

# Design of Tumorigenicity Test for Pluripotent Stem Cell-derived Cell Product

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Foundation for Biomedical Research and Innovation (FBRI)

Center for Cell Therapy

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# Introduction of FBRI

(Foundation for Biomedical Research and Innovation)

- A Leading Translational Research Center in Japan -



# Translational Research Center in Kobe



# Cell therapy developed and conducted in FBRI

## Treatment of ischemic lower limb by mobilized peripheral CD34+ cells

Dr. Kawamoto IBRI Hospital

PIIa investigator-led clinical trial  $\Rightarrow$ TT, sponsored pivotal clinical trial

## Treatment of unhealed fracture with mobilized peripheral CD34+ cells

Dr. Kuroda Kobe Univ. Orthopedic Dept, IBRI hospital

Clinical Study 14 cases  $\Rightarrow$  Investigator-led Clinical Trial PIIa

## Repair of cornea with autologous buccal mucosa sheet

Dr. Sotozono Kyoto Prefectural Medical College, IBRI Hospital

Clinical study  $\Rightarrow$  TT and sponsored clinical trial PIIa

## Treatment traumatic knee with cultured autologous cartilage

Dr. Kuroda Kobe Univ. Orthopedic Dept, IBRI hospital

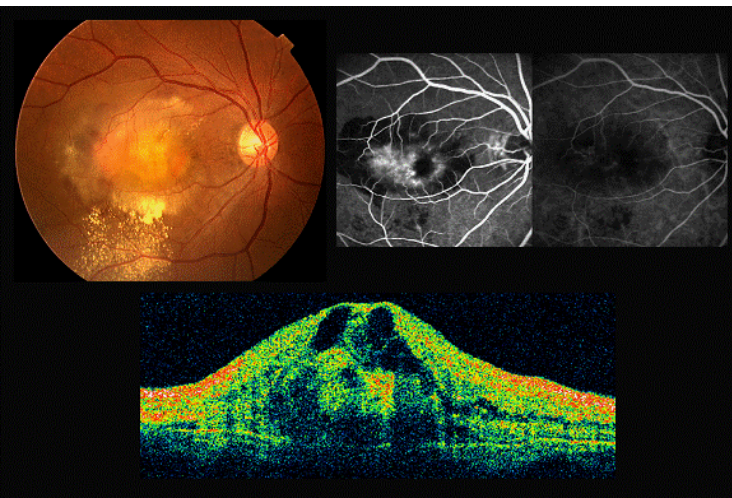
PIIa investigator-led clinical trial  $\Rightarrow$ TT, sponsored pivotal clinical trial

# Treatment of wet type AMD with iPSC derived-RPE sheets

Dr. Takahashi, Team leader of Riken CDB, Dr. Kurimoto IBRI Hospital

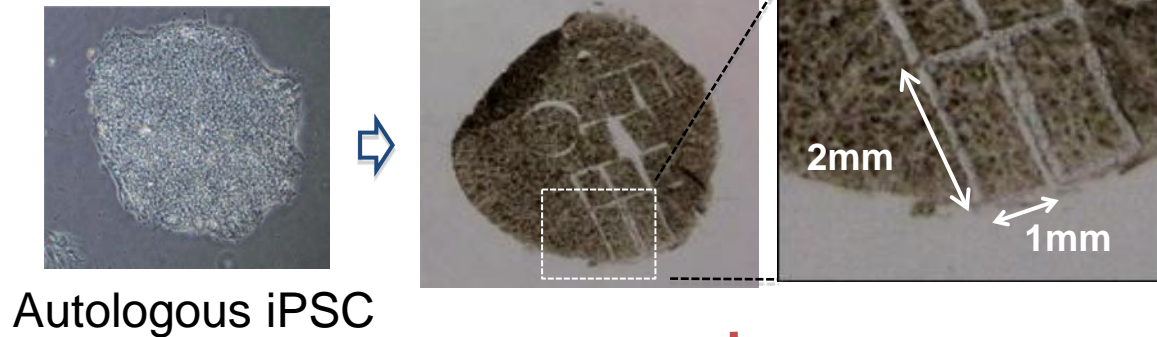
## Target disease

wet type Aged Macula Degeneration

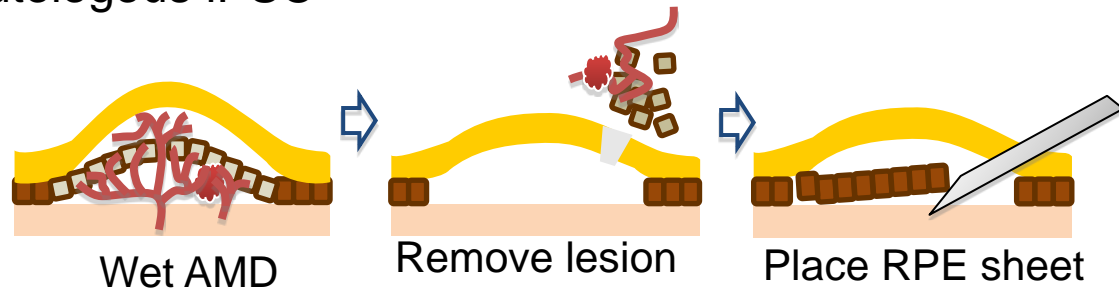


## procedure

Retina Pigment Epithelium (RPE) sheet from autologous iPSC from patient fibroblasts



Autologous iPSC



Tumorigenicity test of iPSC-derived RPE has planned and conducted as preclinical test since 2010

# However

**There is **No** internationally recognized guideline for Tumorigenicity test for cell product used for cell transplantation.**

WHO Technical Report Series No.878, Annex 1. 1998

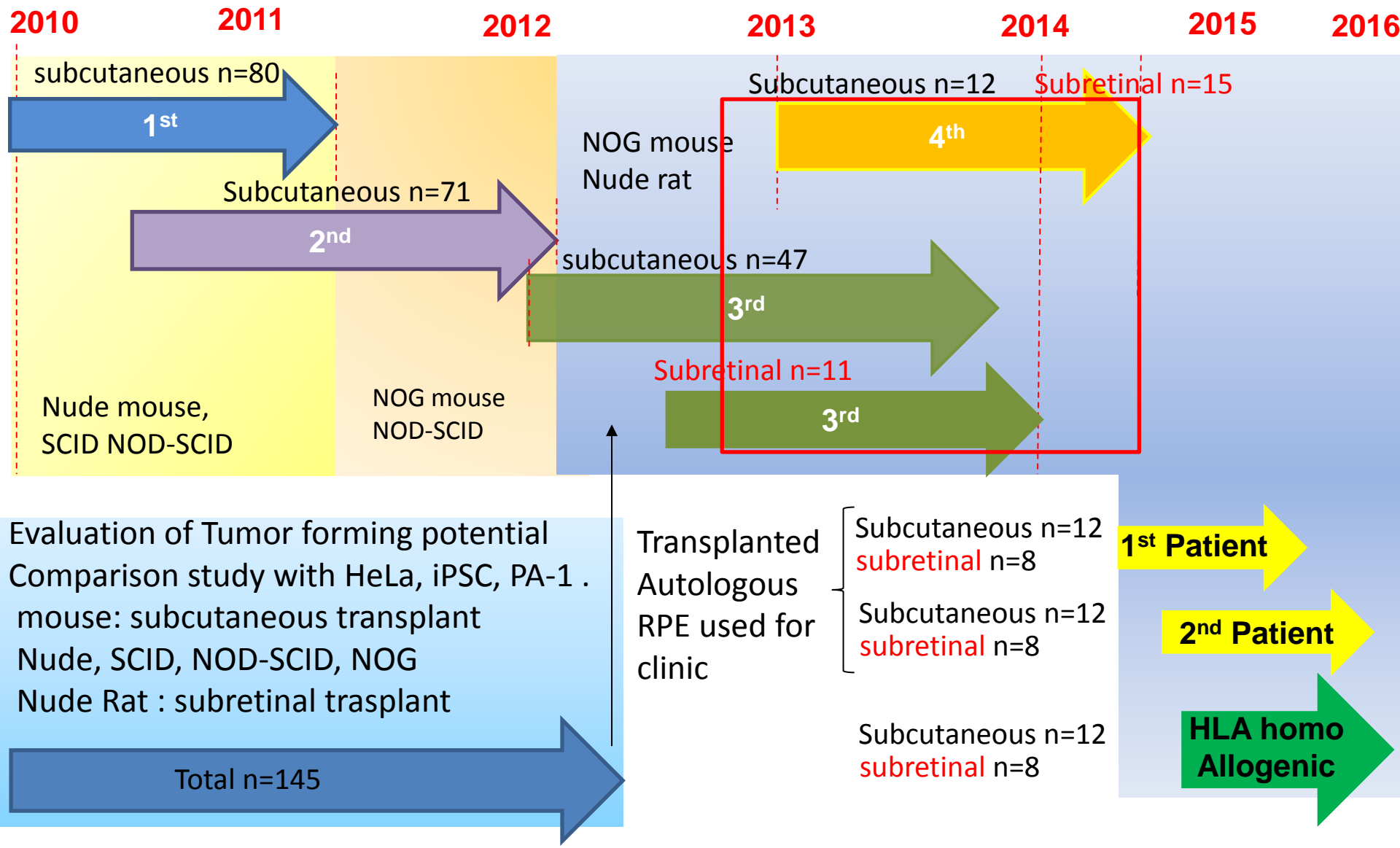
Requirements for the Use of Animal Cells as in Vitro Substrates for the Production of Biologicals.

FDA commentary report

Science Translational Medicine. 2012, 4, 147fs28

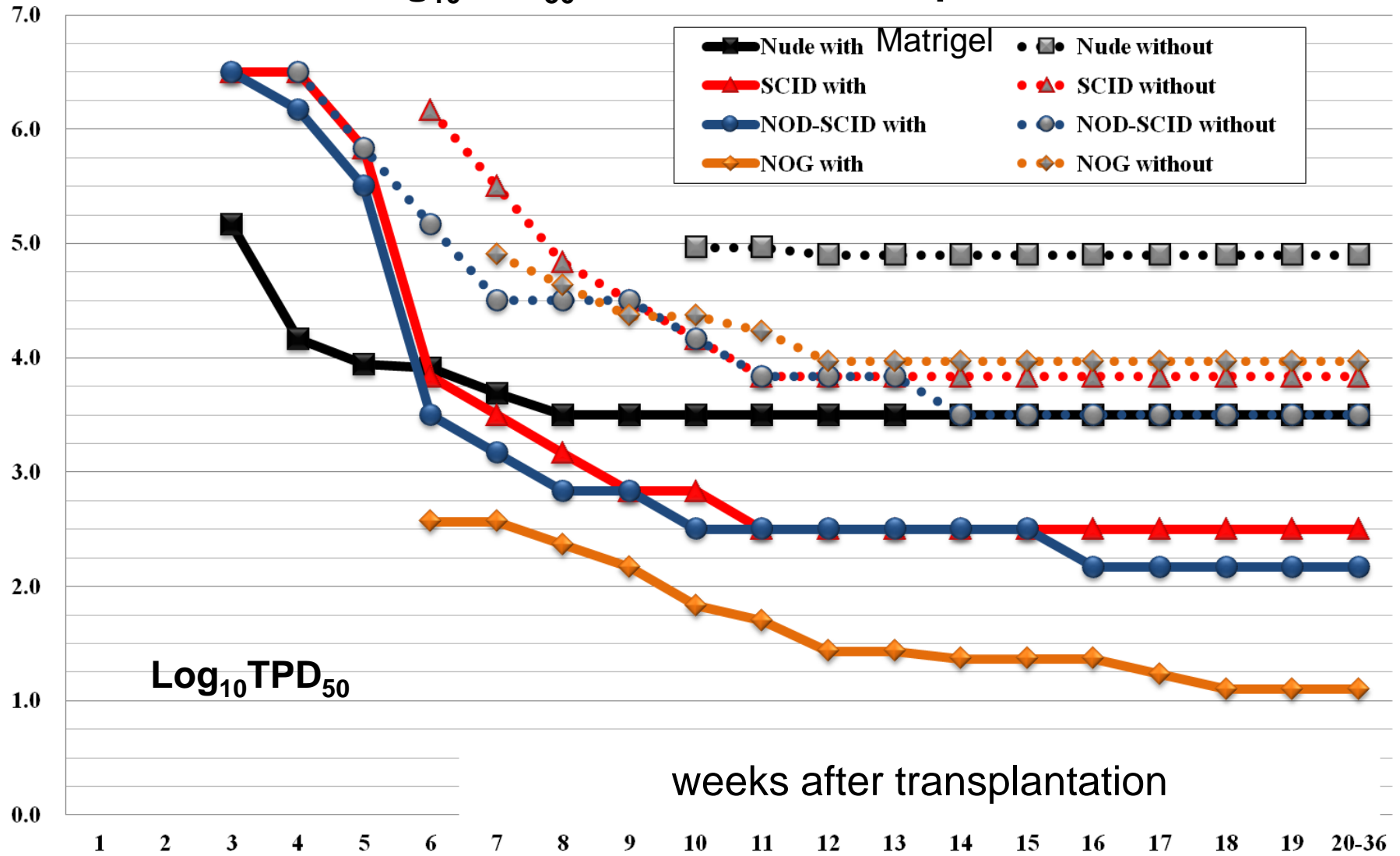
Bailey A.M; Balancing tissue and tumor formation in regenerative medicine. Geron: GRNOPC1, ACT: RPE [case by case appouch](#)

# Series of Tumorigenicity Tests have been conducted for iPS cell-derived RPE since 2010



# Transplantation of HeLa to several immunodeficient mice to examine their tumor forming capability

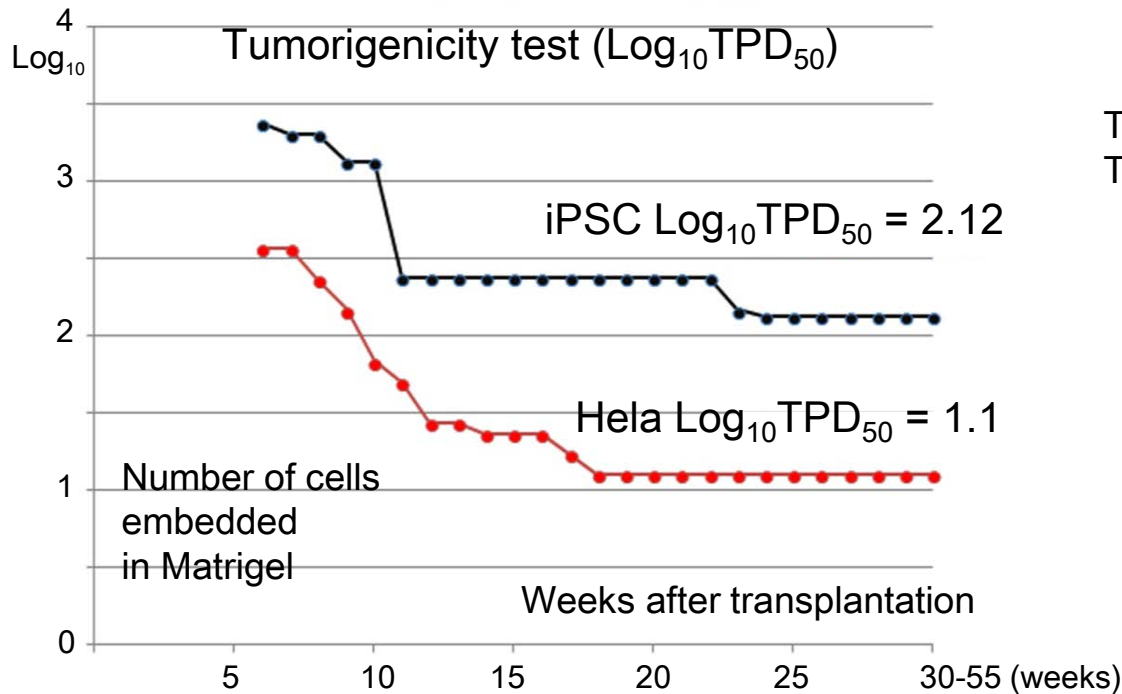
e  $\text{Log}_{10}\text{TPD}_{50}$  curve after transplantation





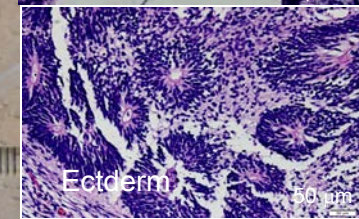
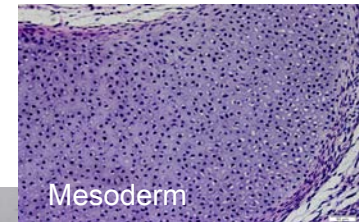
# Subcutaneous tumorigenicity test of positive control iPS cell, HeLa cell with NOG mouse

Cell type	Transplanted cell formula	Minimum number to form tumor	First / last weeks detected tumor	Number of mice	TPD50
iPSC 201B7	Embedded in Matrigel Single-cell suspension	10	5 / 40	30	132
HeLa	Embedded in Matrigel Single-cell suspension	10	5 / 18	75	12.6

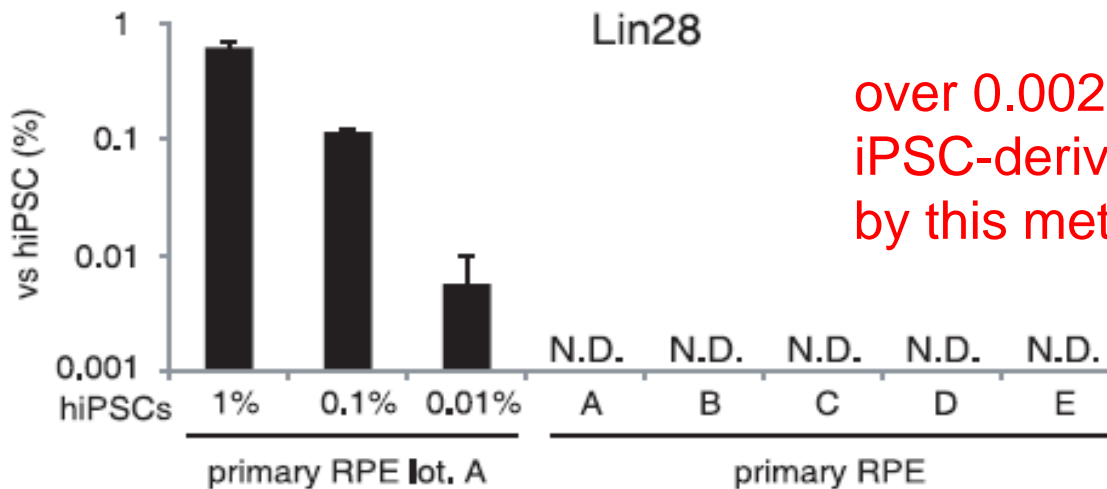
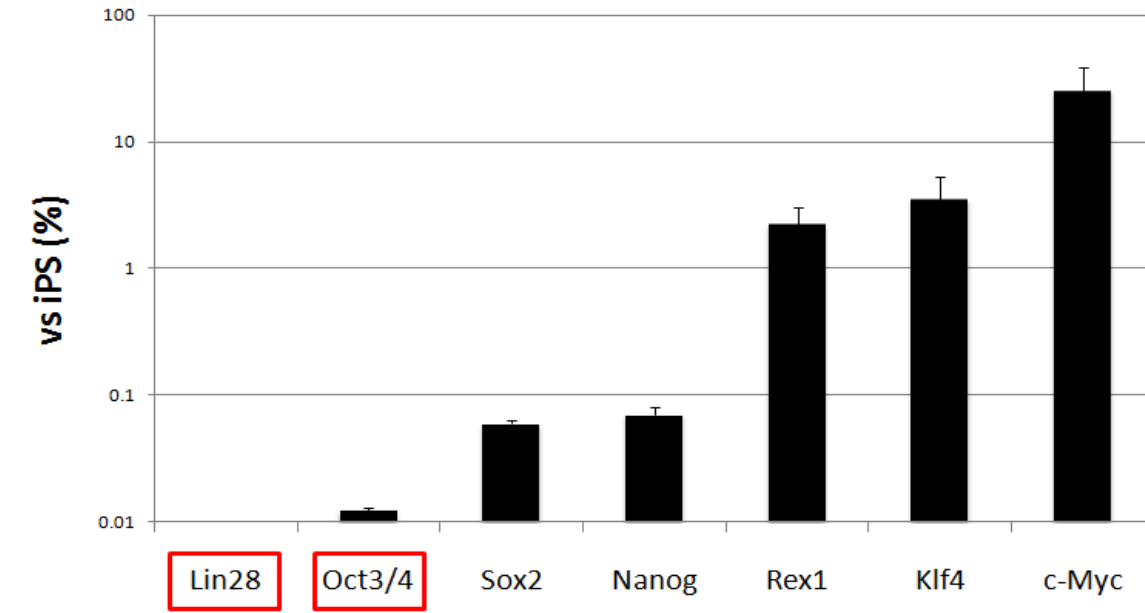


Kawamata et al 2015 JCM , revised

Teratoma generated by Transplantation of iPSC



# Detection of residual iPSC in iPSC-derived RPE by qRT-PCR using primer for Lin28



over 0.002% iPSC inclusion in iPSC-derived RPE is detectable by this method.

Dr. Sato of NIHS

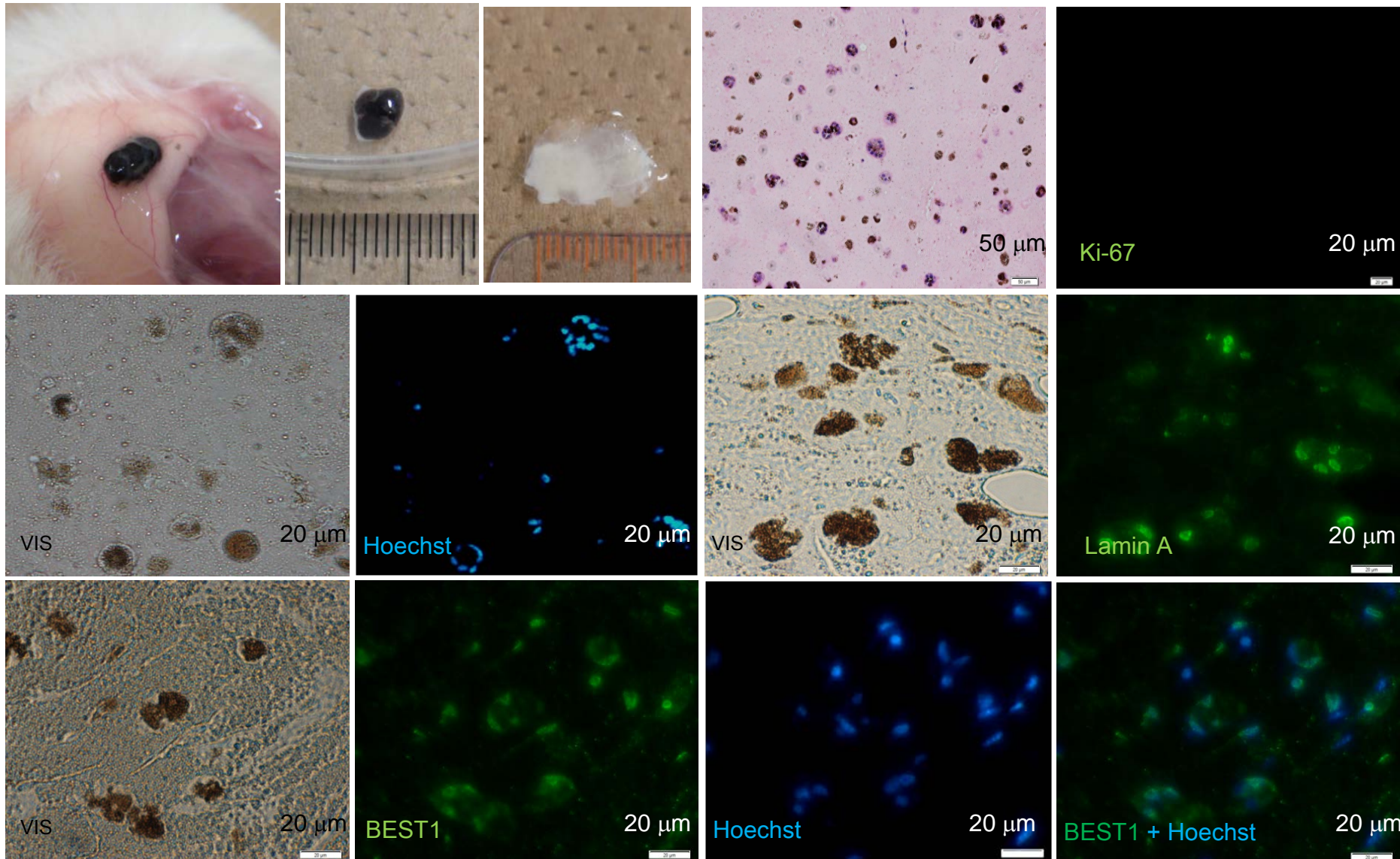
# Result of subcutaneous tumorigenicity test of iPS cell-derived RPE as quality control test

RPE line	Cell formula	Number cells transplanted	monitoring period (weeks)	Number of mice	Incident of tumor formation
59-G3	Embedded in Matrigel RPE-cell suspension	$1 \times 10^6$	60	9	None
K21-G18				8	None
101-G25				10	None
59-G3	Embedded in Matrigel RPE sheet	$1 \times 10^6$	60	5	None
K21-G18				5	None
101-G25				5	None
primary RPE	Embedded in Matrigel RPE-cell suspension	$1 \times 10^6$	52	3	None
primary RPE	Embedded in Matrigel(-) RPE-cell suspension	$1 \times 10^6$	52	2	None

59-G3	Embedded in Matrigel RPE sheet	$1 \times 10^6$	32	3	None
RNT10 RPE			28	3	None
RNT9 RPE			20	3	None
101-EV3	Embedded in Matrigel RPE-cell suspension	$1 \times 10^6$	39	5	None
K11-EV9			39	3	None
K21-EV15	Embedded in Matrigel(-) RPE-cell suspension	$1 \times 10^6$	39	4	None
K11-EV9			39	2	None

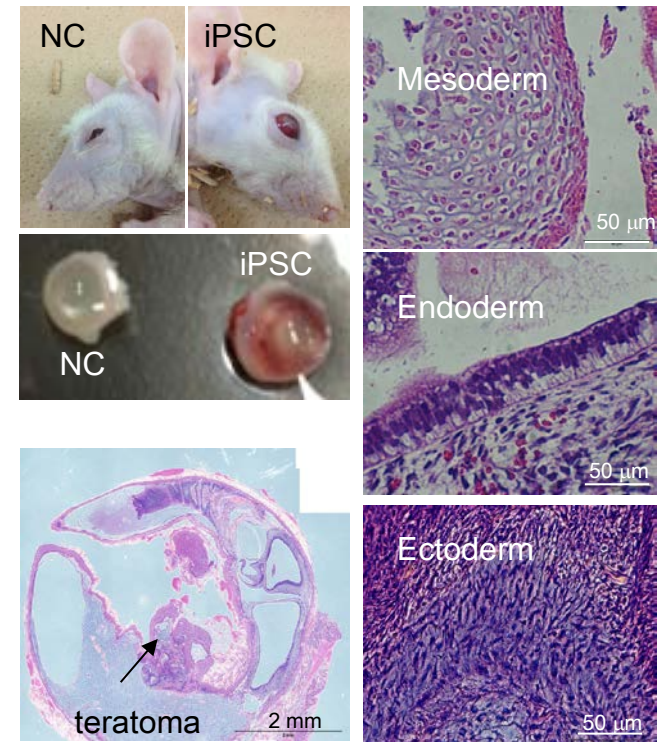
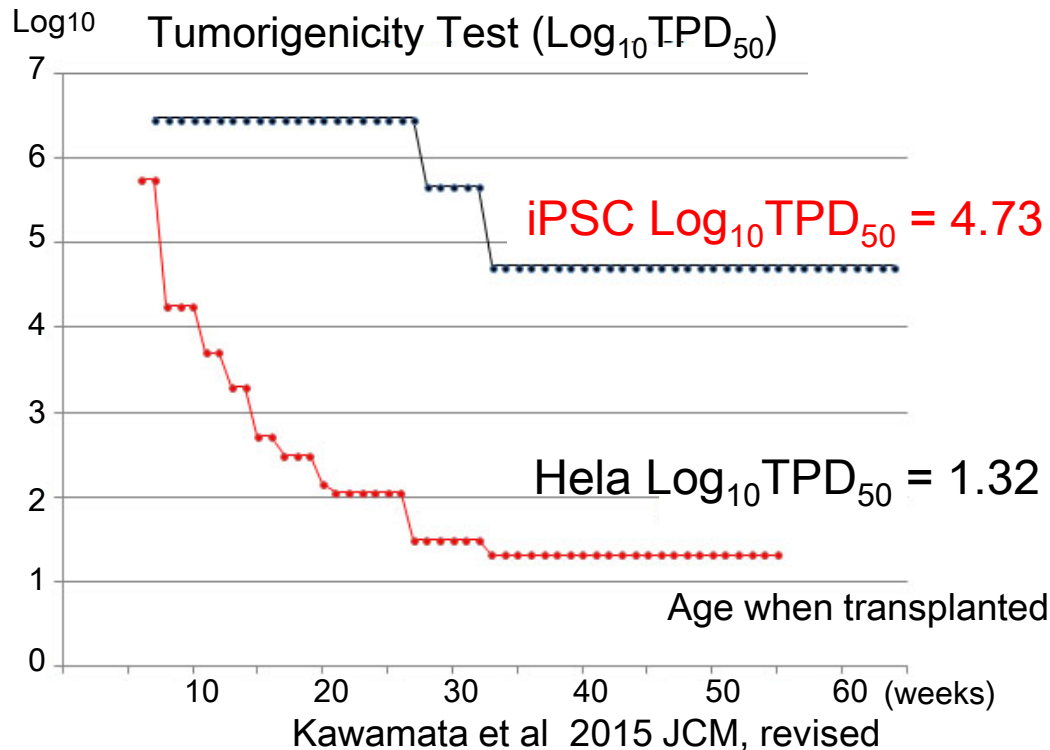


# Histological examination of subcutaneously transplanted iPS cell-derived RPE



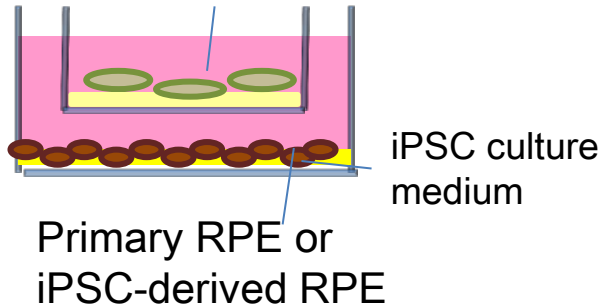
# Subretinal (clinical route) tumorigenicity test with Nude rat for positive control iPSC, HeLa cell

Cell line	Cell formula	Minimum number of cells to generate tumor	First / last week when detect tumor	Number of rats	TPD50
iPSC 201B7	Single-cell suspension	$10^4$	7/ 33	20	$5.0 \times 10^4$
Hela	Single-cell suspension	$10^1$	5 / 33	13	21

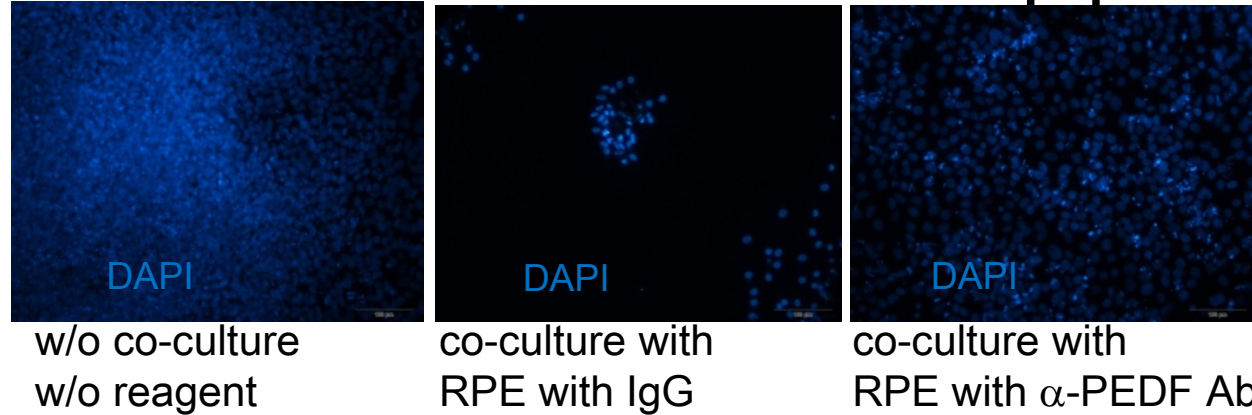


# RPE secretes Pigment Epithelium derived-Factor (PEDF), which induces apoptosis in iPS/ESC

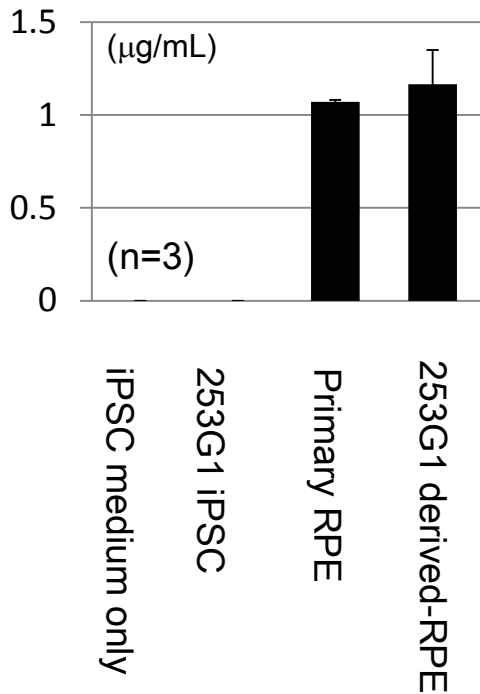
iPSC (in culture insert)



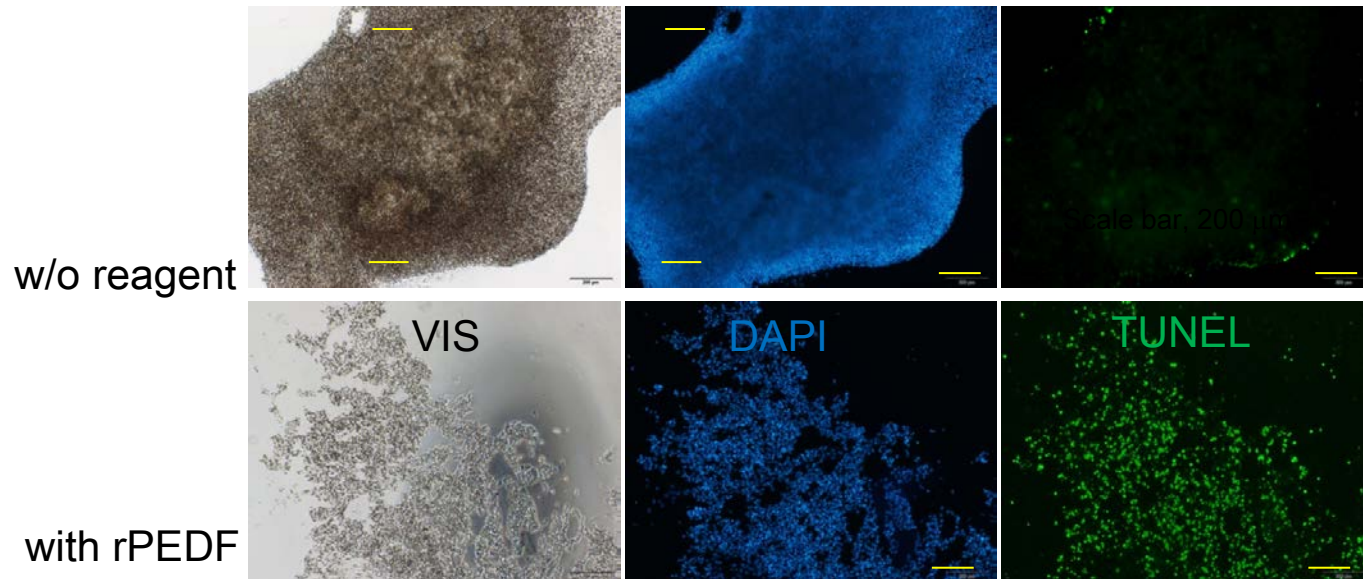
Addition of  $\alpha$ -PEDF Ab in media blocks apoptosis



**PEDF in culture medium**



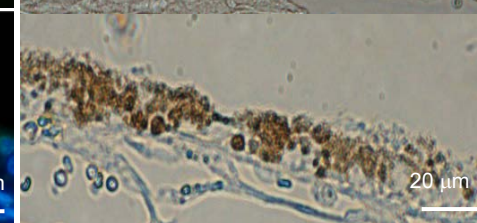
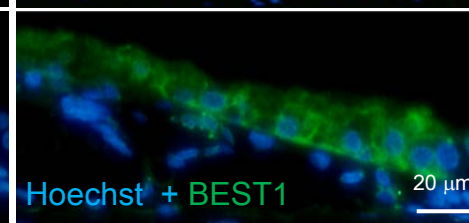
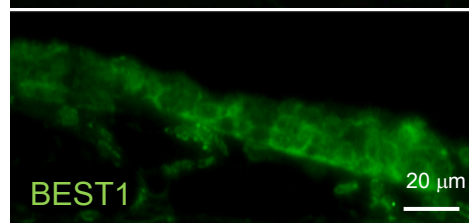
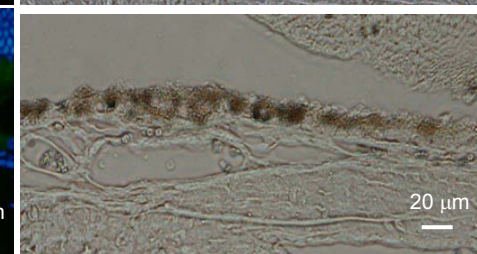
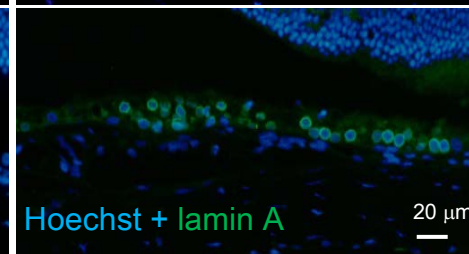
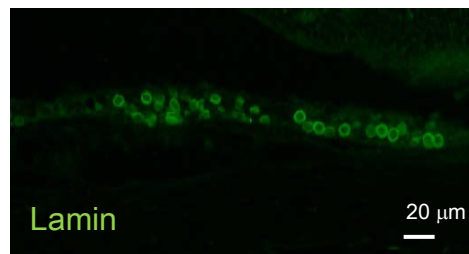
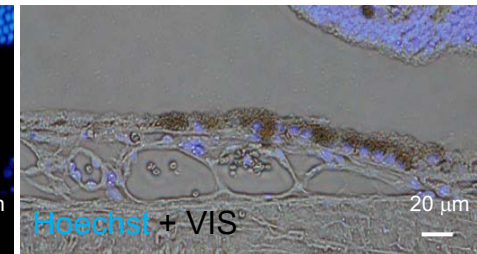
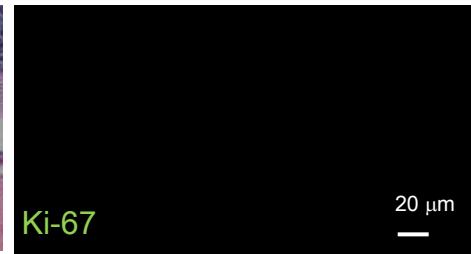
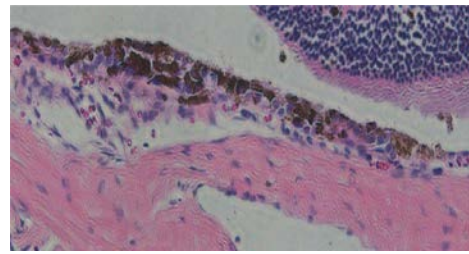
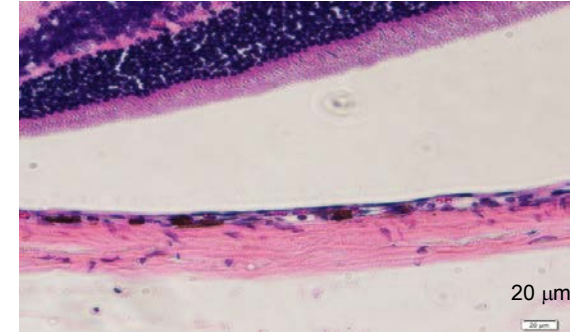
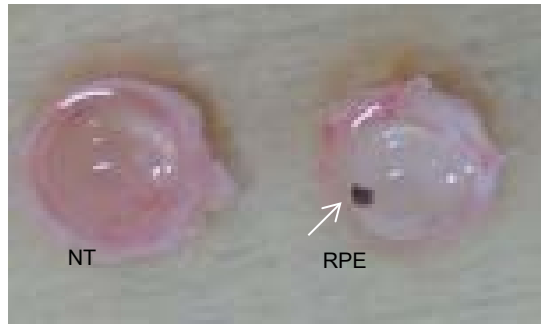
**rhPEDF induces Apoptosis in ESC khES-1**



# Result of subretinal (clinical route) tumorigenicity test of iPS cell-derived RPE

RPE line	Cell formula	Number cells transplanted	monitoring period (weeks)	Number of rats	Incident of Tumor formation
59-G3	RPE cell sheet	0.8-1.5x10 <sup>4</sup>	60	4	None
K21-G18				5	None
101-G25				3	None
59-G3	RPE cell sheet	0.8-1.5x10 <sup>4</sup>	32	8	None
RNT10 RPE			28	8	None
RNT9 RPE			20	8	None

# Histological examination of iPS cell-derived RPE transplanted in subretinal space





# Conclusion of tumorigenic tests of final product

(suppose  $4-8 \times 10^4$  iPSC-derived RPE is transplanted in clinic)

1. Inclusion of residual iPSC in iPSC-derived RPE was evaluated by qPCR. Inclusion will be **less than two iPS cells in transplant in clinic.**

Quality Control Test for Purity of final product prior to shipping

2. Tumorigenic potential of not terminally differentiated cells was evaluated by subcutaneous transplantation using HeLa and iPSC transplantation as positive control. Tumorigenic cell inclusion will be **less than ten cells in transplant in clinic.**

Quality Control Test for Tumorigenic Potential of final product

3. Tumorigenic potential incident from not terminally differentiated cells was evaluated by subretinal transplantation using HeLa and iPSC transplantation as positive control. **Tumorigenic event shall be negligible in clinical setting in the presence of PEDF in clinical route.**

Tumorigenicity Test of final product by animal study

# Then, what we have learned ...

Specific to RPE

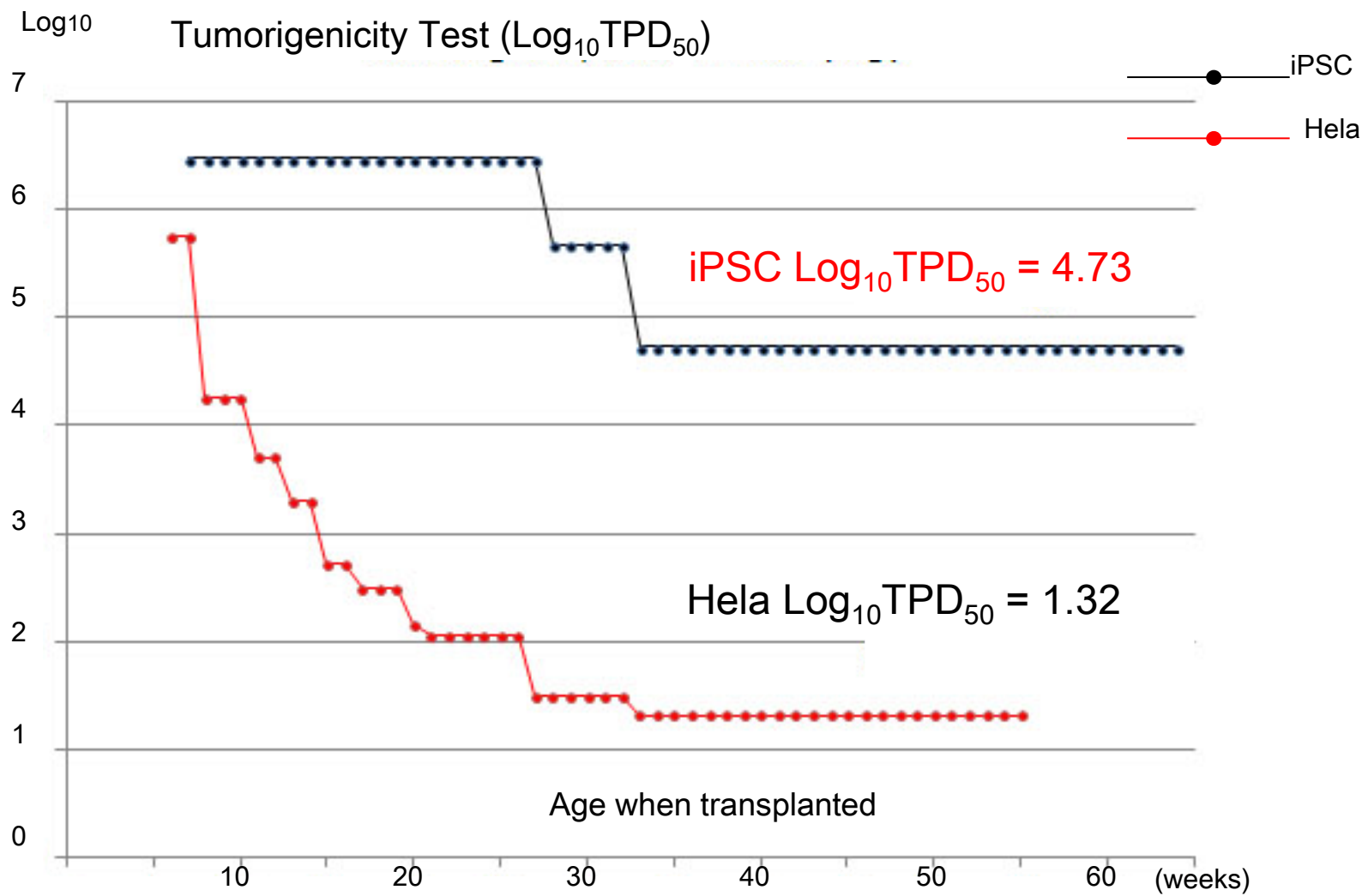
**Subretinal iPSC spike test may not be necessary or not informative at all due to microenvironment in subretinal space .  
(TPD50 for iPSC is  $5 \times 10^4$  cells)...case by case approach is needed.**

---

In general

- 1. We need genetic info for reprogrammed , final product cell, and also epigenetic and phenotypic info and residual PSC number info of final product prior to conducting tumorigenicity tests.**
- 2. We need to conduct a pilot study for tumorigenicity test to provide rationale for the design of tests and its interpretation of the result when extrapolate it to human.**

# Subretinal tumorigenicity test may not be informative to address iPSC/ESC contamination in final product



# Then, what we have learned ...

Specific to RPE

**Subretinal iPSC spike test may not be necessary or not informative at all due to microenvironment in subretinal space .  
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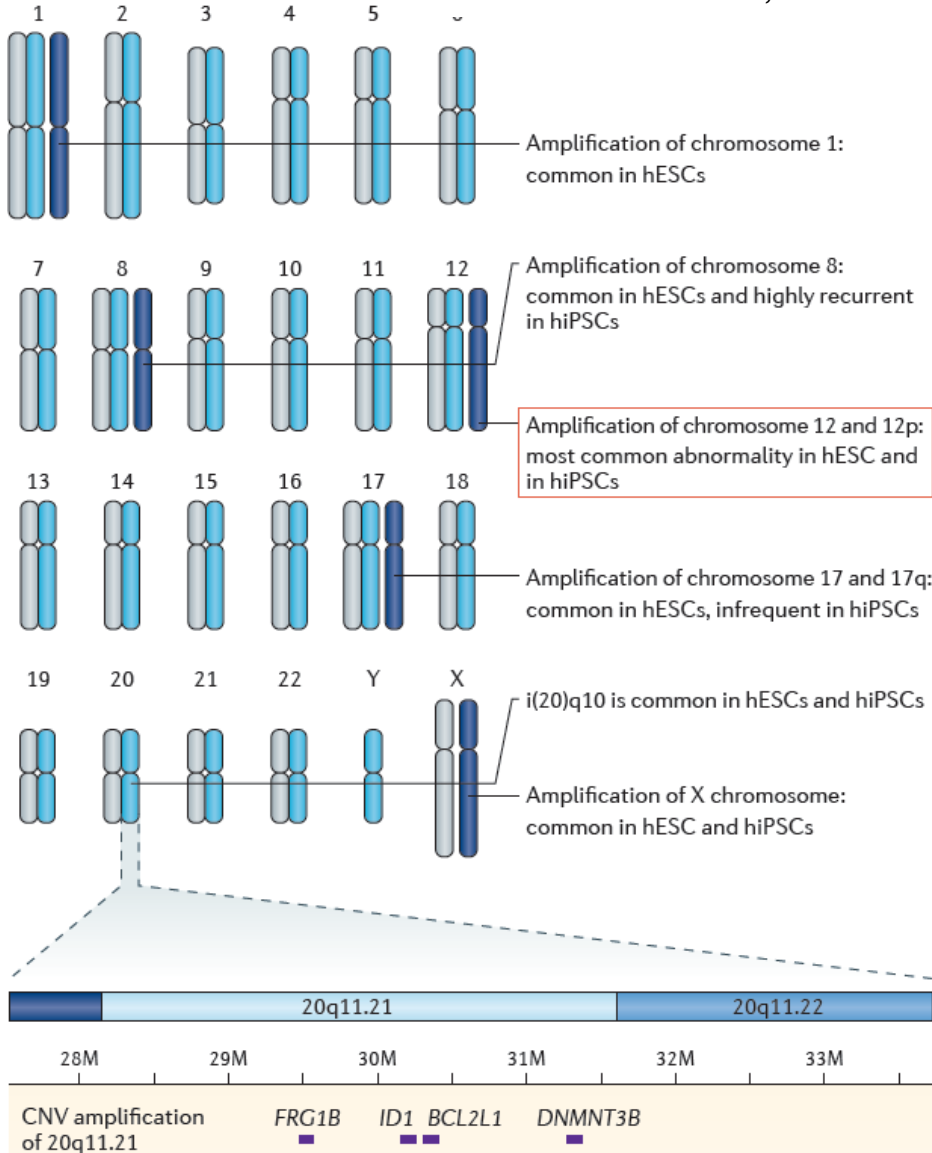
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# Instability of Gene in Pluripotent Stem Cell

*Nat Rev Gen 2012:13, 732-744*



552 iPS cells out of  
those derived from 219 lines  
1,163 Es cells out of  
those derived from 40 lines

**Chromosomal abnormality**  
12.9% of ES cells  
12.5% of iPS cells  
(normal ) 250-300 sites  
variation.



Testing criteria proposed.  
Ex seq. G-band, mFISH  
CGH array, hot spot genomic PCR.  
No episode of tumor, family history  
Refer to cancer / gene Data base  
No additional genomic aberration  
during manipulation and culture

# Then, what we have learned ...

Specific to RPE

**Subretinal iPSC spike test may not be necessary or not informative at all due to microenvironment in subretinal space .  
(TPD50 for iPSC is  $5 \times 10^4$  cells)...case by case approach is needed.**

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In general

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- 2. We need to conduct a pilot study for tumorigenicity test to provide rationale for the design of tests and its interpretation of the result when extrapolate it to human.**

# Risk assessment based POC for Tumorigenicity Test of PSC derived-product

- not to overlook possible tumorigenic event and assess its risk in clinic -

1. Select animal model with rationale: ex. disease model or immuno-deficient rodent.
2. Determine the feasibility of subcutaneous transplantation test that serves as QC test.
- 3. Conduct a series of pilot studies with animal prior to the test to:**
  - 1) Train operators and make sure the delivery of transplant to the designated site.
  - 2) Establish robust IHC to detect live human tissue, assess proliferation and maturation.
  - 3) Set up test design by the assessment of microenvironment, dose, **monitoring period**.
  - 4) Determine the frequency of metastasis and its detection method, if any.

**Set up tests, considering item 1,2, 3, and genetic info and QC info of final product.**

# Acknowledgements

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## **CIEA**

**Central Institute for Experimental Animals**

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