September 28th, 2023

13th Global Summit on Regulatory Science

Safety assessment of cell-based therapeutic products derived from iPS cells

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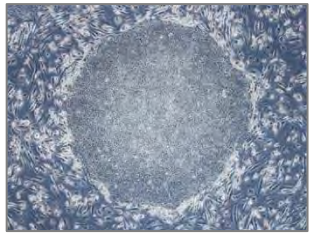
Stem cells used for cell therapy/regenerative medicine

Stem cells

Pluripotent stem cells

Embryonic stem cells (ES cells)
Induced pluripotent stem cells (iPS cells)

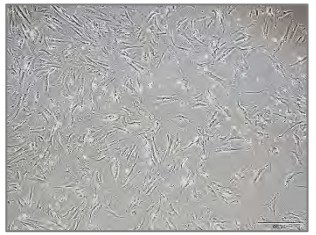
Ability to differentiate into various cell types *in vitro*
Infinite number of cell division



Somatic stem cells

Hematopoietic stem cells
Mesenchymal stem cells
Neural stem cells, etc.

Presence in adult tissues, ability to differentiate into limited types of cells *in vitro*
Limited number of cell division



Development of regenerative medicine using human iPS/ES cells

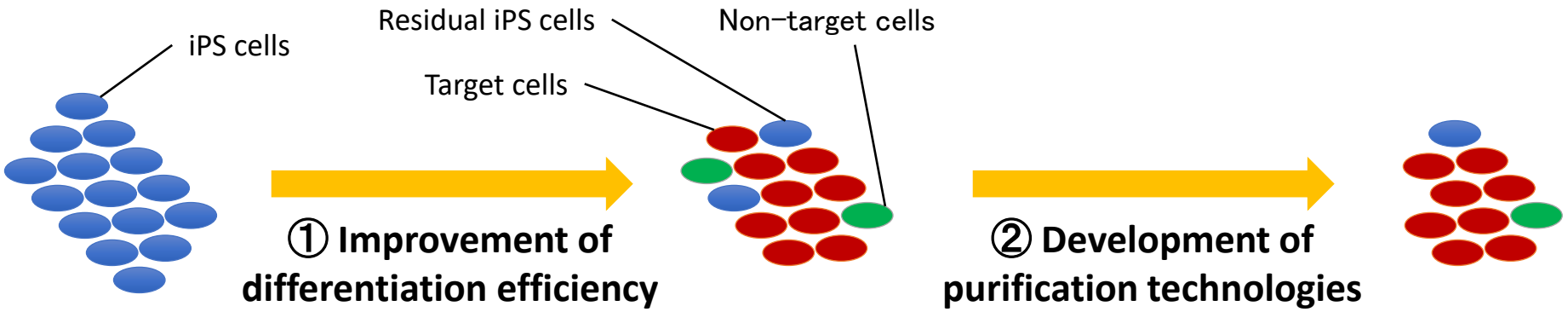
Clinical research and clinical trials using cells derived from human iPS/ES cells approved in Japan ［Mar, 2023］

Transplanted cells	Raw materials	Diseases	Facilities	Clinical research /clinical trials	Approval	First in human
Retinal Pigment Epithelium	Autologous iPS cells	Wet age-related macular degeneration	IBRI	Clinical research	2013	2014
Retinal Pigment Epithelium	Allogeneic iPS cells	Wet age-related macular degeneration	Kobe City Medical Center, *etc.*	Clinical research	2017	2017
Dopaminergic neuron	Allogeneic iPS cells	Parkinson's diseases	Kyoto University	Investigator-initiated trial	2018	2018
Platelets	Autologous iPS cells	Aplastic anemia	Kyoto University	Clinical research	2018	2019
Corneal epithelial cells	Allogeneic iPS cells	Limbal Stem Cell Deficiency	Osaka University	Clinical research	2019	2019
Hepatocytes	ES cells（Allogeneic）	Congenital urea cycle disorder	NCCHD	Investigator-initiated trial	2019	2019
Cardiomyocytes	Allogeneic iPS cells	Ischemic cardiomyopathy	Osaka University	Investigator-initiated trial	2019	2020
Neural progenitor cells	Allogeneic iPS cells	Spinal cord injury	Keio University, *etc.*	Clinical research	2019	2021
Retinal photoreceptor cells	Allogeneic iPS cells	Retinitis pigmentosa	Kobe City Eye Hospital	Clinical research	2020	2020
NKT cells	Allogeneic iPS cells	Relapsed/advanced head and neck cancer	Chiba University, RIKEN	Investigator-initiated trial	2020	2020
Cartilage cells	Allogeneic iPS cells	Knee cartilage injury	Kyoto University	Clinical research	2020	2021
Cardiomyocytes	Allogeneic iPS cells	Dilated cardiomyopathy	Keio University	Clinical research	2020	–
Retinal Pigment Epithelium	Allogeneic iPS cells	Retinal pigment epithelial insufficiency	Kobe City Eye Hospital	Clinical research	2021	2022
AntiGPC3-CAR NK cells	Allogeneic iPS cells	Ovary cancer	Kyoto University, NCC	Investigator-initiated trial	2021	2021
Corneal endothelial cells	Allogeneic iPS cells	Bullous keratopathy	Keio University	Clinical research	2021	2023
Platelets	Allogeneic iPS cells	Thrombocytopenia	Megakaryon, Kyoto University, CiRA-F	Sponsor-initiated trial	2021	2022
Cardiomyocytes	Allogeneic iPS cells	Ischemic heart diseases	Heartseed, Keio University	Sponsor-initiated trial	2021	2023

Safety and quality of cell therapy products derived from human iPS cells

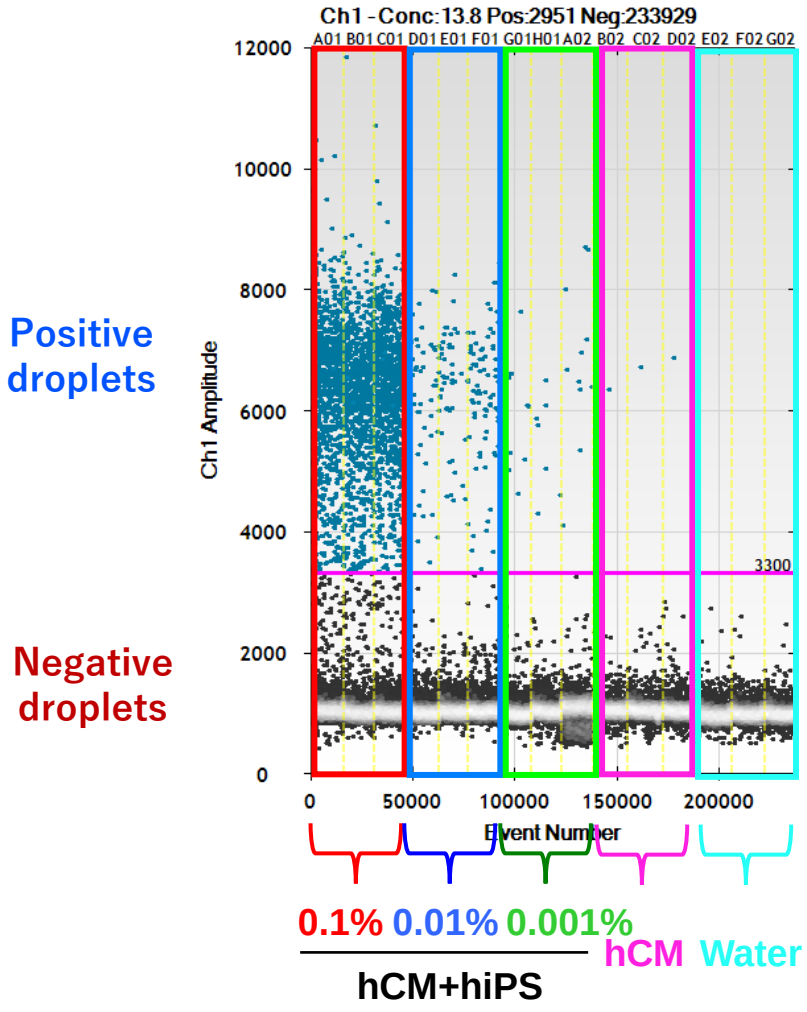
- Since undifferentiated ES/iPS cells have **the ability to form teratoma**, **residual ES/iPS cells** have a potential risk of tumorigenicity.
- Tumorigenic **transformed cells** possibly appear, associated with cell processing.

It needs to prevent contamination with residual undifferentiated ES/iPS cells and tumorigenic cells.

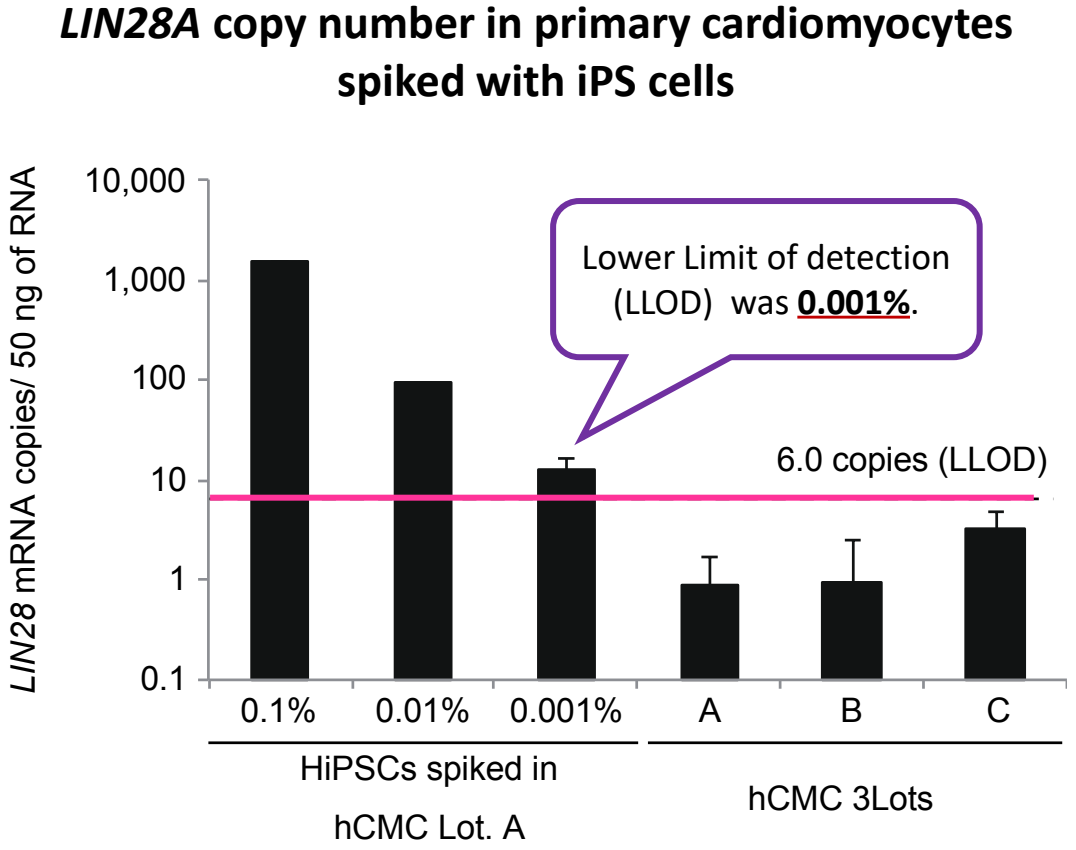


③ Testing methods to check removal/residue of undifferentiated ES/iPS cells and tumorigenic cells are essential for development of products.

Detection of *LIN28A* mRNA using droplet digital PCR (ddPCR) assay



Kuroda et al., Regen Ther. 2015



To confirm validity of the testing methods, the variability among laboratories needs to be examined in multisite studies at the international level.

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.

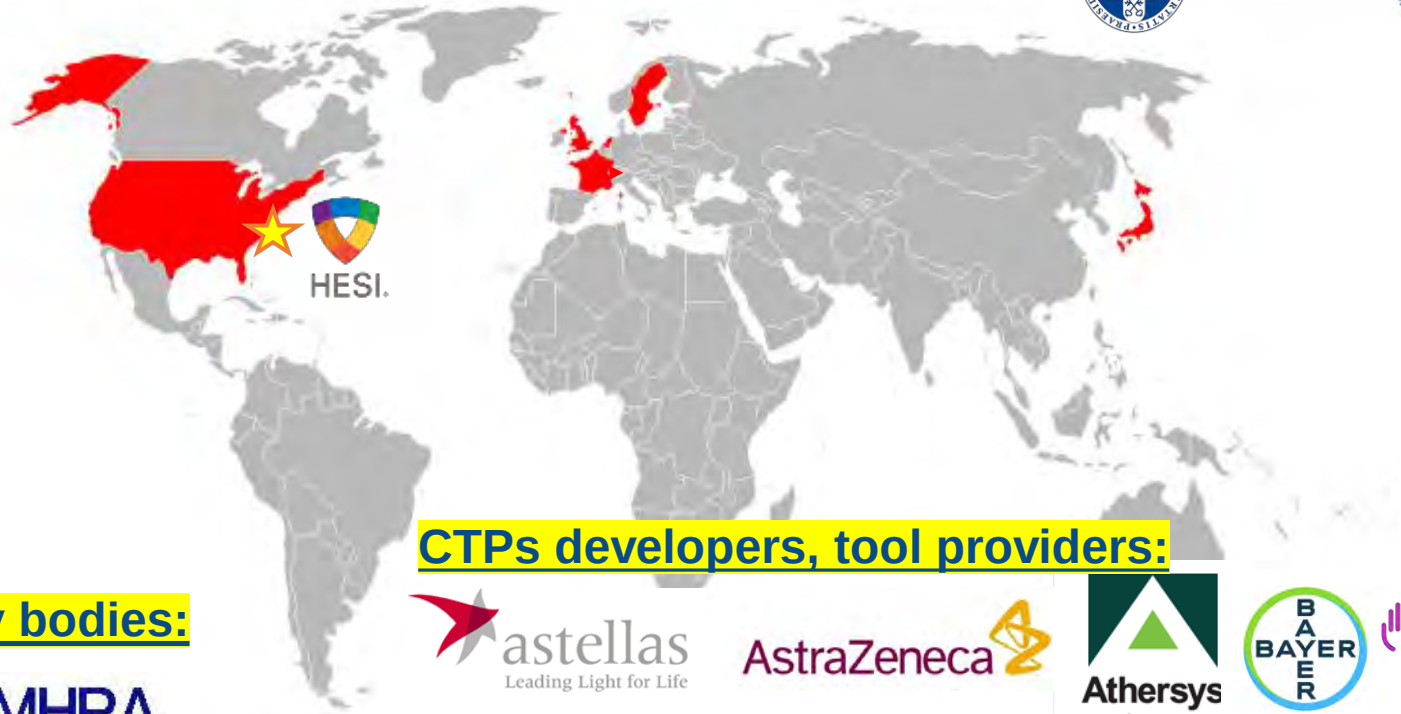
Stanford Cardiovascular Institute

UCL

Universiteit Leiden

Wageningen University & Research

The University of Sydney



HESI.

CT-TRACS Members

(2022 data)

>100 Participants

>35 Organizations

Government & Regulatory bodies:

FDA

cBG MEB Medicines Evaluation Board

MHRA

NIH National Institutes of Health

NIHS

NIST National Institute of Standards and Technology

CTPs developers, tool providers:

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

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
Ridhi Buhania (Bayer)
Vladimir Ponomarev (MSKCC)

Collaboration with Japanese Consortium
FIRM-CoNCEPT & AMED MEASURE

TUMORIGENICITY WG
Silvana Libertini (Novartis)
Charlotte de Wolf (CBG-MEB)

- Cell Tracking DB
- Education & Outreach
- Research:PhD studentship (co-sponsored)
- Multi-site studies for residual iPSCs
  - ddPCR
  - HEC assay
- Advanced sequencing for off target (CRISPR)
- IL-2 for CAR-T
- SACF/GILA (CRISPR)

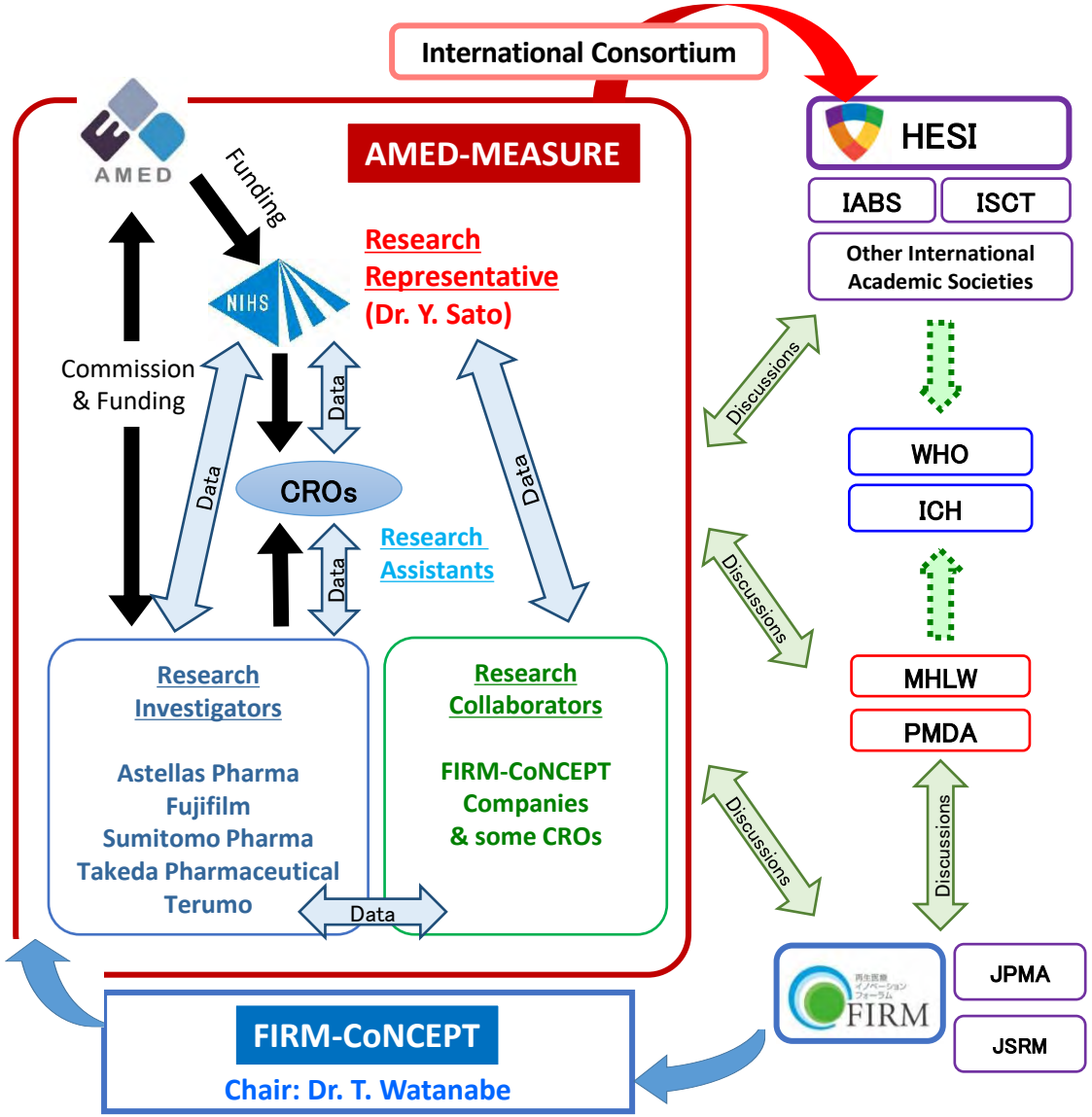
https://hesiglobal.org/cell-therapy-tracking-circulation-safety-ct-tracs/

Courtesy of Dr. Lucilia Mouriès, HESI

FIRM-CoNCEPT & AMED-MEASURE

What is “MEASURE”?

AMED-supported Japanese **public-private partnership research** on standardization and validation of **methods for tumorigenicity assessment** of cell therapy products



HESI CT-TRACS ddPCR team participants

International multi-site studies for validation of the ddPCR method to detect residual undifferentiated iPSCs in cell therapy products

- Using iPSC-derived differentiated cells (iCell cardiomyocytes, FCDI) and iPSCs (ChiPSC18, Takara Bio) for spiking experiments
- Selecting several target genes expressed in undifferentiated iPSCs but not in iPSC-derived CMs as markers with ddPCR analysis on top of LIN28A

ddPCR_Step-1

Objectives

To confirm the variance regarding ddPCR analytical process at multiple sites through using the same samples prepared at one site (NIHS).

Actions

- Purchase iPSC and iCell® (Cardiomyocyte)
- Prepare spiked cells
- Conduct preliminary studies to estimate as follows
  - condition to prepare spiked cells
  - spiked iPSC conc. range,
  - optimal biomarkers (>3) on top of LIN28.
- Deliver RNA samples isolated from spiked cells, human heart total RNA 3 lots, and probe & primer sets to each facility

Actions

- Agree the number of biomarker
- Receive RNA samples isolated from spiked cells, human heart total RNA 3 lots, and probe & primer sets from NIHS
- Analyze samples prepared at NIHS based on the protocol
- Keep each probe & primer set for study-2

iPSC marker gene identification

Cytotherapy 25 (2023) 59–67

Contents lists available at ScienceDirect

CYTOTHERAPY

journal homepage: www.isct-cytotherapy.org

FULL-LENGTH ARTICLE
Basic Research

Identification of marker genes to monitor residual iPSCs in iPSC-derived products

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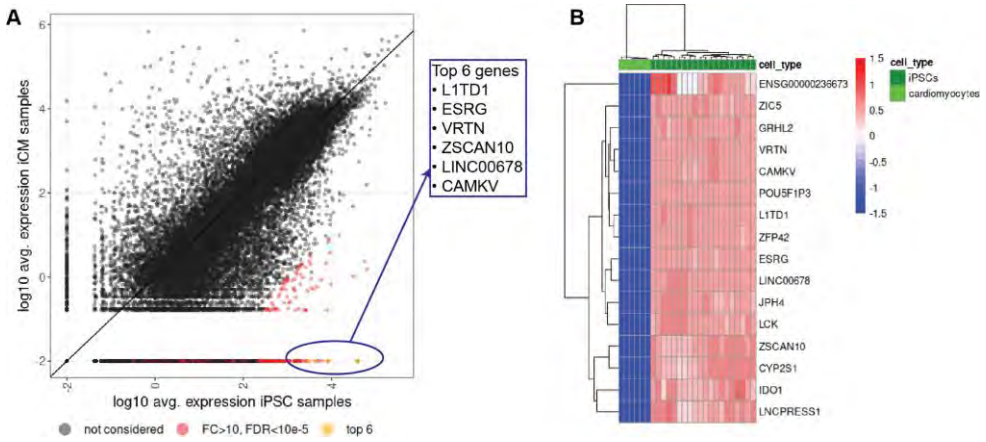


RT-qPCR analysis

	Samples	*LIN28A*	*OCT3/4*	*NANOG*	*ESRG*	*L1TD1*	*ZSCAN10*	*CNMD*	*LINC00678*	*VRTN*	*CAMK5*	*EPHA1*
iPSCs	ChiPSC18	100	100	100	100	100	100	100	100	100	100	100
	201B7	121	111	89	76	119	134	306	102	121	136	122
iPSC-derived cells	iCell CM	0.012	0.005	0.137	ND	ND	0.002	ND	ND	ND	ND	ND
	iCell Neurons	2.668	0.008	0.060	ND	0.024	0.031	0.047	ND	0.092	60.82	ND
	iCell MSC	ND	0.007	0.018	ND	0.413	0.005	0.270	ND	ND	0.037	ND
Tissues & somatic stem cells	hHeart	ND	0.006	0.016	ND	ND	ND	ND	ND	ND	ND	ND
	hSpinal cord	ND	0.013	0.069	ND	0.014	ND	0.292	ND	0.045	3.047	ND
	hMSC	ND	0.005	0.006	ND	ND	ND	ND	ND	ND	ND	ND

ESRG, CNMD, LINC00678, L1TD1, VRTN, ZSCAN10 & LIN28A

Performance of ddPCR assay targeting iPSC marker genes

	CNMD	*ESRG*	*LINC00678*	*LIN28A*	*L1TD1*	*VRTN*	*ZSCAN10*
Relationship with iPSC standards	++	+++	+++	++	+++	+++	++
Relationship with spiked iPSC conc.	++	+++	+++	+++	++	+	++
Precision of spiked iPSCs	++	+++	++	+++	+++	+++	++
Limit of detection (LOD)	++	+++	+++	+++	+	+	++
Limit of quantification (LOQ)	++	+++	+++	+++	+	+	++
Signals of iCell CM as the negative control	++	+++	+++	+++	+	+	+++
Precision of positive control	++	++	++	+++	+++	++	++
Target abundance	+	+++	+	++	++	+	+
Overall score	15	**23**	**20**	**22**	16	13	15

***ESRG*, *LINC00678* and *LIN28A* were ranked as the top 3 marker genes.**

Repeatability and reproducibility of the results in Step-1

$$s_R^2 = s_L^2 + s_r^2$$

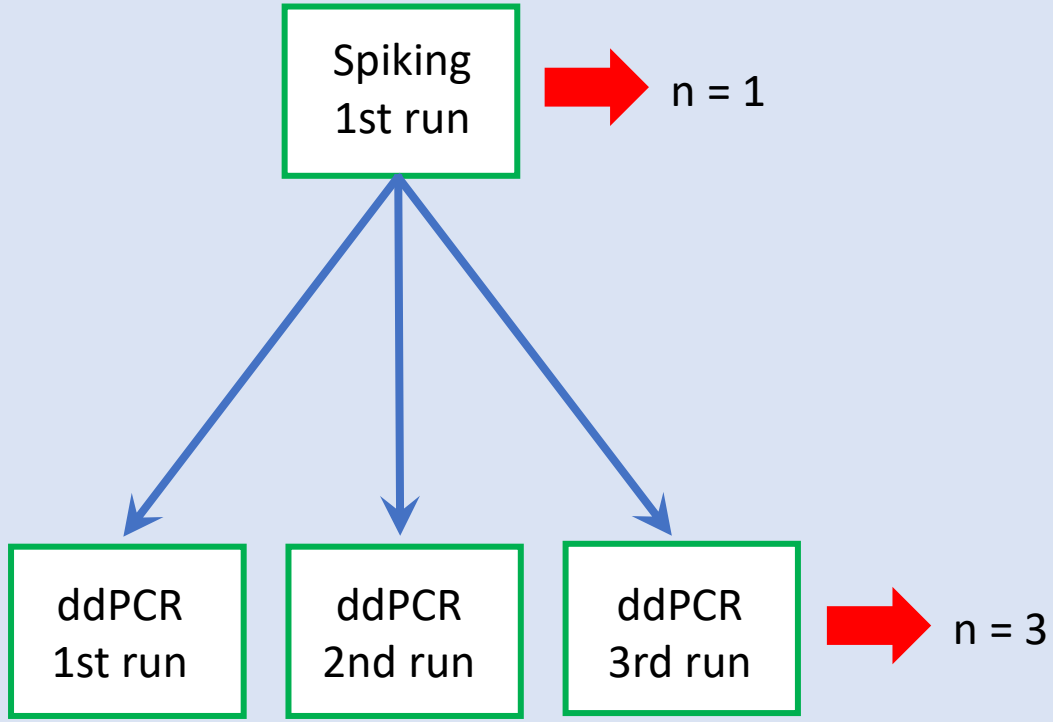
s_R^2: reproducibility variance
s_L^2: between-laboratory variance
s_r^2: repeatability variance

	Repeatability CV			Reproducibility CV		
Gene name	***ESRG***	***LINC00678***	***LIN28A***	***ESRG***	***LINC00678***	***LIN28A***
iCell CM 0.1%	4%	9%	12%	4%	9%	15%
iCell CM 0.03%	7%	9%	9%	9%	10%	18%
iCell CM 0.01%	7%	17%	13%	11%	17%	13%
iCell CM 0.003%	10%	**23%**	11%	12%	**29%**	15%
iCell CM 0.001%	11%	38%	29%	**14%**	38%	30%
iCell CM 0.0003%	**29%**	65%	**19%**	47%	86%	**24%**
iCell CM 0%	35%	79%	27%	79%	106%	27%

≤ 30% of coefficient of variation (CV) values are highlighted in blue or red.

To evaluate repeatability and reproducibility of sample preparation

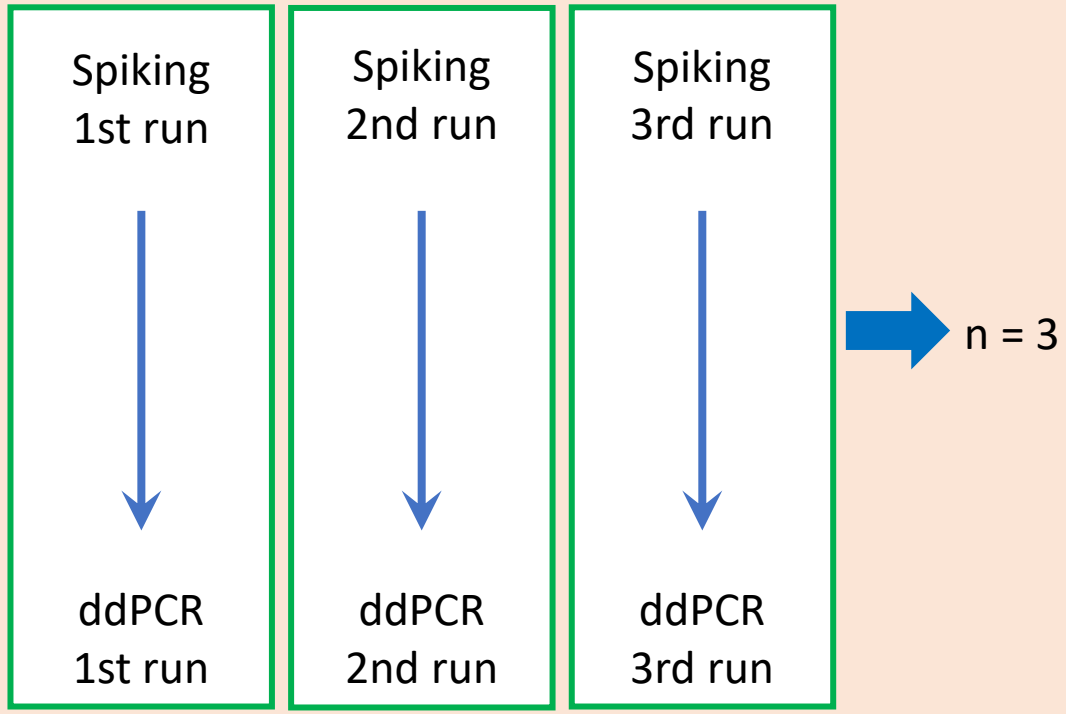
Step 1



Variation of 3 runs of ddPCR with the same samples was observed in Step-1 study.

Repeatability and reproducibility of sample preparation is not evaluated.

Step 2



Variation of 3 runs of ddPCR measurement is known in Step-1 study. Therefore, one run of spiking should correspond to one run of ddPCR measurement.

Repeatability and reproducibility of sample preparation can be evaluated.

ddPCR_Step-2

Arm 1

To focus on variability of spiking (validation of the test method)

NIHS

Sumitomo Pharma

Takeda

Arm 2

To explore more PSC markers suitable for PSC-derived cardiomyocytes and housekeeping genes (establishing a panel of markers)

NOVARTIS

CATAPULT Cell and Gene Therapy

AstraZeneca

Bristol Myers Squibb™

BAYER

Variability regarding entire analytical process

	ESRG	Spiked iPSCs (%)		*LINC00678*	Spiked iPSCs (%)		*LIN28A*	Spiked iPSCs (%)	
		0.01	0.003	0.001	0.01	0.003	0.001	0.01	0.003	0.001
iPSC spiking at each run	**Repeatability CV**	**15.1%**	**32.7%**	**37.1%**	**21.1%**	**38.4%**	**38.4%**	**20.1%**	**27.8%**	**41.0%**
	Reproducibility CV	**47.8%**	**41.1%**	**45.0%**	**55.9%**	**53.5%**	**51.6%**	**44.5%**	**32.5%**	**56.6%**
Using the same samples	Repeatability CV_step1	6.8%	9.6%	10.8%	16.7%	23.4%	37.7%	12.9%	11.0%	29.4%
	Reproducibility CV_step1	10.6%	12.2%	14.2%	16.7%	28.8%	37.7%	12.9%	15.0%	29.8%

- iPSC spiking at each run in 3 facilities increased CV values of repeatability and reproducibility.
- Repeatability CV showed dependency of iPSC doses, whereas reproducibility CV was relatively constant in any doses.
- These results suggest that contribution of between laboratory variance to reproducibility in Step 2 is high compared to Step 1 study.

Summary

- In Step 1 study, different concentrations of iPSCs were spiked into iPSC-derived cardiomyocytes at 1 site, and the same RNA samples were analyzed by ddPCR at multiple sites.

- The ddPCR assay targeting to *ESRG*, *LINC00678*, and *LIN28A* detected undifferentiated iPSCs in CTPs with LOD of 0.001%, 0.003% and 0.0003%, respectively, and offers a highly sensitive and robust detection.

- In Step 2 study, the entire process, including spiking, was conducted at each facility. The CV values of repeatability and reproducibility in Step 2 study were consistently higher compared to Step 1 study.

- The main contribution to variability between laboratories is the iPSC-spiking procedure, and not the ddPCR measurement.

- The validated ddPCR assay would be generally applicable for tumorigenicity evaluation of iPSC-derived CTPs with appropriate marker genes.

Acknowledgements

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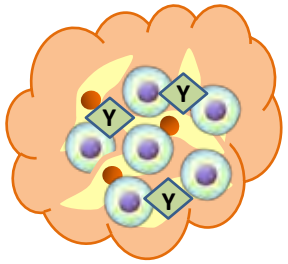
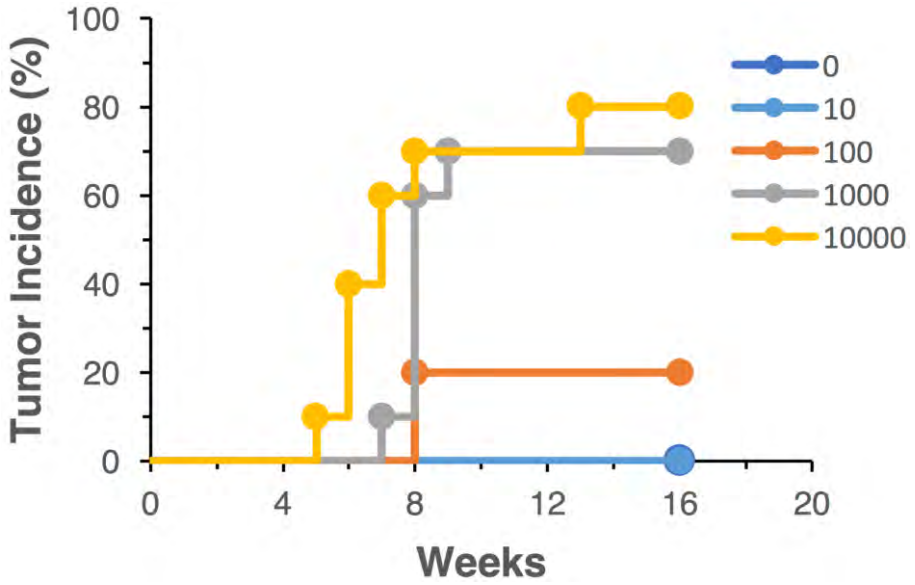
Supplementary

Tumorgenicity testing using NOG mice subcutaneously transplanted with iPSCs



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% and 0.006%, respectively.

Yasuda et al., PLoS One 2018