\log_2$FC
LRRC75A	1.0357
KRT7	0.8382
KRT16	0.7902
C16orf56	0.7815
FLG	0.6049
SH3BGRL3	0.5970
TPM2	0.5859
POLR2L	0.5555
GADD45B	0.5543

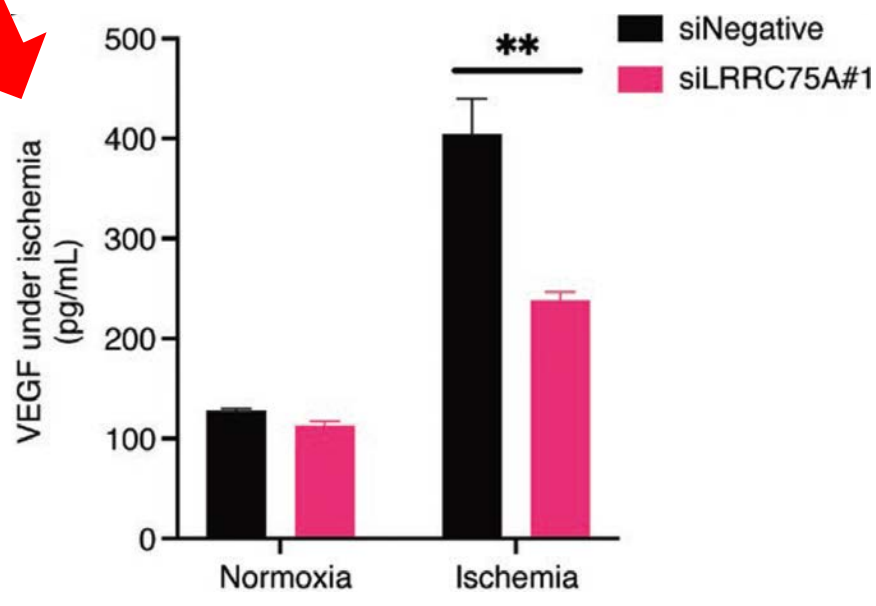
Reseach applications

- Identifying cell subpopulations and biomarkers that correlate with therapeutic efficacy of MSC-based therapeutic products
- Identifying critical quality attributes and setting specifications for MSC-based therapeutic products

Hidden CQAs



***LRRC75A* is functionally involved in VEGF secretion during ischemia.**



Science

HYPOTHESIS
The expression of *LRRC75A* in CL3 regulates the VEGF secretion from hMSCs under ischemia.

The Populations of hMSCs
(the Ideas in Plato's Philosophy)

hMSCs | ***Bone Marrow-Derived hMSCs*** | ***Adipose Tissue-Derived hMSCs*** | ***Umbilical Cord-Derived hMSCs*** | …

Specific Samples
(Our Study)

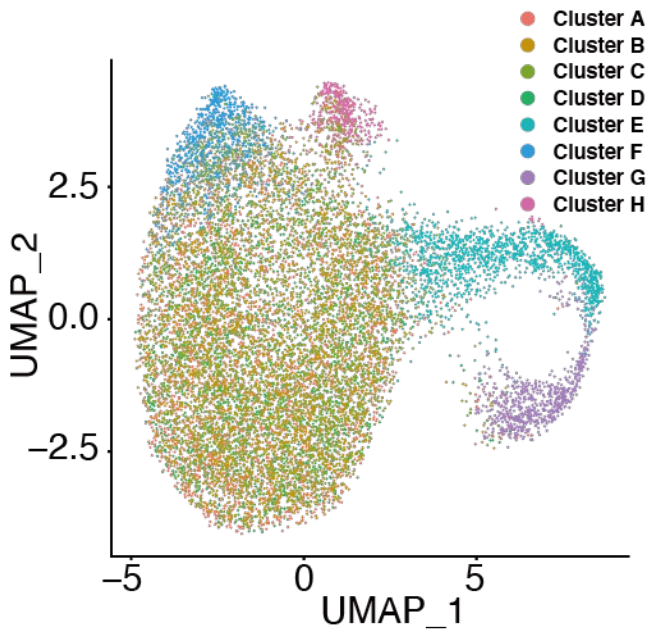
11 Lines of Bone Marrow-Derived hMSCs ↑ a training set

4 Lines of Bone Marrow-Derived hMSCs ↑ a test set

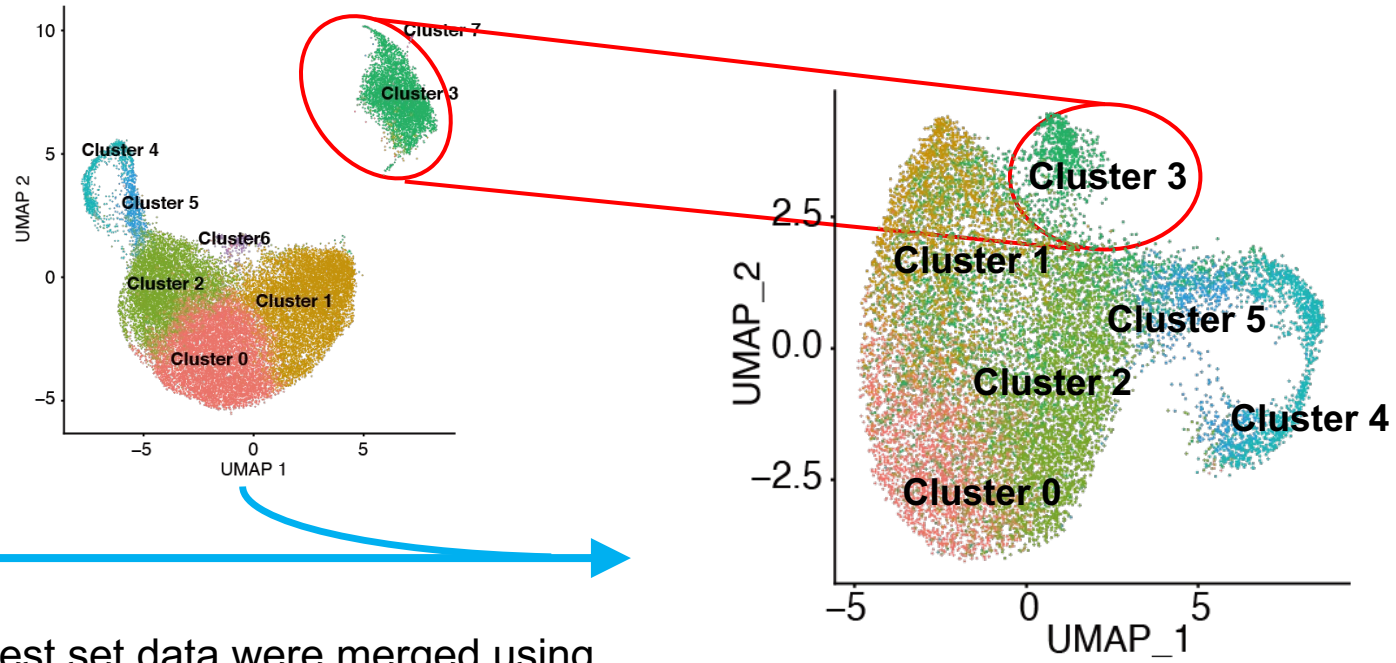
Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

Identification of CL3-like cells in another set of BM-MSCs

scRNA-Seq Analysis for the Test Set (4 lines: L15 –L18)



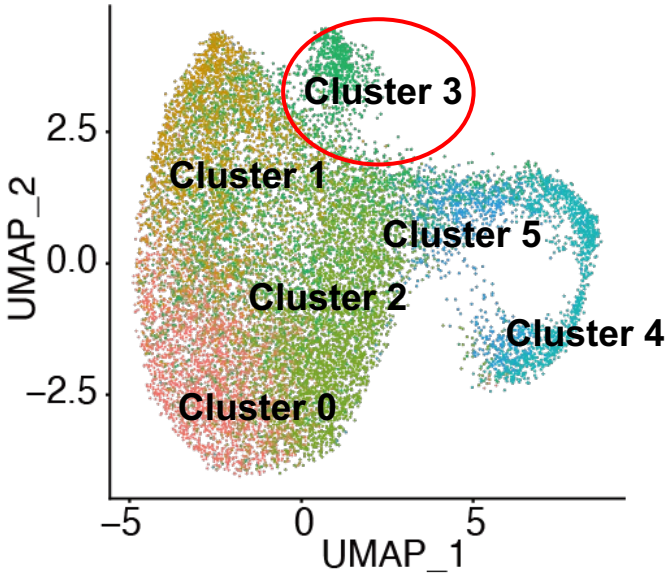
Reference Data Set (L2–SB1)



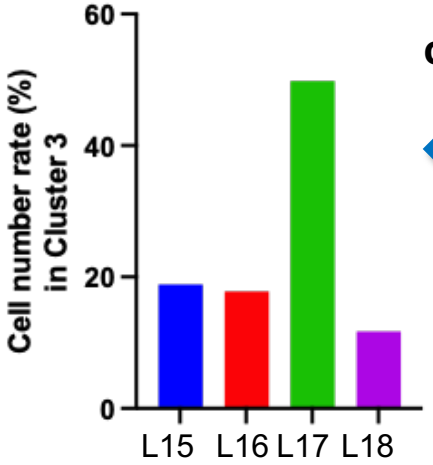
The test set data were merged using *FindTransferAnchors* function with the reference set and identified a CL3-like population.

Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

Identification of CL3-like cells in another set of BM-MSCs



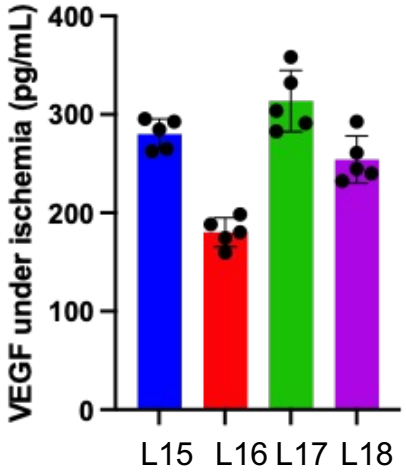
% Distribution of Cells in Each Line of the Test Set to CL3



BM-MSC lines

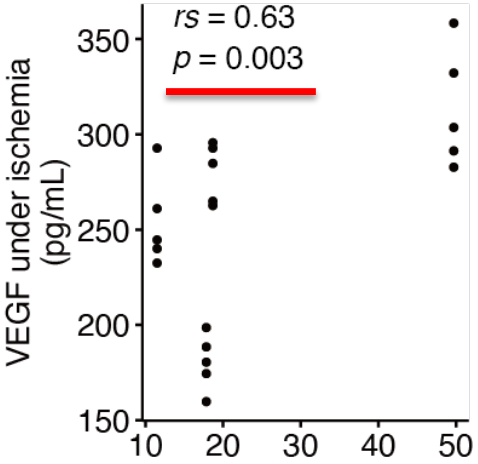
correlation

VEGF Production in Each Line (During Ischemia)



BM-MSC lines

Spearman's Rank Correlation Coefficient (% Cell Distribution *vs.* VEGF Production During Ischemia)



% Cell Distribution to CL3

Science

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The expression of *LRRC75A* in CL3 regulates the VEGF secretion from hMSCs under ischemia.

The Populations of hMSCs
(the Ideas in Plato's Philosophy)

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(Our Study)

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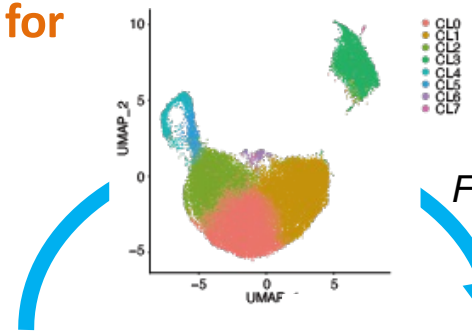
4 Lines of Bone Marrow-Derived hMSCs ↑ a test set

4 Lines of Adipose Tissue-Derived hMSCs

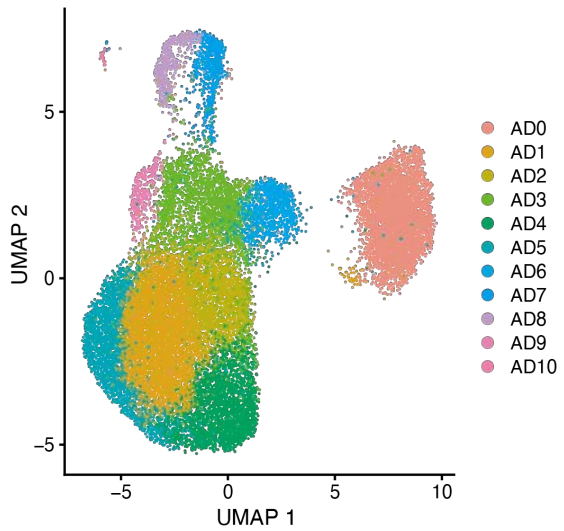
Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

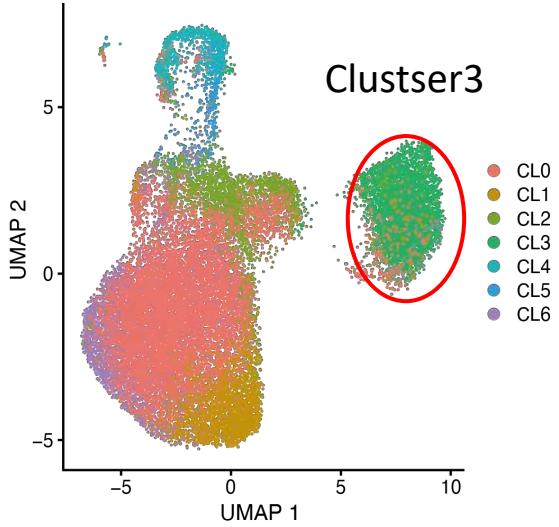
CL3-like cells in adipose-derived MSCs (AD-MSCs)

scRNA-Seq Analysis for AD-MSCs (4 Lines)

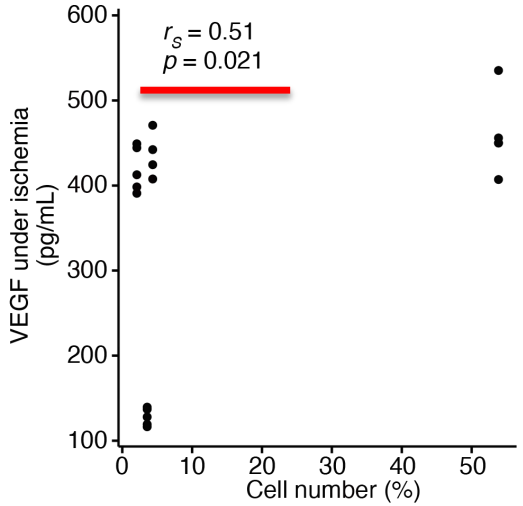


FindTransferAnchors function

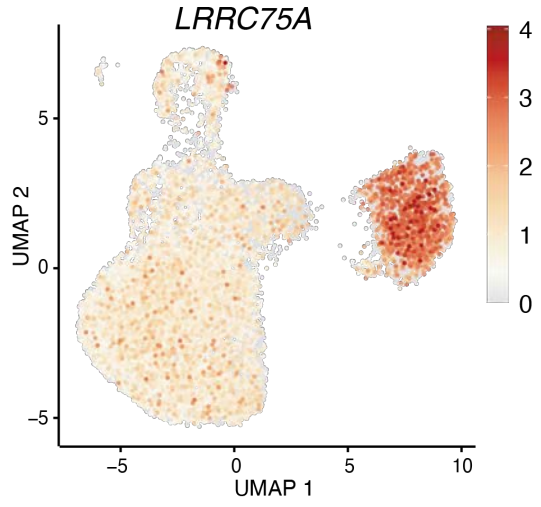




Spearman's Rank Correlation Coefficient (% Cell Distribution *vs.* VEGF Production During Ischemia)



Expression of *LRRC75A* in AD-MSCs



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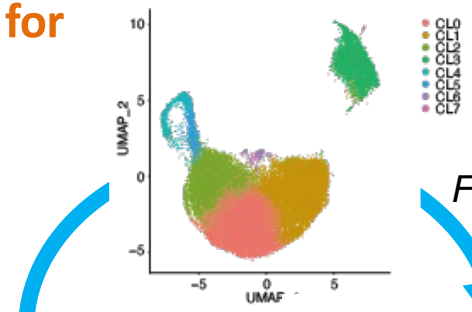
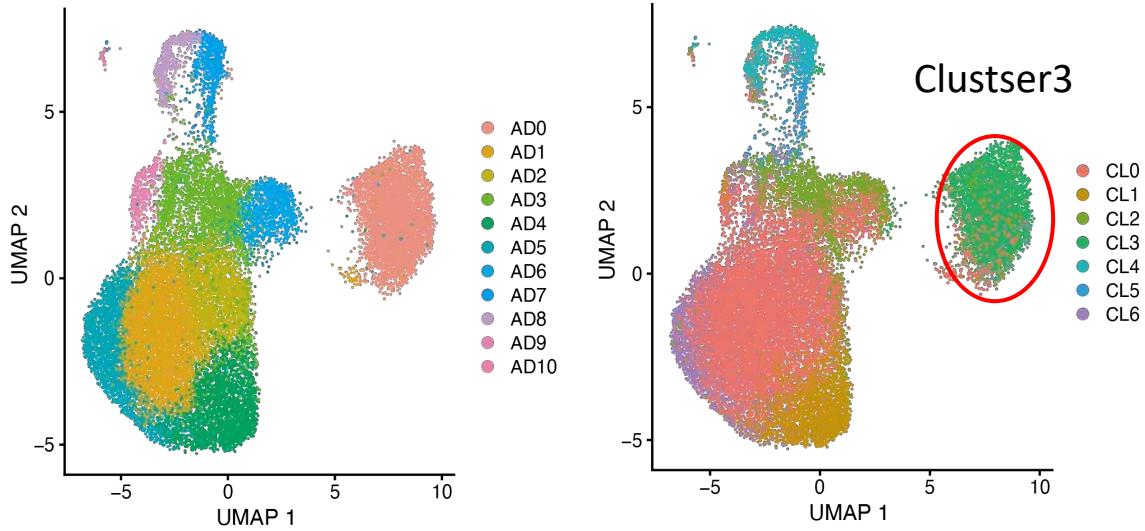
CL3-like cells in adipose-derived MSCs (AD-MSCs)

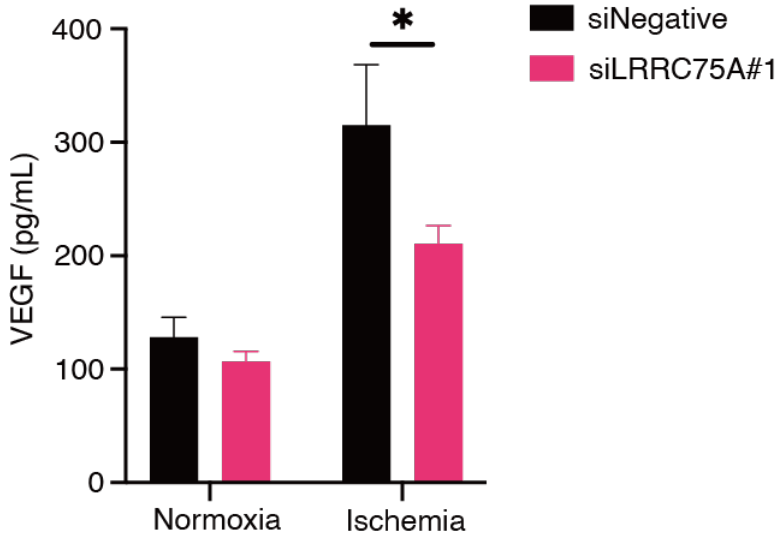
scRNA-Seq Analysis for AD-MSCs (4 Lines)



FindTransferAnchors function



Suppression of VEGF secretion in AD-MSCs by *LRRC75A* siRNA



In AD-MSCs,

- **CL3-like cells contribute to VEGF production under ischemia.**
- **The expression of *LRRC75A* is high in CL3-like cells.**
- **KD of *LRRC75A* suppresses the secretion of VEGF.**

Science

HYPOTHESIS
The expression of *LRRC75A* in CL3 regulates the VEGF secretion from hMSCs under ischemia.

The Populations of hMSCs
(the Ideas in Plato's Philosophy)

hMSCs | ***Bone Marrow-Derived hMSCs*** | ***Adipose Tissue-Derived hMSCs*** | ***Umbilical Cord-Derived hMSCs*** | ***...***

Specific Samples
(Our Study)

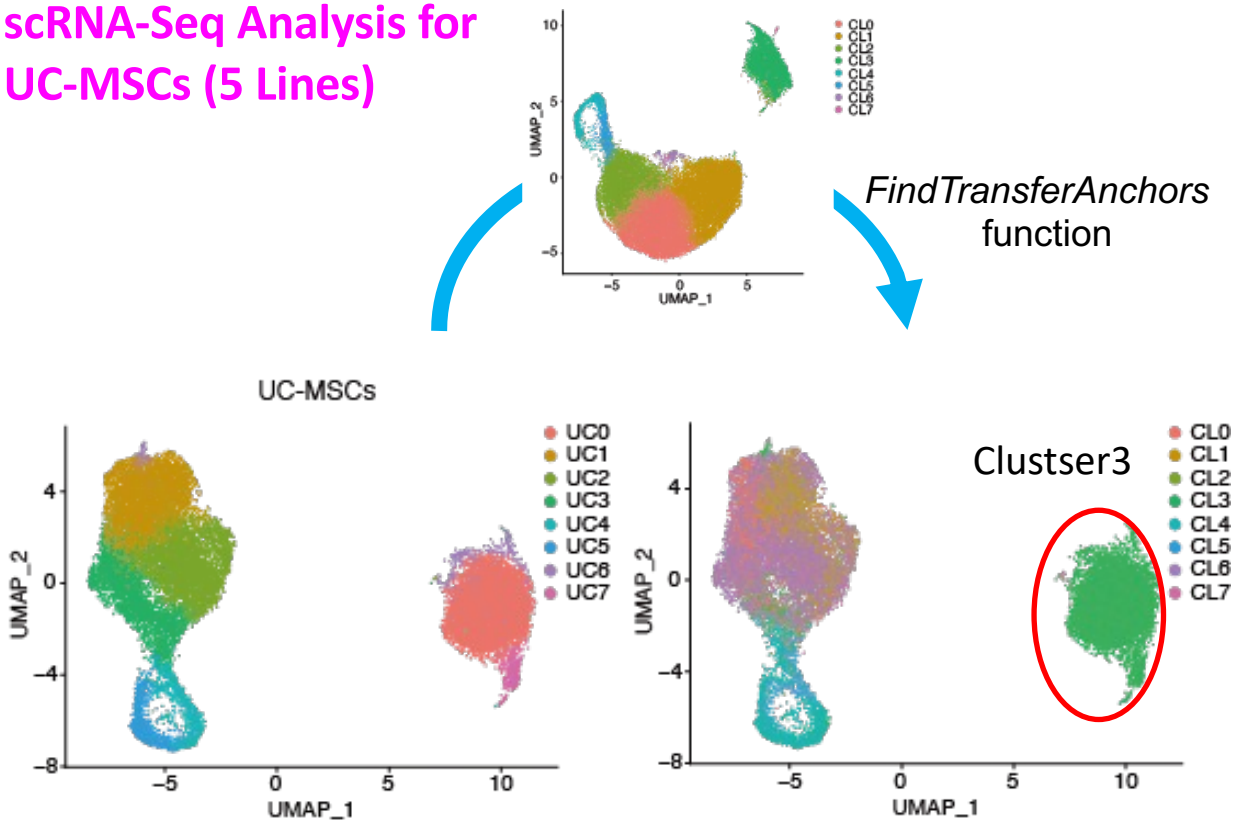
- 11 Lines of Bone Marrow-Derived hMSCs
- 4 Lines of Bone Marrow-Derived hMSCs
- 4 Lines of Adipose Tissue-Derived hMSCs
- 5 Lines of Umbilical Cord-Derived hMSCs

↑ a training set ↑ a test set

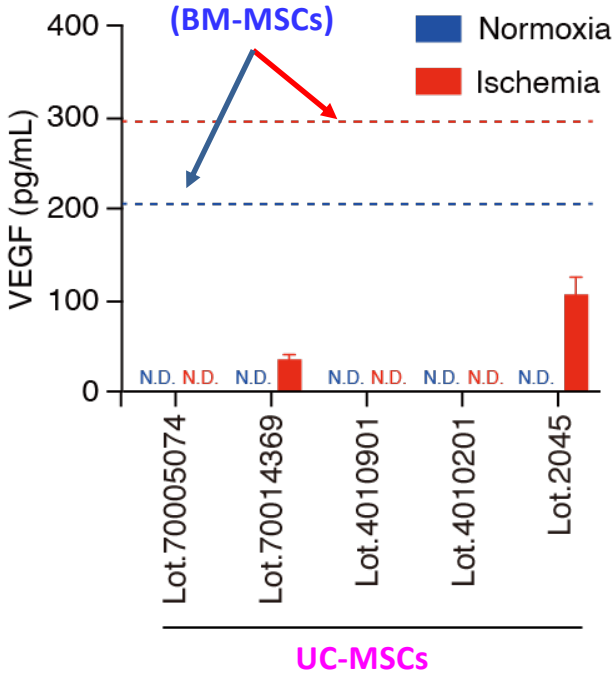
Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

CL3-like cells in umbilical cord-derived MSCs (UC-MSCs)

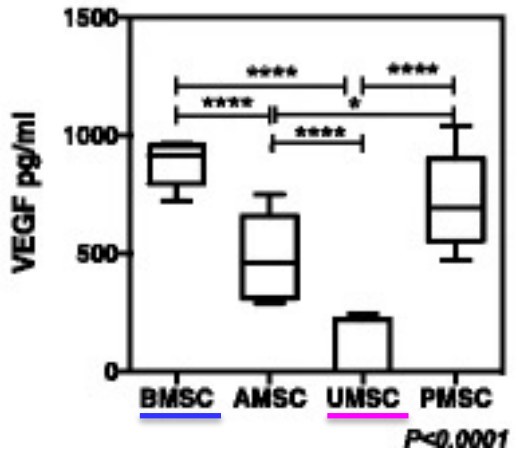
scRNA-Seq Analysis for UC-MSCs (5 Lines)



VEGF Production in Each Line (Normoxia/Ischemia)



Data from Another Group (Normoxia)



Du WJ, *et al. Stem Cell Res Ther.* 2016;7:163.

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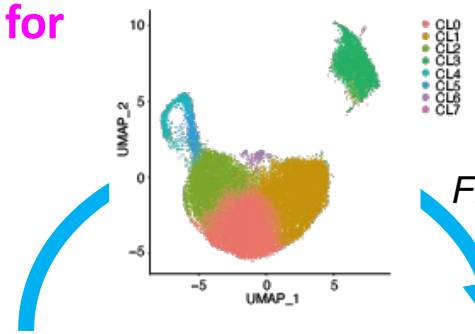
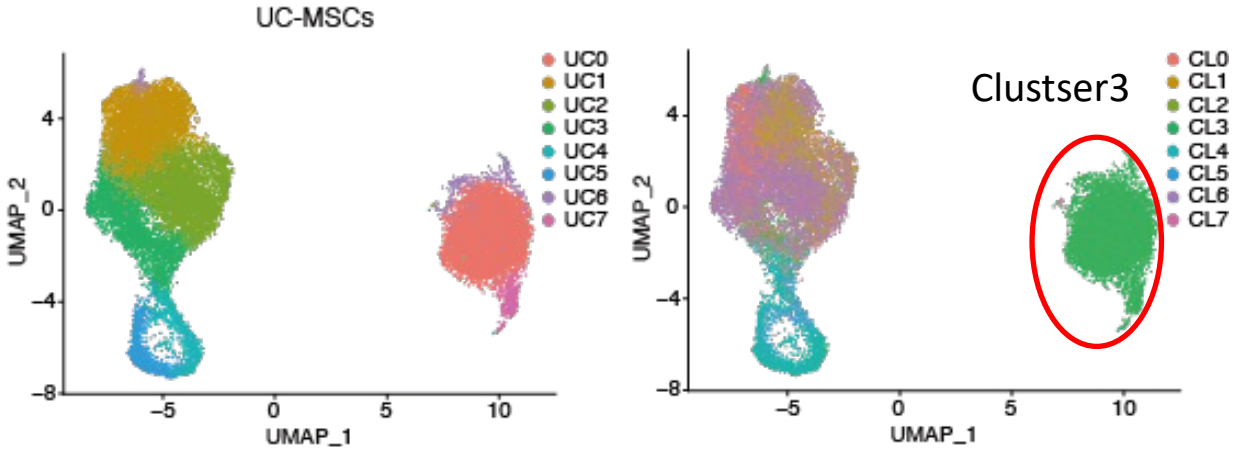
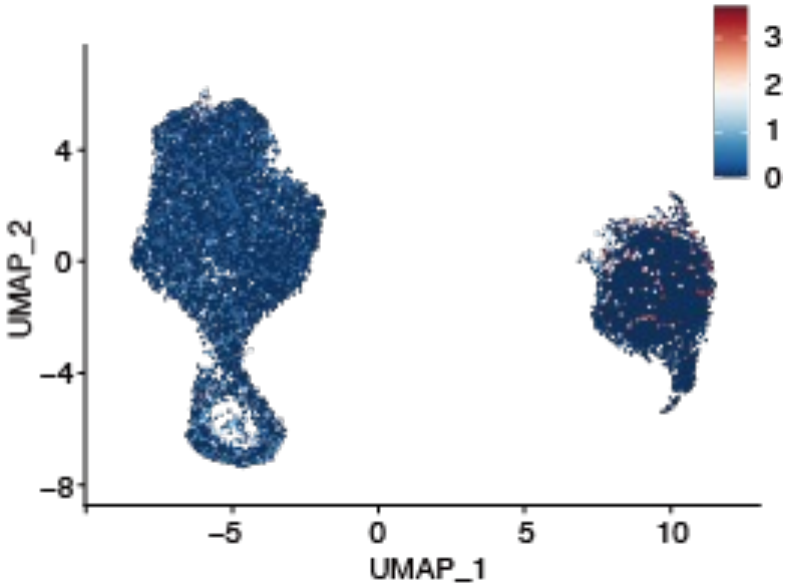


FindTransferAnchors function



Expression of *LRRC75A* in UC-MSCs



Significantly fewer cells express high levels of *LRRC75A* in CL3-like cells.

Science

HYPOTHESIS
The expression of *LRRC75A* in CL3 regulates the VEGF secretion from hMSCs under ischemia.

The Populations of hMSCs
(the Ideas in Plato's Philosophy)

hMSCs | ***Bone Marrow-Derived hMSCs*** | ***Adipose Tissue-Derived hMSCs*** | ***Umbilical Cord-Derived hMSCs*** | ***...***

Specific Samples
(Our Study)

- 11 Lines of Bone Marrow-Derived hMSCs
- 4 Lines of Bone Marrow-Derived hMSCs
- 4 Lines of Adipose Tissue-Derived hMSCs
- 5 Lines of Umbilical Cord-Derived hMSCs

↑ a training set ↑ a test set

Science

VERY LIKELY TO BE TRUE

HYPOTHESIS

The expression of *LRRC75A* in CL3 regulates the VEGF secretion from hMSCs under ischemia.

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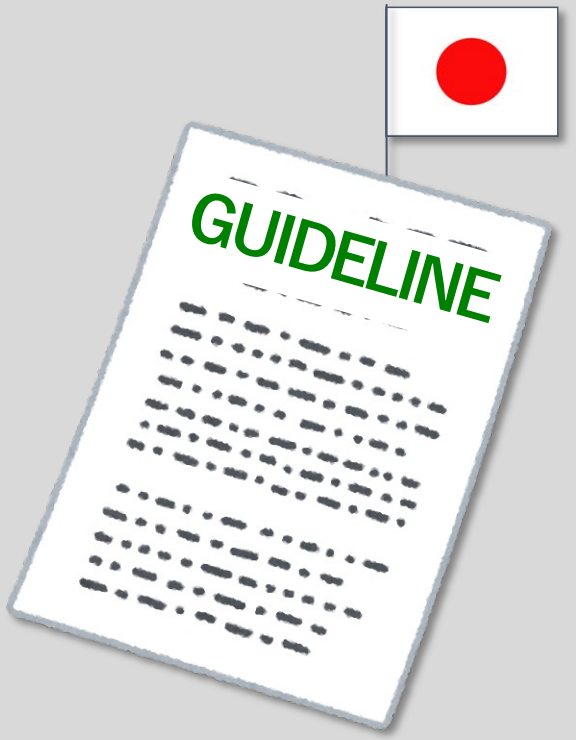
AGENDA (2)

1. **What is Comparability? – An Essential Requirement for Quality when Changing the Manufacturing Process of Cell Therapy Products –**

2. **CQA Mining – A New Approach for Stem Cell Pharmacotaxonomy –**

3. **MHLW Guideline for Comparability of Human Cell-Processed Products Subject to Changes in Their Manufacturing Process**

MHLW Guideline for Comparability of Human Cell-Processed Products Subject to Changes in Their Manufacturing Process

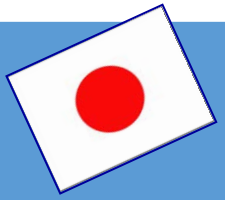


MHLW/PSEHB/MDED Notifications No. 0329-1

[The original Japanese docuememt is already available at: https://www.pmda.go.jp/files/000267916.pdf]

（Draft）Guidance/Guideline Documents on the Comparability Assessment of Cell Therapy Products Subject to Changes in Their Manufacturing Process”

細胞治療製品の製造工程の変更に伴う同等性／同質性評価に関するガイダンス／ガイドライン（案）

Country or Region	EU/UK 	US 	JP 
Title	Questions and answers: Comparability considerations for Advanced Therapy Medicinal Products (ATMP)	Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products [Draft]	Gudeline for Comparability of Human Cell-Processed Products Subject to Changes in Their Manufacturing Process
Source	EMA/CAT/499821/2019 https://www.ema.europa.eu/en/documents/other/questions-answers-comparability-considerations-advanced-therapy-medicinal-products-atmp_en.pdf	FDA/CBER https://www.fda.gov/media/170198/download	MHLW/PSEHB/MDED Notifications No. 0329-1 https://www.pmda.go.jp/files/000267916.pdf [in Japanese]
Issued or Published	December 2019	July 2023	March 2024

AMED Research Project (FY2019-FY2021)
“Research on the Comparability Assessment of Cell-Processed Products Subject to Changes in Their Manufacturing Process”

AMED研究事業（2019年度–2021年度）
「細胞加工製品の製造工程の変更に伴う同等性／同質性評価のあり方に関する研究」

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GUIDELINE FOR COMPARABILITY OF HUMAN CELL-PROCESSED PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

ヒト細胞加工製品の製造工程の変更に伴う同等性／同質性評価に関する指針

1.2 背景

・・・ 既存のICHガイドラインや国内関連法令等には、ヒト細胞加工製品の製造工程変更前後の製品の同等性／同質性を実証するために考慮すべき事項に焦点をあてた記載はなされていない。しかしいくつかのICHガイドラインや国内関連法令等においては、参考となる技術的情報が示されており、これらはヒト細胞加工製品の製造工程変更に伴う評価に際しても有用と考えられる（本文書「参考文献」の項に代表例を示す）。本文書は、主にICH Q5Eガイドライン「生物薬品（バイオテクノロジー応用医薬品／生物起源由来医薬品）の製造工程の変更にともなう同等性／同質性評価」の内容を踏まえつつ、ヒト細胞加工製品の製造工程変更前後の製品の同等性／同質性を実証するために品質特性評価の面からアプローチを行う際に必要な指針を提供するものである。

1.2 Background

… The existing ICH documents and relevant domestic laws and regulations have not specifically addressed considerations for demonstrating comparability of human cell-processed products before and after a change to the manufacturing process. However, several ICH documents and relevant domestic laws and regulations have provided referential technical information that can also be useful for assessing process changes for human cell-processed product. (Representative examples are shown in the "References" section of this document.) This document is intended to provide the guidelines necessary to take an approach in terms of quality characterization to demonstrate the comparability of human cell-processed products before and after a change to the manufacturing process, mainly based on the ICH Q5E guideline "Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process."

ICH Q5E: COMPARABILITY OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

ICH Q5E：生物薬品（バイオテクノロジー応用医薬品／生物起源由 来医薬品）の製造工程の変更にともなう同等性／同質性評価

1.0 緒言
- 1.1 本ガイドラインの目的
- 1.2 背景
- 1.3 適用対象
- 1.4 一般原則

2.0 ガイドライン
- 2.1 同等性／同質性評価作業に関する留意事項
- 2.2 品質に関する留意事項
  - *2.2.1 分析法*
  - *2.2.2 特性解析*

1.0 INTRODUCTION
- 1.1 Objectives of the Guideline
- 1.2 Background
- 1.3 Scope
- 1.4 General Principles

2.0 GUIDELINES
- 2.1 Considerations for the Comparability Exercise
- 2.2 Quality Considerations
  - *2.2.1 Analytical Techniques*
  - *2.2.2 Characterisation*

GUIDELINE FOR COMPARABILITY OF HUMAN CELL-PROCESSED PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

ヒト細胞加工製品の製造工程の変更に伴う同等性／同質性評価に関する指針

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2.2.3 規格及び試験方法

2.2.4 安定性

2.3 製造工程に関する留意事項

2.4 開発段階における製造工程変更時の同等性／同質性

2.5 非臨床試験及び臨床試験に関する留意事項

2.5.1 非臨床試験及び臨床試験を計画する際考慮すべき要素

2.5.2 試験の種類

3.0 用語集

4.0 参考文献

2.2.3 Specifications

2.2.4 Stability

2.3 Manufacturing Process Considerations

2.4 Demonstration of Comparability during Development

2.5 Nonclinical and Clinical Considerations

2.5.1 Factors to be Considered in Planning Nonclinical and Clinical Studies

2.5.2 Type of Studies

3.0 GLOSSARY

4.0 REFERENCES

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1.3.2 適用対象製品の特徴（抜粋）

「適用対象となるヒト細胞加工製品は、『医薬品，医療機器等の品質，有効性及び安全性の確保等に関する法律』に定められる再生医療等製品のうち、人の細胞に培養その他の加工を施すことにより製造されるものを指す。ヒト細胞加工製品は複雑で不均一な生細胞を成分として含むため、CQAを網羅的に観察することができるとは限らないこと、及び遺伝子組換え体細胞又は非組換え体細胞のタンパク質発現系から培養により産生されて高度に精製されることにより製造される生物薬品（バイオテクノロジー応用医薬品／生物起源由来医薬品）のように既存の一連の分析方法を用いての特性解析が可能であるとは限らないことに留意する必要がある。一方、ヒト細胞加工製品の同等性／同質性評価においては、特性解析のみならず、他の要因（例えば変更する製造工程の原理的な差分の説明を含めた評価を加えて判断することもありうる。個別製品の製造工程の変更に伴う同等性／同質性評価の充足性については、製造販売業者は規制当局に相談すること。・・・」

1.3.2 Characteristics of Applicable Products (excerpts)

"Applicable human cell-processed products shall refer to regenerative medicine products specified in the "Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices" that are manufactured by culturing or otherwise processing human cells. Because human cell-processed products contain complex and heterogeneous viable cell components, it should be noted that **their CQAs cannot always be observed comprehensively**, and that **they cannot always be characterized using an existing set of analytical procedures like biopharmaceuticals (biotechnological/biological products)**, which are produced from recombinant or non-recombinant somatic cell protein expression systems by culture and highly purified. **On the other hand, it is also possible that, in the evaluation of the comparability of human cell-processed products, the decision may be made not only on the basis of characterization, but also on other factors (e.g., rationale differences in the manufacturing process to be changed).** As for the sufficiency of the comparability assessment following changes in the manufacturing process of individual products, the manufacturer should consult with the relevant regulatory authority. …"

GUIDELINE FOR COMPARABILITY OF HUMAN CELL-PROCESSED PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

ヒト細胞加工製品の製造工程の変更に伴う同等性／同質性評価に関する指針

GUIDELINE FOR COMPARABILITY OF HUMAN CELL-PROCESSED PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

ヒト細胞加工製品の製造工程の変更に伴う同等性／同質性評価に関する指針

1. 4. 2 ヒト細胞加工製品の同等性／同質性評価作業における基本的考え方（抜粋）

「低分子医薬品やICH Q5Eが対象とするバイオテクノロジー応用医薬品とは異なり、ヒト細胞加工製品の場合、有効成分である細胞の品質特性を分子レベルで網羅的に解析及び提示することが著しく困難であり、その一方で細胞集団の不均一性、並びに周辺環境の影響による細胞の形質の変化（例えば分化や脱分化）及び周辺環境に対する細胞の応答（例えば生理活性物質の放出）などを検討することが重要である。

従って、ヒト細胞加工製品では、現時点の技術で測定可能な品質特性をすべて挙げたとしても、有効性及び安全性の同等性／同質性を十分に保証するために必要な必須品質特性すべてを完全に網羅・同定できているとは限らない。 ・・・」

1.4.2 Basic Concepts for Comparability Exercise of Human Cell-Processed Products (excerpts)

“Unlike low-molecular-weight pharmaceuticals and biotechnological products subject to ICH Q5E, **for human cell-processed products, there are significant difficulties in comprehensively analyzing and presenting the quality attributes of cells as the active ingredient at a molecular level**, whereas **it is important to examine the heterogeneity of cell population, phenotypical changes attributable to the surrounding environment (e.g., differentiation and dedifferentiation), and cellular responses to the surrounding environment (e.g., release of bioactive substances)**.

Therefore, **even if all quality attributes measurable with current technology are listed for human cell-processed products, it may not always be assured that all critical quality attributes necessary to fully assure the comparability of efficacy and safety have been completely covered and identified.** …”

Conclusions 2

- Because of the complexity and heterogeneity of the cells as the active ingredient of cell therapy products (CTPs), even if we list all of the quality attributes that we can recognize, it may not be possible to fully identify and encompass all of the CQAs necessary to assure the efficacy and safety of the CTPs after their manufacturing changes.
- **Avoidance of false negatives is critical in the evaluation of safety-related CQAs, and it is important to understand the sensitivity and specificity of the test methods.**
- **Identification of cell subpopulations and biomarkers that correlate with potency/efficacy through single-cell transcriptome analysis and other methods, and use of these as CQAs, will help establish manufacturing methods to reproducibly produce effective CTPs.**
- **In Japan, the guideline document for the comparability assessment of CTPs subject to changes in their manufacturing process, which is based on ICH Q5E, has just been issued.**

- 細胞治療製品（CTP）の有効成分である細胞は複雑で不均質であるため、認知しうる品質特性をすべて列挙したとしても、製造変更後のCTPの有効性と安全性を保証するために必要なCQAをすべて特定・網羅することはできない可能性がある。
- **安全性関連のCQAの評価においては偽陰性の回避が最重要課題であり、試験法の感度や特異度を把握することが重要である。**
- **シングル・セル・トランスクリプトーム解析などにより、力価／有効性と相関する細胞亜集団やバイオマーカーを同定し、これらをCQAとすることは、有効な細胞治療製品を再現性高く製造する製法の確立に役立つと考えられる。**
- **日本では、ICH Q5Eをもとに、CTPの製法変更前後の品質の同等性評価に関するガイドラインが最近発出された。**

ACKNOWLEDGMENTS (Tumorigenicity)

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- **The Secretariat of the Forum for Innovation in Regenerative Medicine (FIRM)**
- **AMED Regulatory Science Division and Regenerative Medicine R&D Division**
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- Rumi Sawada[1], Satoko Matsuyama[1,5], Hideya Kawaji[3,6], Takeya Kasukawa[3], Masayoshi Itoh[3],
- Akifumi Matsuyama[5], Jay W. Shin[3,7], Akihiro Umezawa[2], Jun Kawai[3,8]

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- [7] Genomic Institute of Singapore, Agency for Science, Technology and Research, Singapore
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- **Funded by**

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Thank you for your attention!

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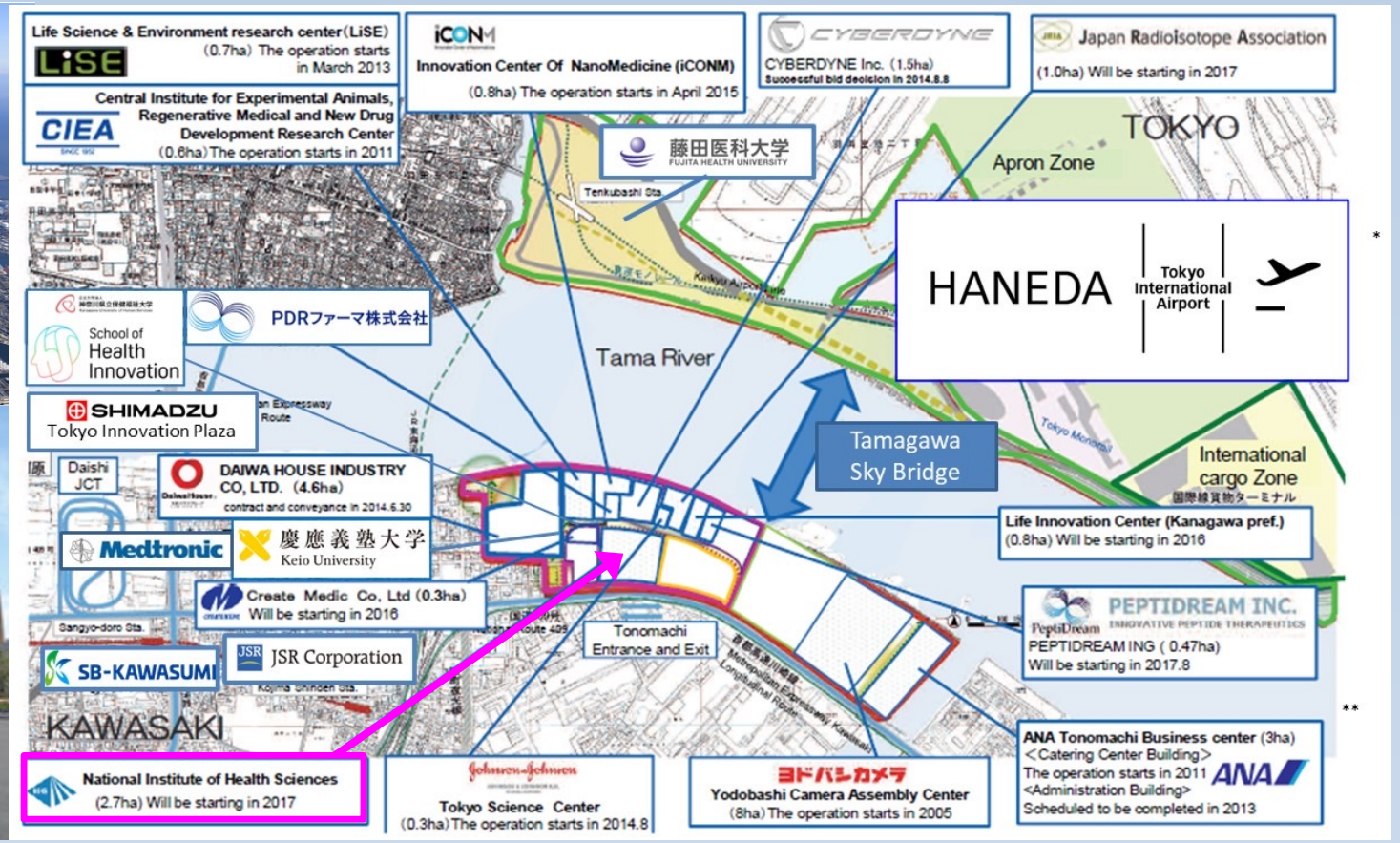

* https://www.oag.com/hubfs/air-canada-787.jpg

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