Current status and future vision of retinal cell therapy

Masayo Takahashi MD, PhD

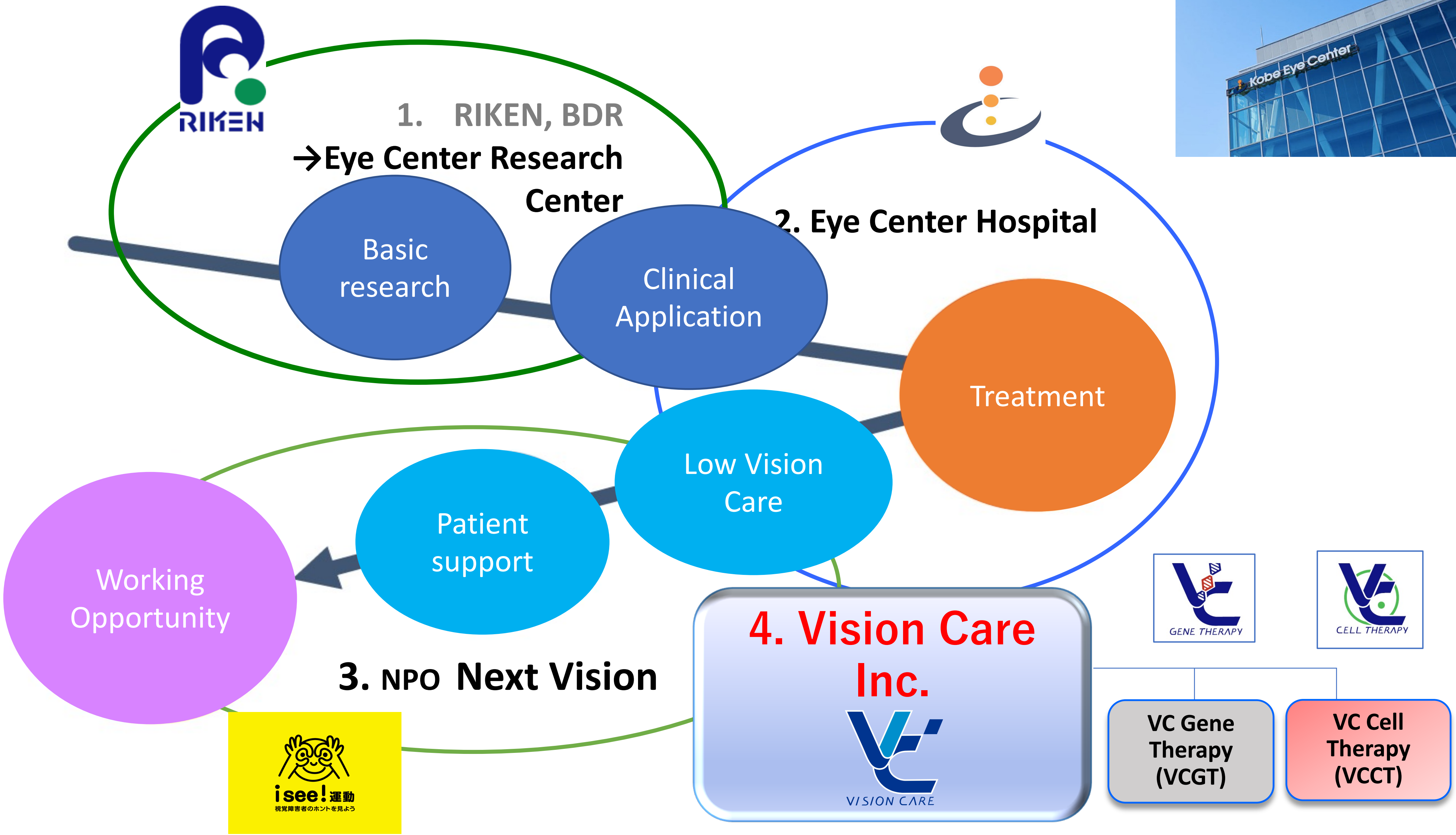
2019.8~ Vision Care Inc. (President)

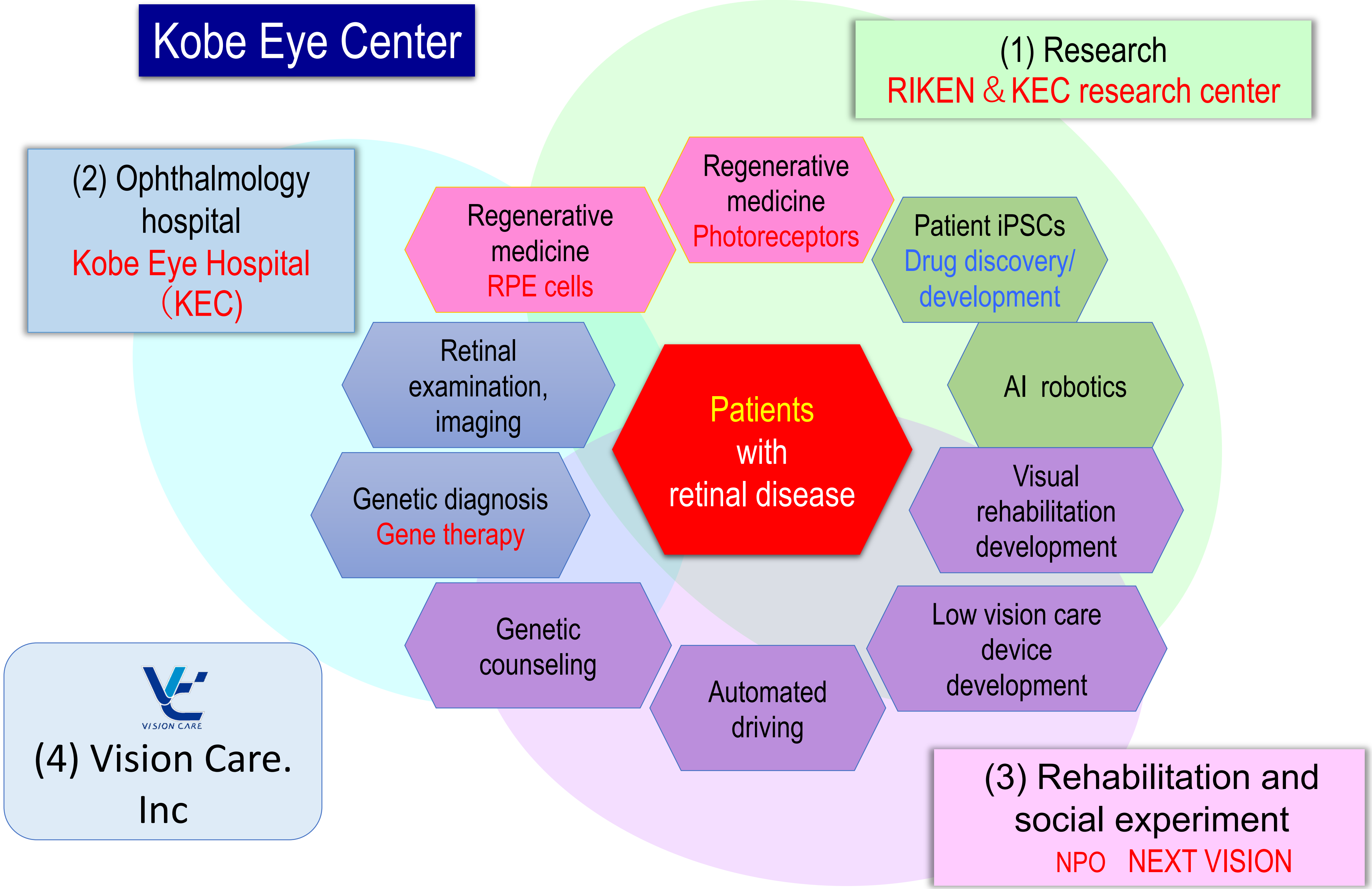
Kobe Eye Center Hospital

RIKEN Center for Biosystems Dynamics Research

NPO NEXT VISION

Kobe eye center total solutions for every patient




Kobe Eye Center
(1) Research
RIKEN & KEC research center
(2) Ophthalmology hospital
Kobe Eye Hospital (KEC)
Regenerative medicine
RPE cells
Regenerative medicine
Photoreceptors
Patient iPSCs
Drug discovery/ development
Retinal examination, imaging
AI robotics
Patients with retinal disease
Genetic diagnosis
Gene therapy
Visual rehabilitation development
Genetic counseling
Low vision care device development
Automated driving
VISION CARE
(4) Vision Care. Inc
(3) Rehabilitation and social experiment
NPO NEXT VISION

Masayo Takahashi MD, PhD

Academia

2018 ～ USA NAM (National Academy of Medicine) member

Board member :

～2018 ISSCR (International Society for Stem Cells and Regeneration)

2015～ Japanese Society for Ophthalmology

2018～ Japanese Vitreoretinal Society

2012～ Japanese Society for Regenerative Medicine

Ministry

Committee members of

Ministry of Education, Culture, Sports, Science and Technology

Ministry of Health, Labor and Welfare

Ministry of Economy

PMDA (Japanese FDA) Board member (~2020)

Company

Board member : Sysmex, S'UIMIN

Advisor : IPGI Technologies

Founder : Healios, VC' group

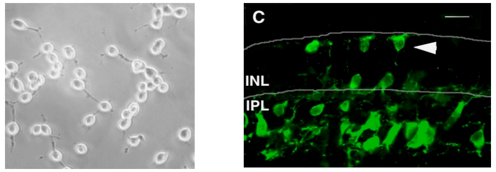
Masayo Takahashi MD, PhD

Two "first in the world" works @ the Salk Institute (1995-1996) in Fred Gage lab

First in human

Neural stem cells : Usage of the stem cell for retinal transplantation

M Takahashi et al. Molecular Cellular Neurosci. 1998



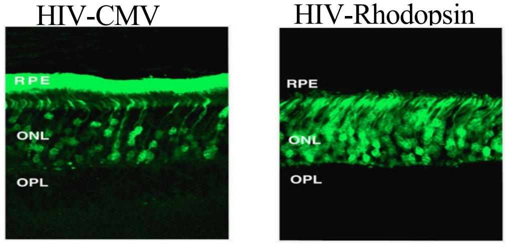
→ Retinal cell therapy using iPS cells

VC cell therapy (VCCT)

HIV vector: Animal experiments of the lentiviral vector

H Miyoshi, M Takahashi (Co-first) et al. PNAS 1998

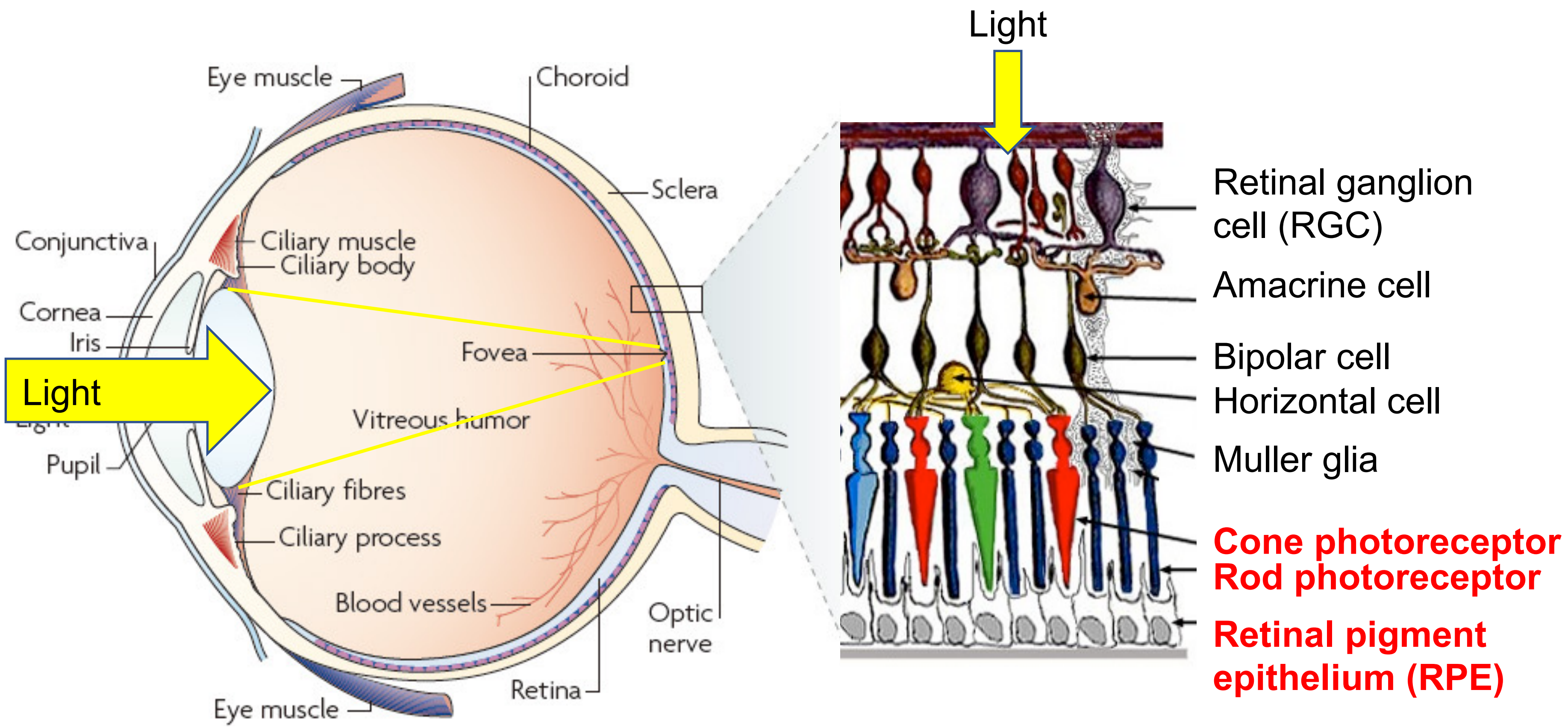
M Takahashi, H Miyoshi et al. J Vil 1998



→ Gene therapy for Retinal degeneration

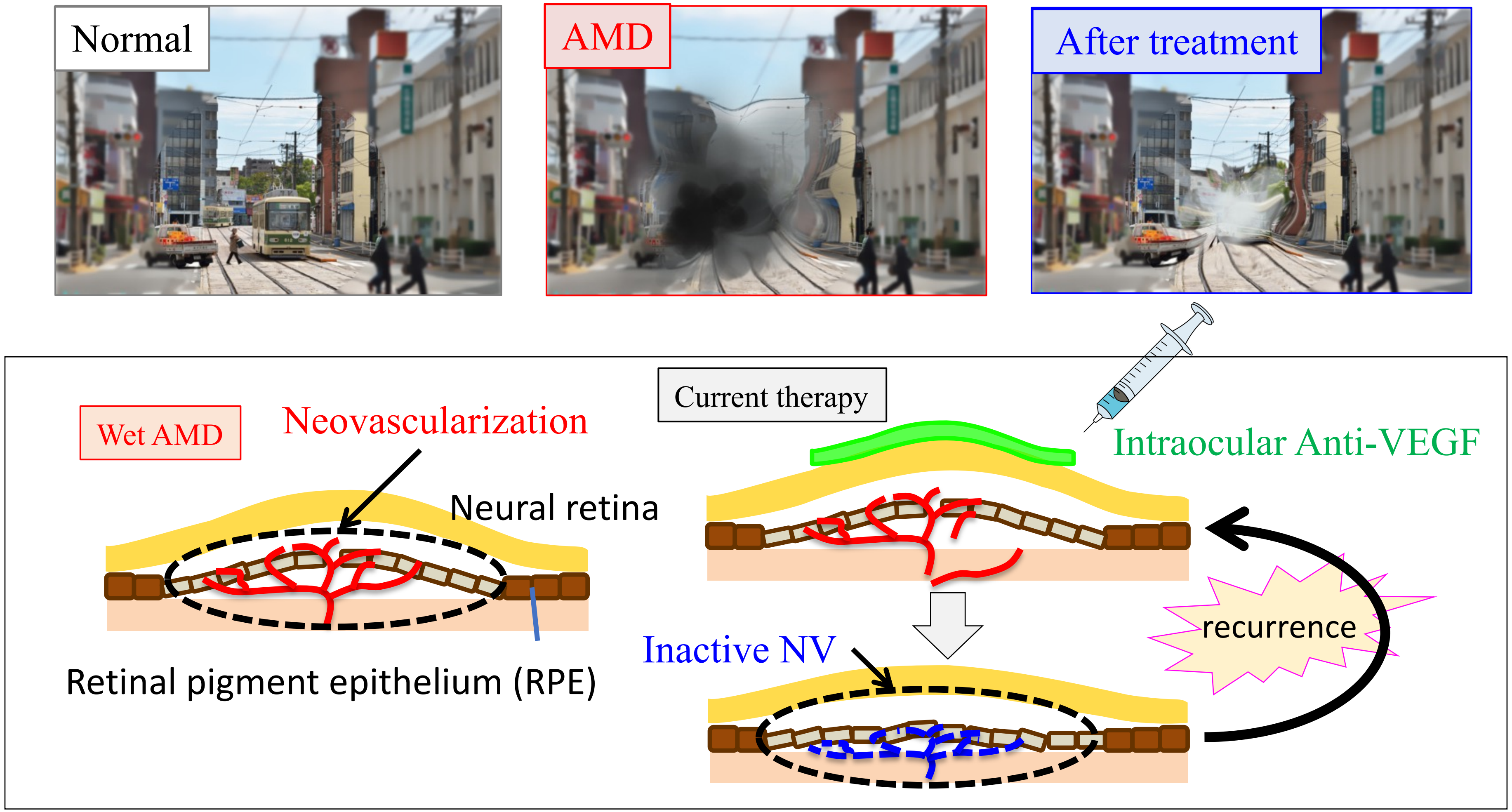
VC gene therapy (VCGT)

Retina

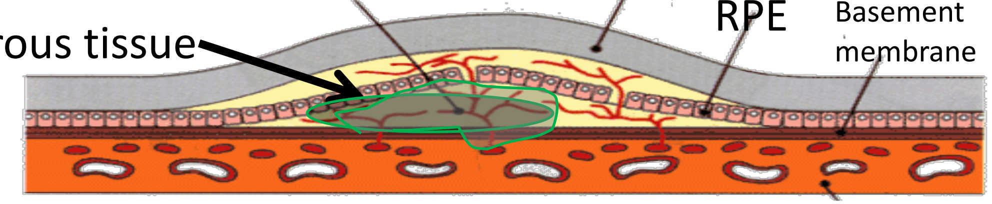


Modified from Nat. Rev. Genet. 11, 273-284 2010

Age related macular degeneration (AMD) & Current therapy



Stages of AMD & suitable treatment



Neovascularization — **Existing treatment** PDT, anti-VEGF drugs

Fibrous membrane — CNV removal

RPE damage — RPE transplantation

Photoreceptor damage — RPE + photoreceptor transplantation

Choroidal damage — RPE + photoreceptor + Choroid transplantation

Wet type AMD

Dry type AMD

Regenerative medicine

RPE transplantation : wet AMD

Human embryo **RPE**

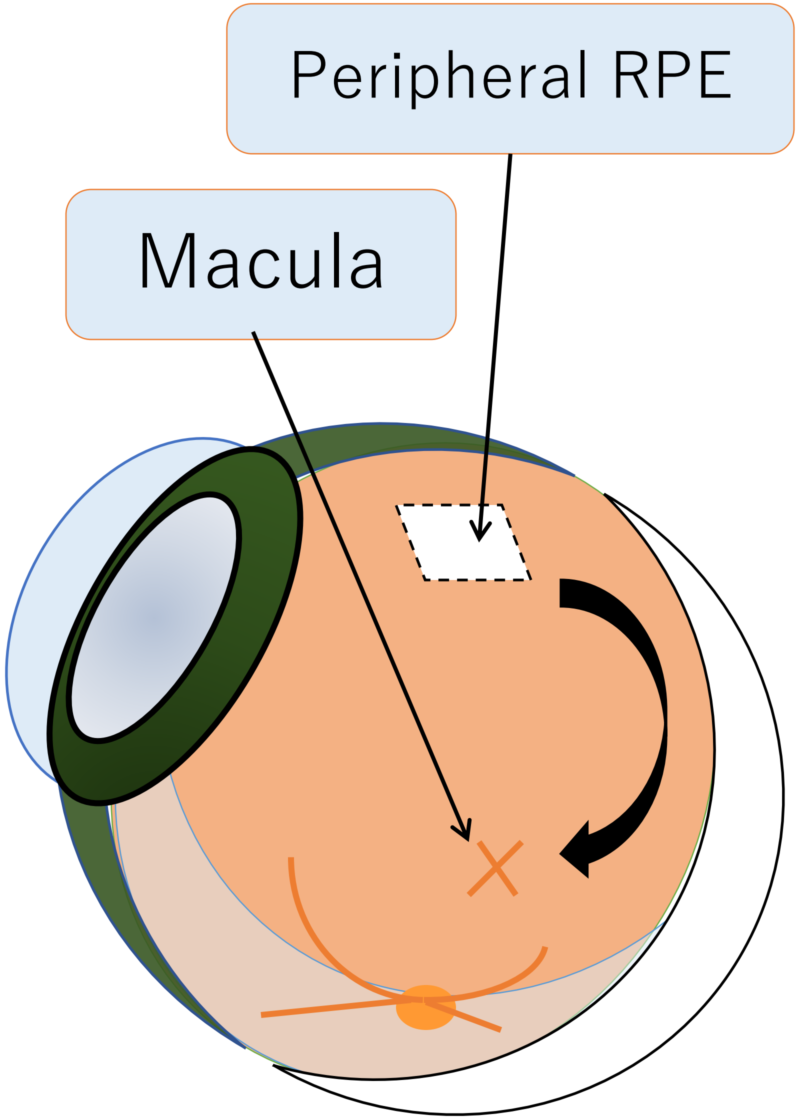
- Algvere PV (1999)
- Allo - Rejection

Auto **RPE suspension**

- van Meurs JC (2004)
- Cell suspension
  - Low survival ratio
- Risk of cell harvest

Auto RPE sheet

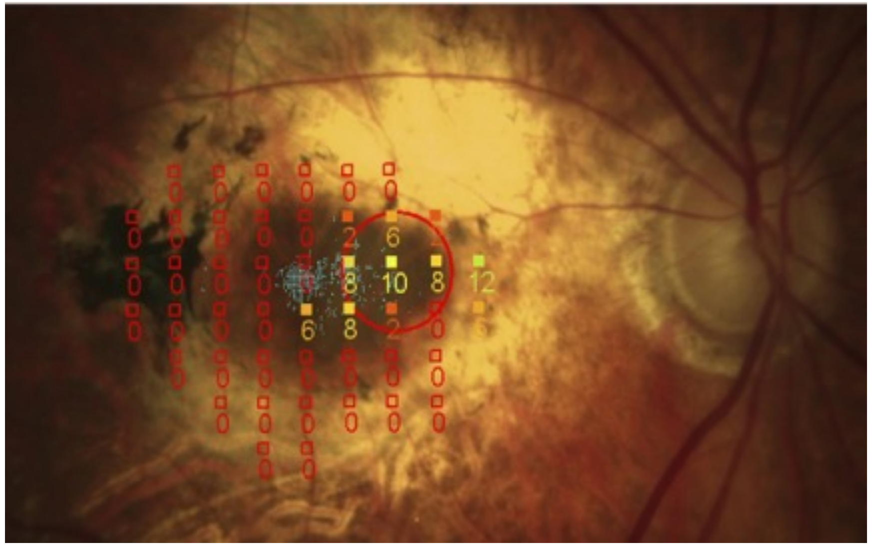
- 2006～7年4 report：effective for 58% patients （total 73 cases）
- Cell harvest High risk 40%



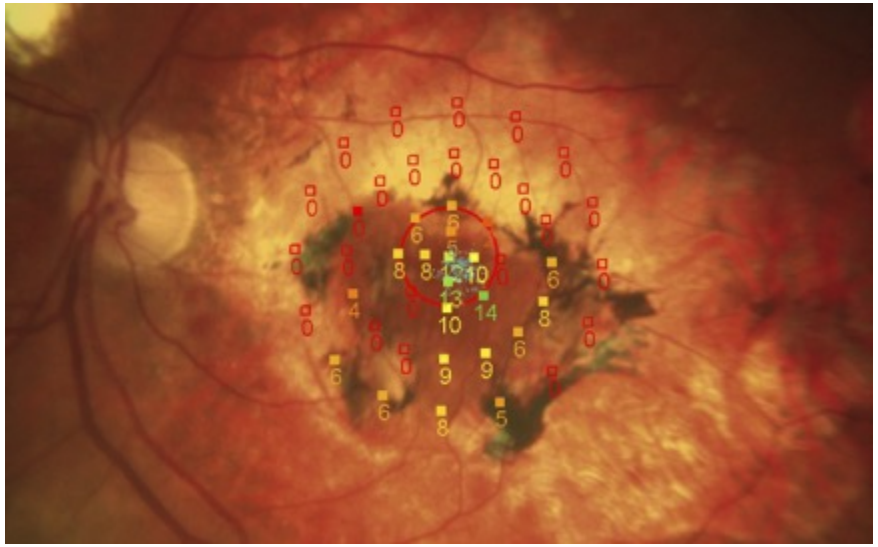
A Free Retinal Pigment Epithelium–Choroid Graft in Patients With Exudative Age-Related Macular Degeneration: Results up to 7 Years

ELSBETH J.T. VAN ZEEBURG, KRISTEL J. M. MAAIJWEE, TOM O. A.

Am J Ophthalmol 2012;153:120–127.



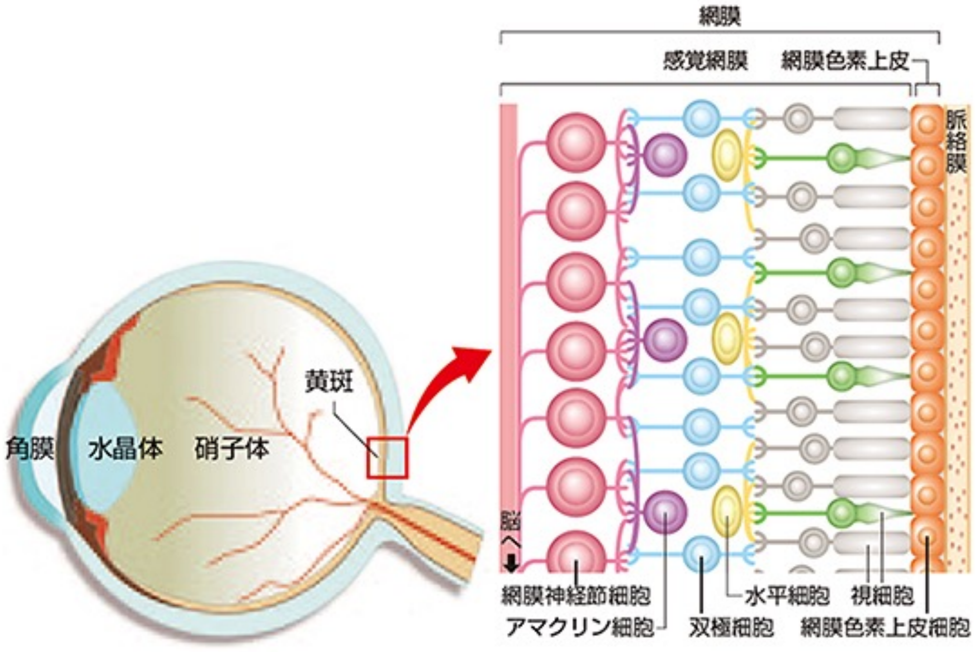
7 yrs after surgery:
visual acuity 20/32 (= 0.6) ,
fixation on the graft.



6 yrs and 7 mo after surgery:
visual acuity 20/50 (= 0.4) ,
fixation on the graft.

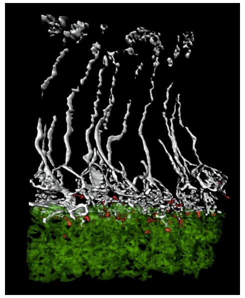
Retinal Cell Therapy

- iPSC-RPE (Phase 1,2)
  - RPE impairment diseases
- iPSC-photoreceptor cells (Phase 1)
  - Retinal degenerative diseases



RPE

photoreceptor



Clinical study

RPE:
#1 2013-15（auto）
#2 2017-18（HLA matched）
#3 2020 - （Allo）

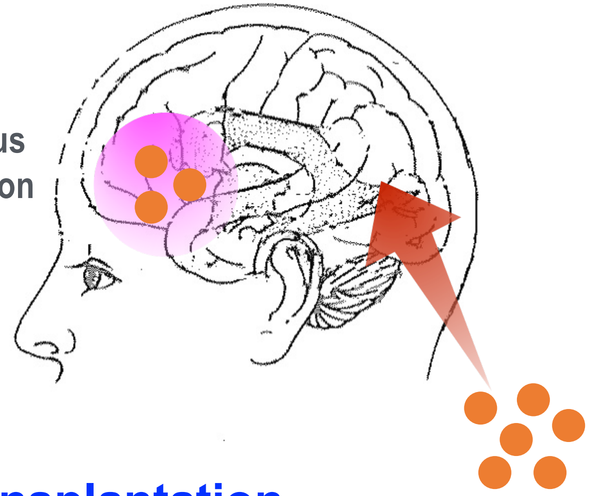
Photoreceptor:
#1 2020 -

Categories of Regenerative Medicine

Classification by Action Mechanism

1. Endogenous stem cell activation

Endogenous
stem cell activation



2. Cell Transplantation

Fetal cells
Stem cells
Differentiated
cells

A. Replacement therapy

B. Trophic effect

Classification by treatment method

- Regeneration promoters
- Cell therapy
  - 1. i.v. — Systemic
  - 2. Surgery — Local

Graft survival

Short	Days〜weeks	MSC
Long No division	Several division (mature cells)	RPE
Long Division	Continuous division (Stem/immature cells)	Neural retina

Progress of retinal cell therapy

Safety

Efficacy,
System

1case

#1 Auto RPE

2013.8~
2015.9

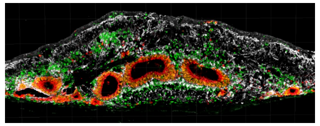
5 cases

#2 Allo RPE

2017.3~
2018.9

2 cases

#3 photoreceptor
Retinal sheet



2020.6 ~

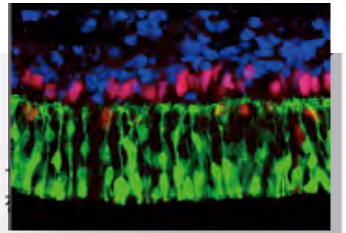
1/50 cases

#4 RPE
multicenter

2020.1~

CELL THERAPY

#5 2nd generation
Retinal sheet



CELL THERAPY

Now

時 間

時 間

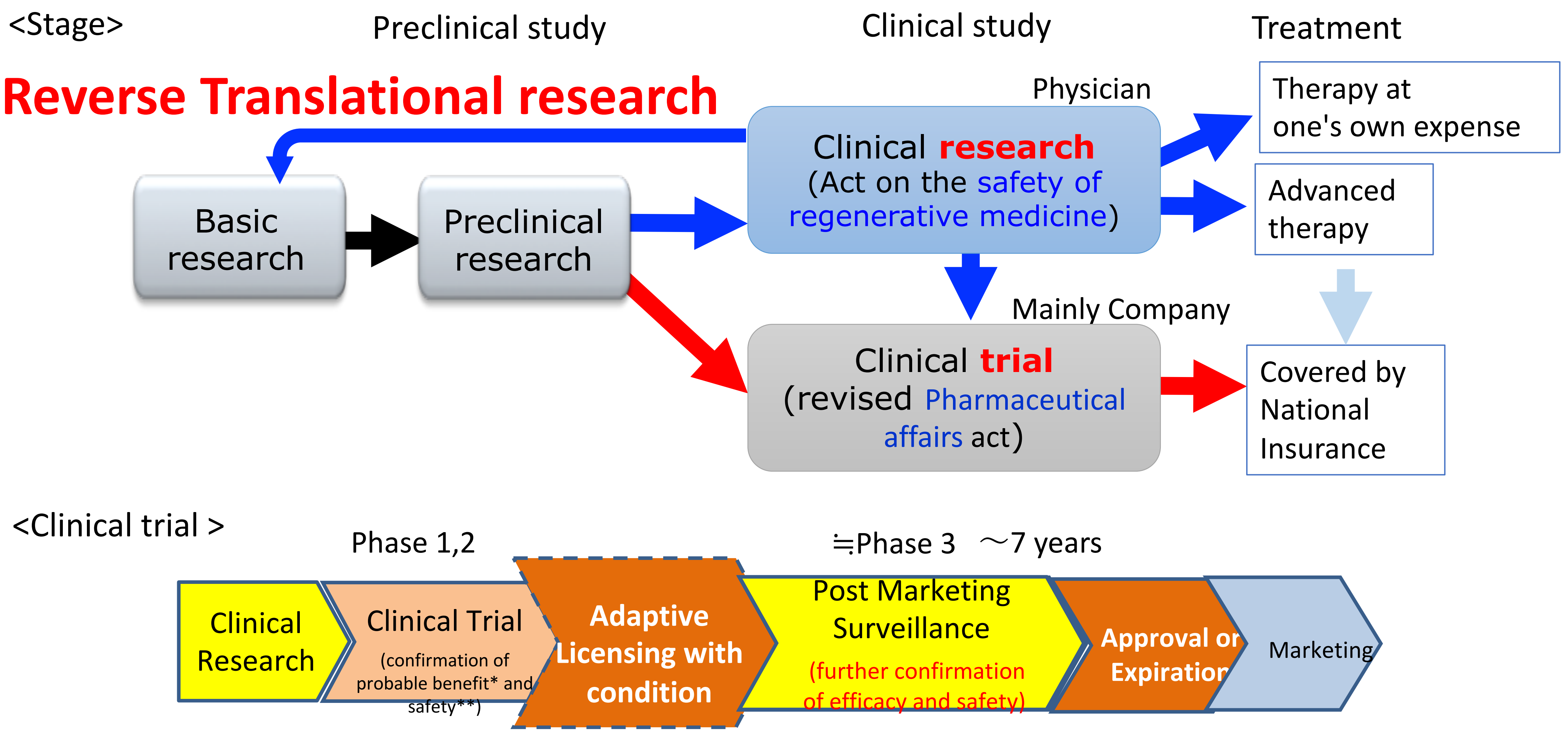
The ideal state of regenerative medicine from the patients' & doctors' perspective

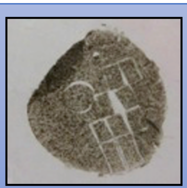
- Optimal treatment for each case
  - Reconsideration of disease names
  - Various forms (suspension & sheet)
- Reduce treatment costs
  - Regulation, CPF
- Sustainable treatment as a medical system
  - Consider hospitals profits (Japan)
- From cell products to therapy
  - Surgery

Around the treatment

- Evaluation tests
- QA of genetic diagnosis

Regulatory system in Japan





RPE cells
(for RPE impaired diseases)

1st clinical research

using iPS cells

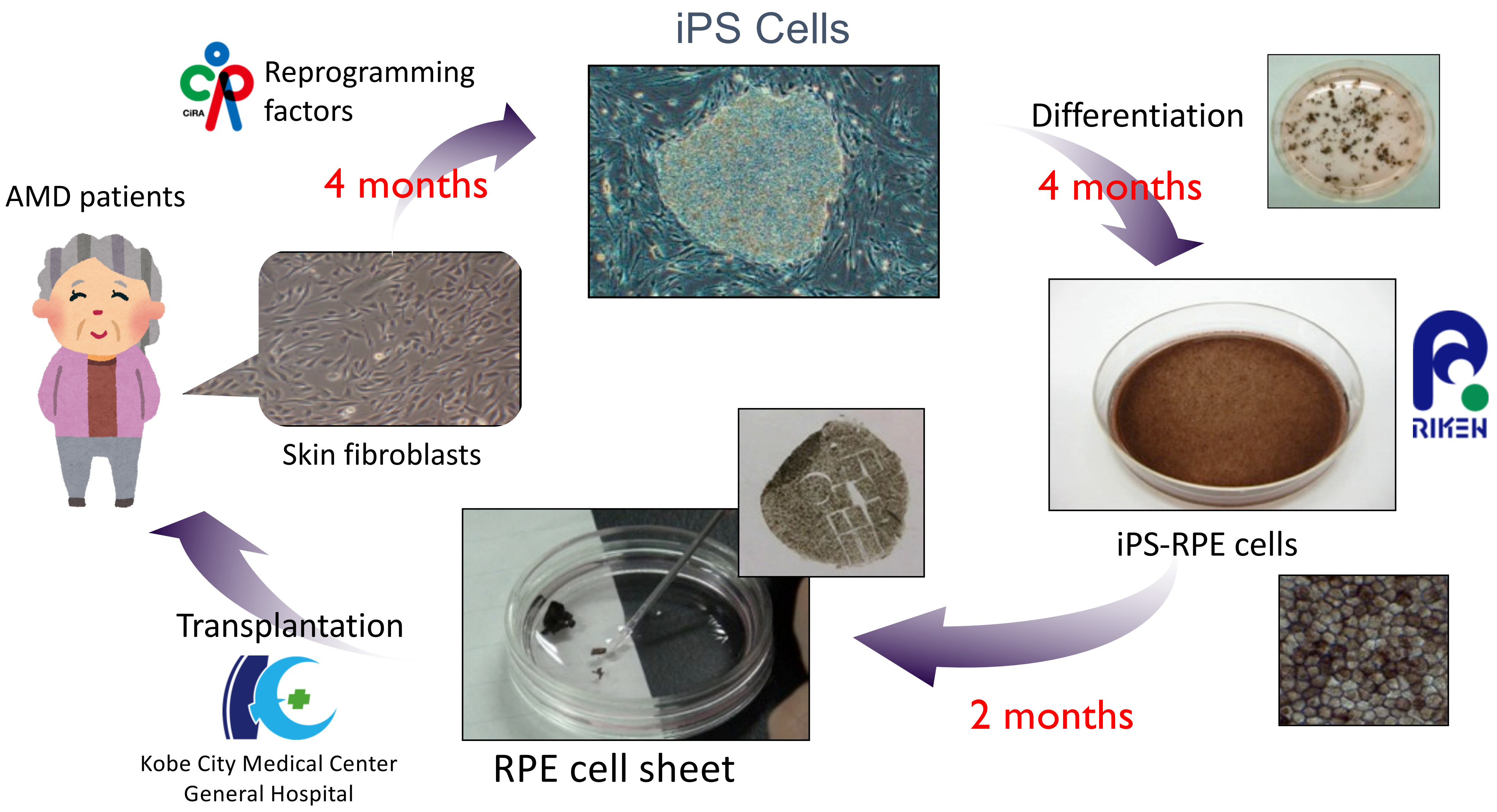
<u>Autologous</u> iPSC-RPE transplantation
(1 case: 2013~2015)

To show **<u>a safe way</u>** of clinical use

Mandai et al. New Engl J Med 2017

1st clnical research 2013～2015

Autologous iPS-RPE transplantation to AMD patients



RPE & Tumors

- No report of metastatic tumor ever in the history

  Even in the **familial tumor** patients only hyperplasia of RPE occurs

  = with oncogene (ex. p53) mutations

- PEDF (pigment epithelial derived factor)= strong antitumor factor

Cells

- Eye ball is full of Retinoic Acid = strong inducing factor for differentiation

Environment

Why the RPE cell is the first one

Mature cells ; less proliferation

Purification

Kuroda et al. 2012 PlosONE

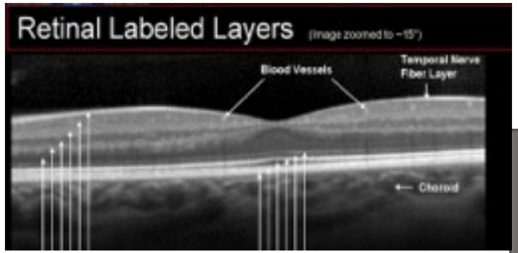
Small amount of cells

PEDF ; anti-tumor factor

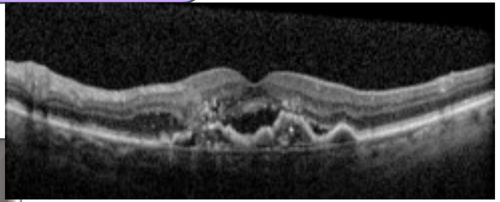
Kanemura et al. 2013 Scientific report

OCT ; fine examination

OCT



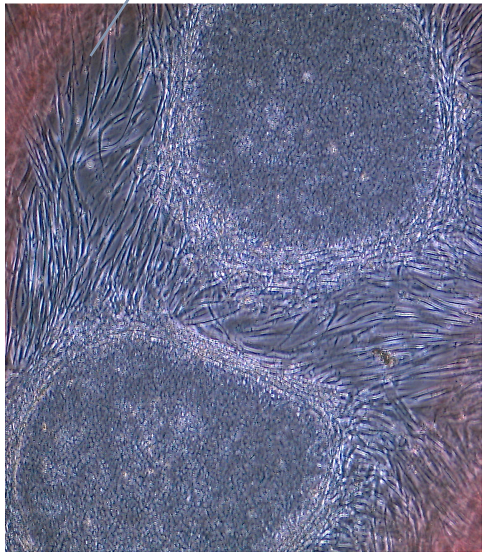
OCT

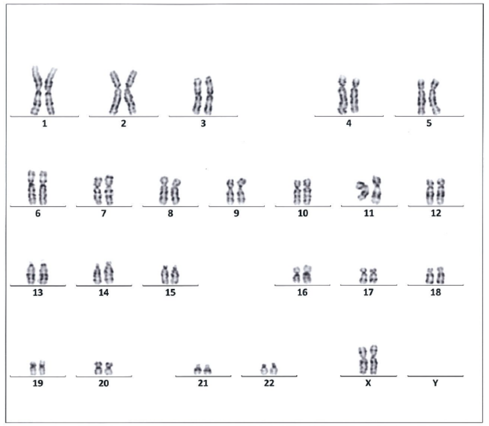


Safety

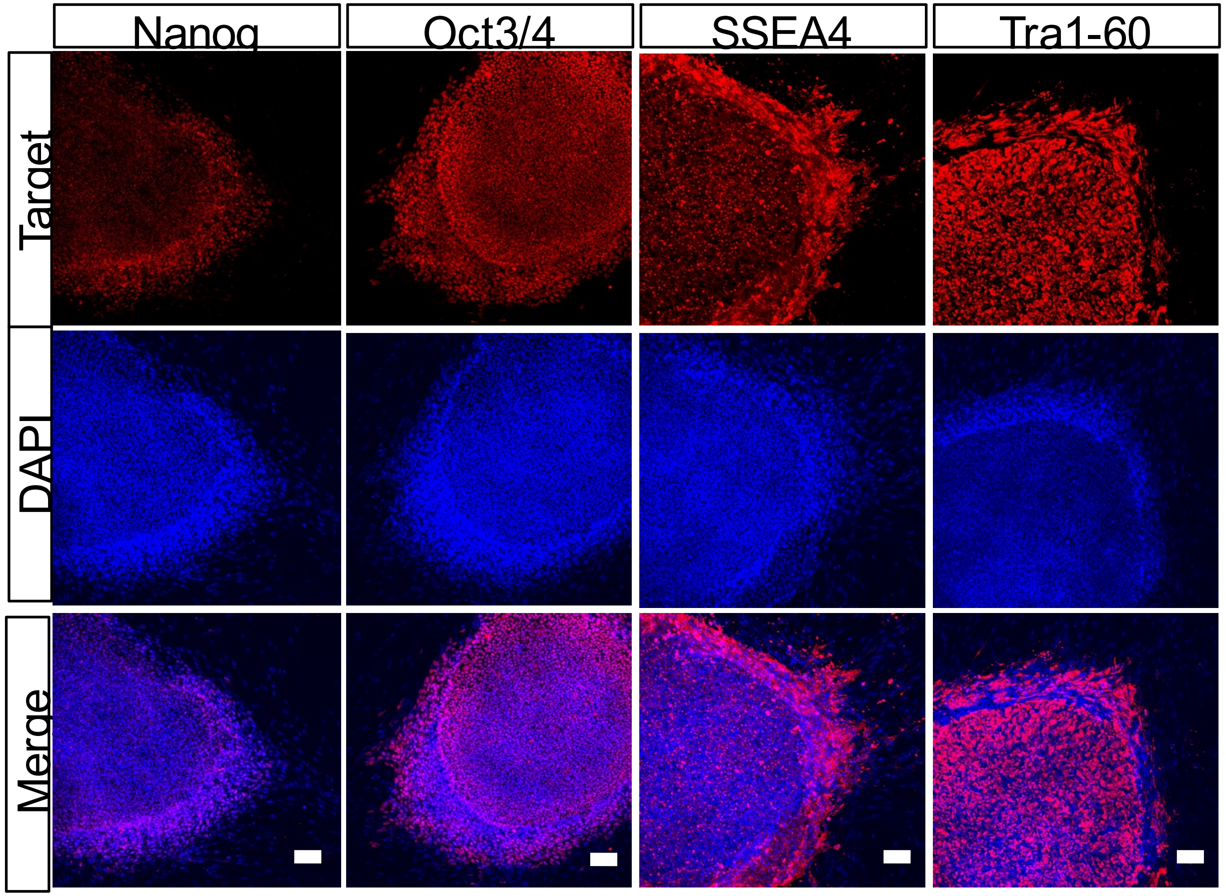
1st patient's iPS cells

Feeder cells: the patient's fibroblast





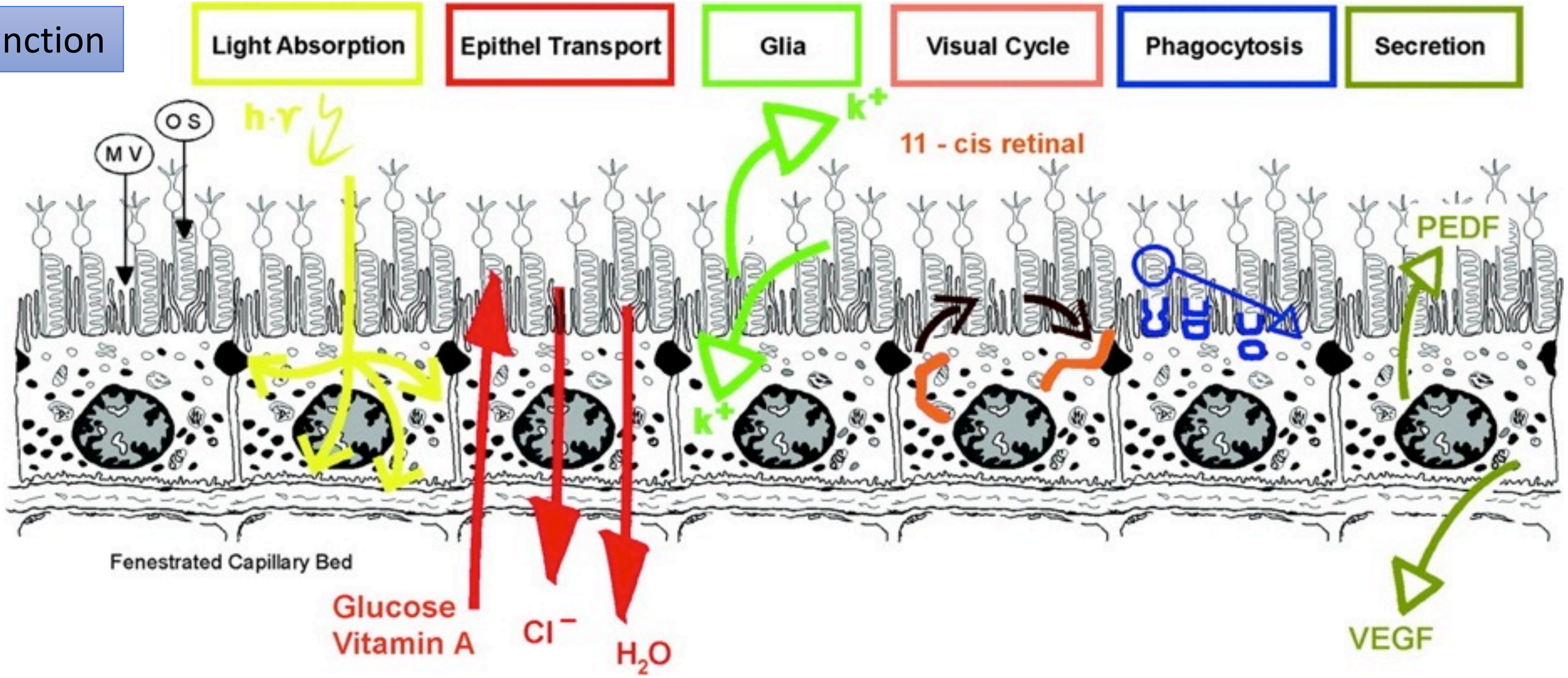
Karyotype: normal



Function of RPE sheet → Quality Control (QC)

Quality control	pigment		Best1 CRALBP	RPE65	Outer segment phagocitose test	PEDF VEGF

Function

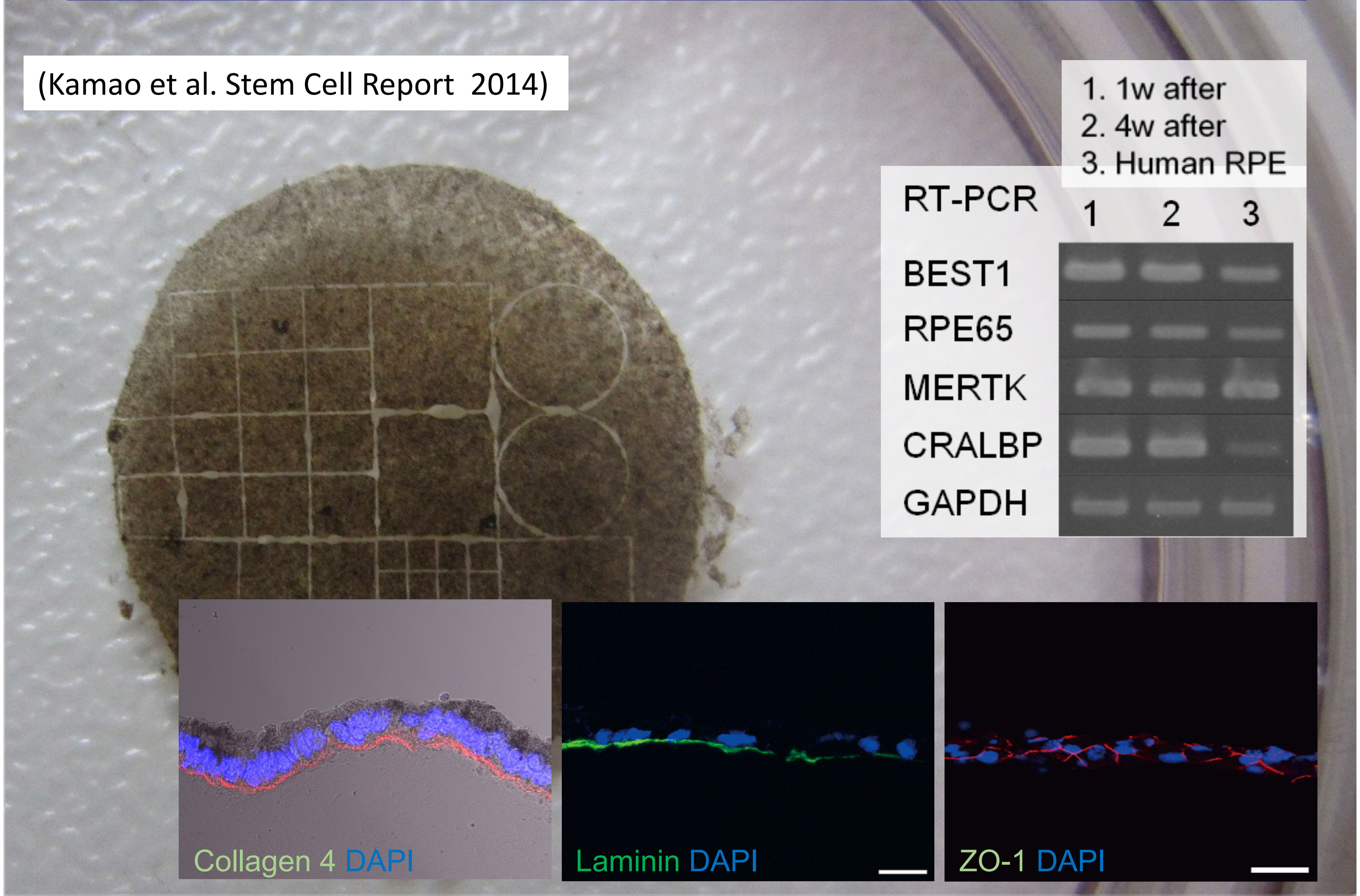


Strauss O Physiol Rev 2005;85:845-881

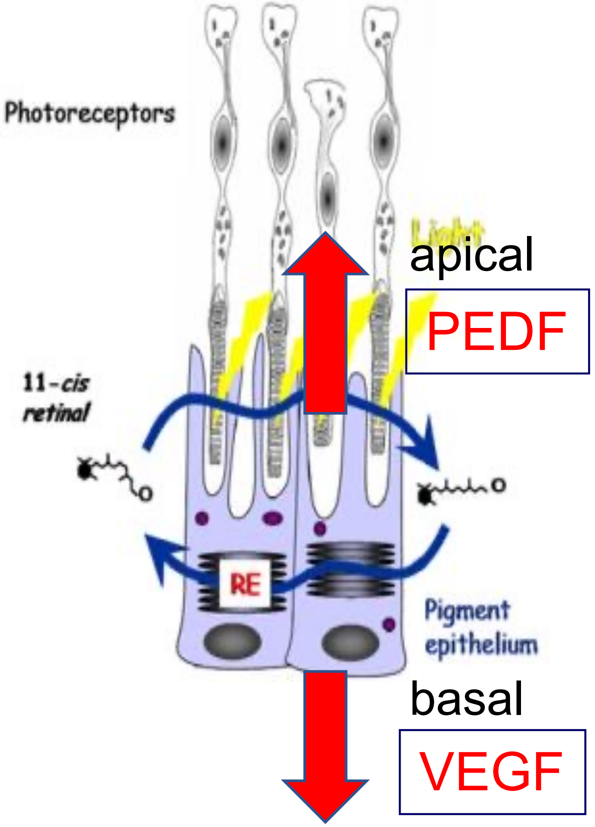
Physiological Reviews

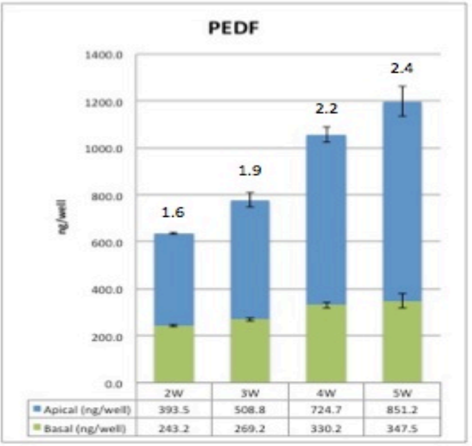
Quality of hiPSC-RPE cell-sheets

(Kamao et al. Stem Cell Report 2014)

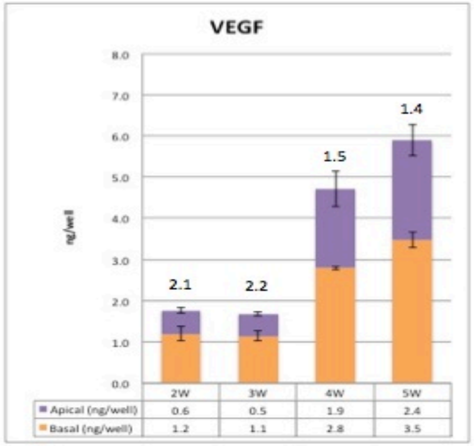


Monitoring points : RPE function



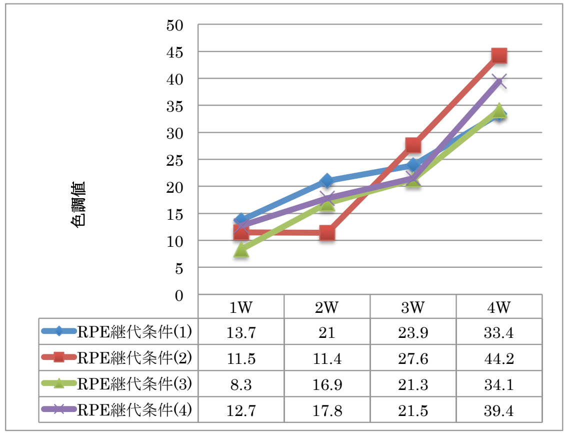


	2W	3W	4W	5W
Apical (ng/well)	393.5	508.8	724.7	851.2
Basal (ng/well)	243.2	269.2	330.2	347.5



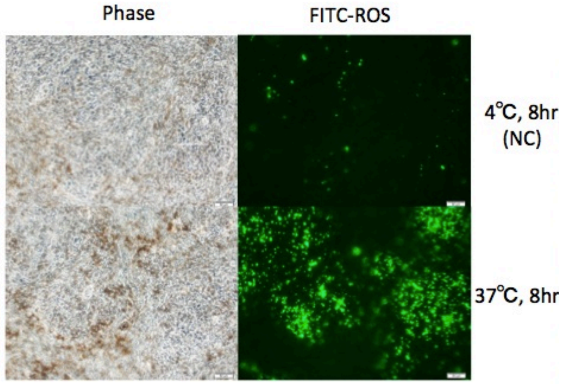
	2W	3W	4W	5W
Apical (ng/well)	0.6	0.5	1.9	2.4
Basal (ng/well)	1.2	1.1	2.8	3.5

PEDF Growth factors VEGF



	1W	2W	3W	4W
RPE継代条件(1)	13.7	21	23.9	33.4
RPE継代条件(2)	11.5	11.4	27.6	44.2
RPE継代条件(3)	8.3	16.9	21.3	34.1
RPE継代条件(4)	12.7	17.8	21.5	39.4

Color

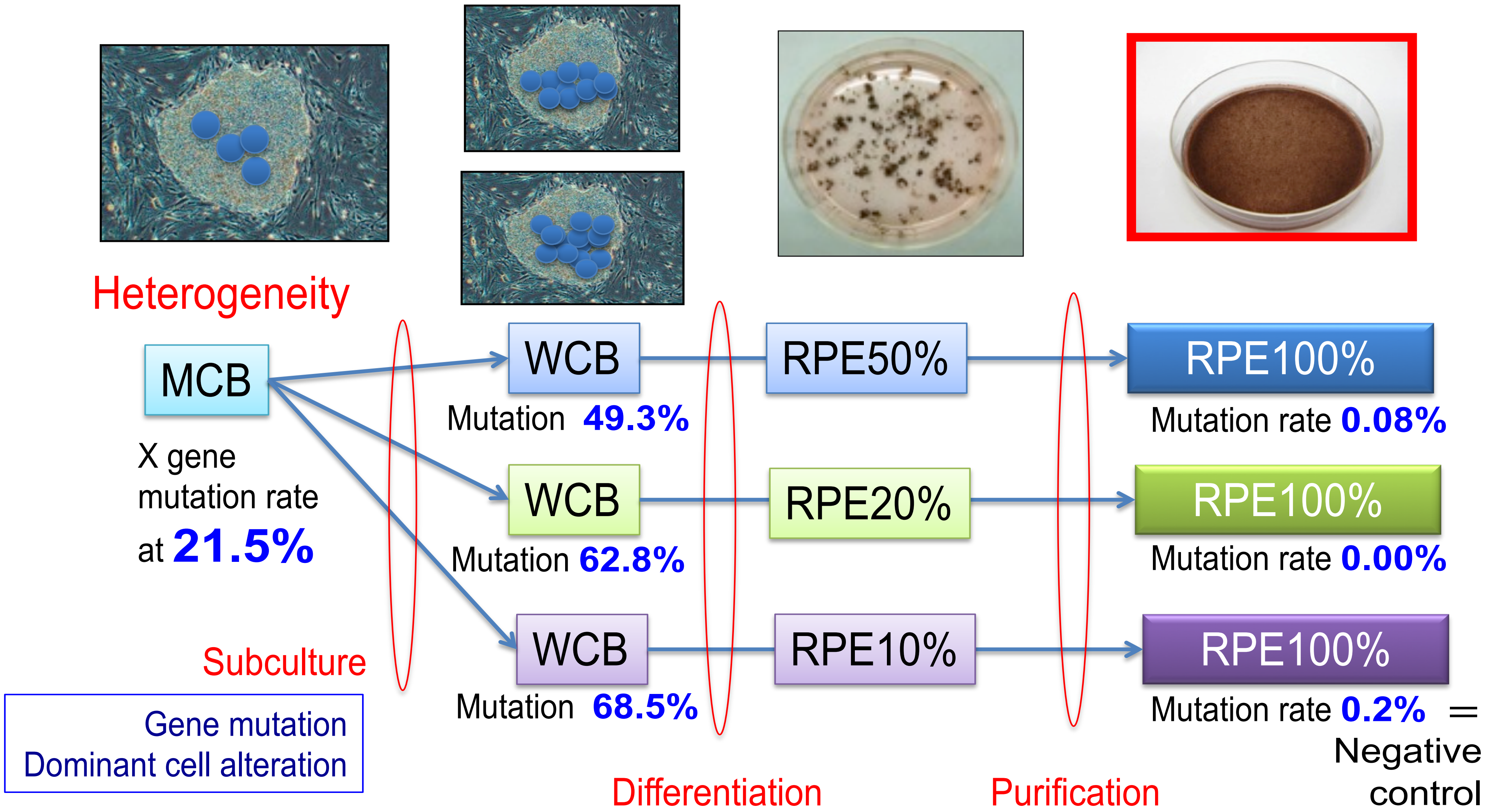


Phagocytosis

QC: Quality control of hiPS-RPE

		methods		Case 1
Quality /function	1) Appearance of sheets	Observation by eye and under microscopy	No cell defect No contamination of odd materials No discolor	Good
	2) Structure of sheets	Z-Stack observation of confocal microscopy		
	3) Live cell ratio & density of cells	Toripane blue staining after tripsinization of sheet	>= 70% >= 4,500 cells/mm^2	96.8% 16,200
	4) RPE specific gene	RT-PCR (RPE65, CRALBP, MERTK, BEST1)	Positive band	positive
safety	5) purity	①immunocytochemistry & pigmenatiton ②tight attachment cell	① >= 95% ② >= 99.9%	100% >=99.9%
	6) stem or immature cell marker	qRT-PCR (Lin28)	Not detected (= less than 1/50000 cells)	None
	7) bacteria・fungus	薬局方（mambrane filter method）	none	None
	8) micoplasma	薬局方（PCR、immunostaining）	none	None
	9) endotoxine	薬局方	<= 3EU	<=3EU

Heterogeneity of cells in the iPSC colonies



MCB: Master Cell Bank WCB: Working Cell Bank

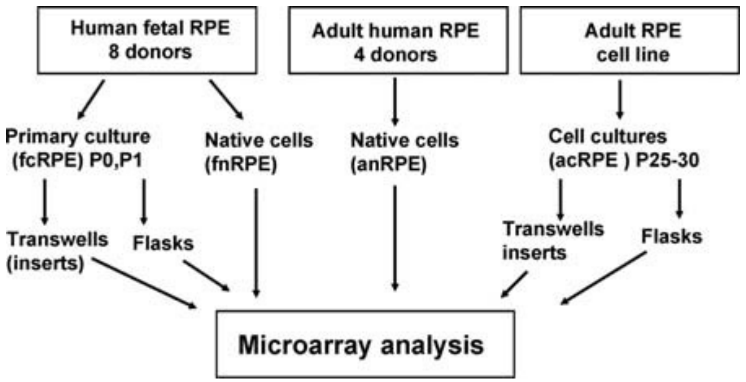
RPE signature genes

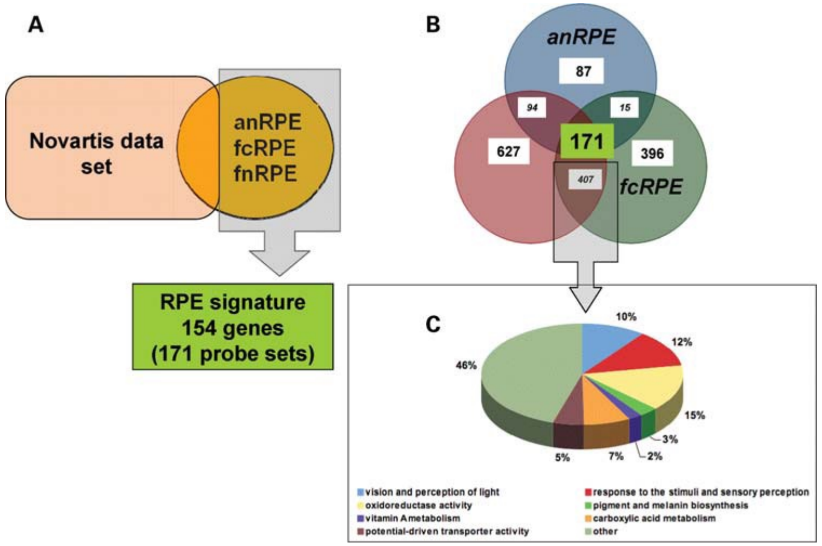
RPE signature gene

Human Molecular Genetics, 2010, Vol. 19, No. 12 2468–2486

Transcriptome analysis and molecular signature of human retinal pigment epithelium

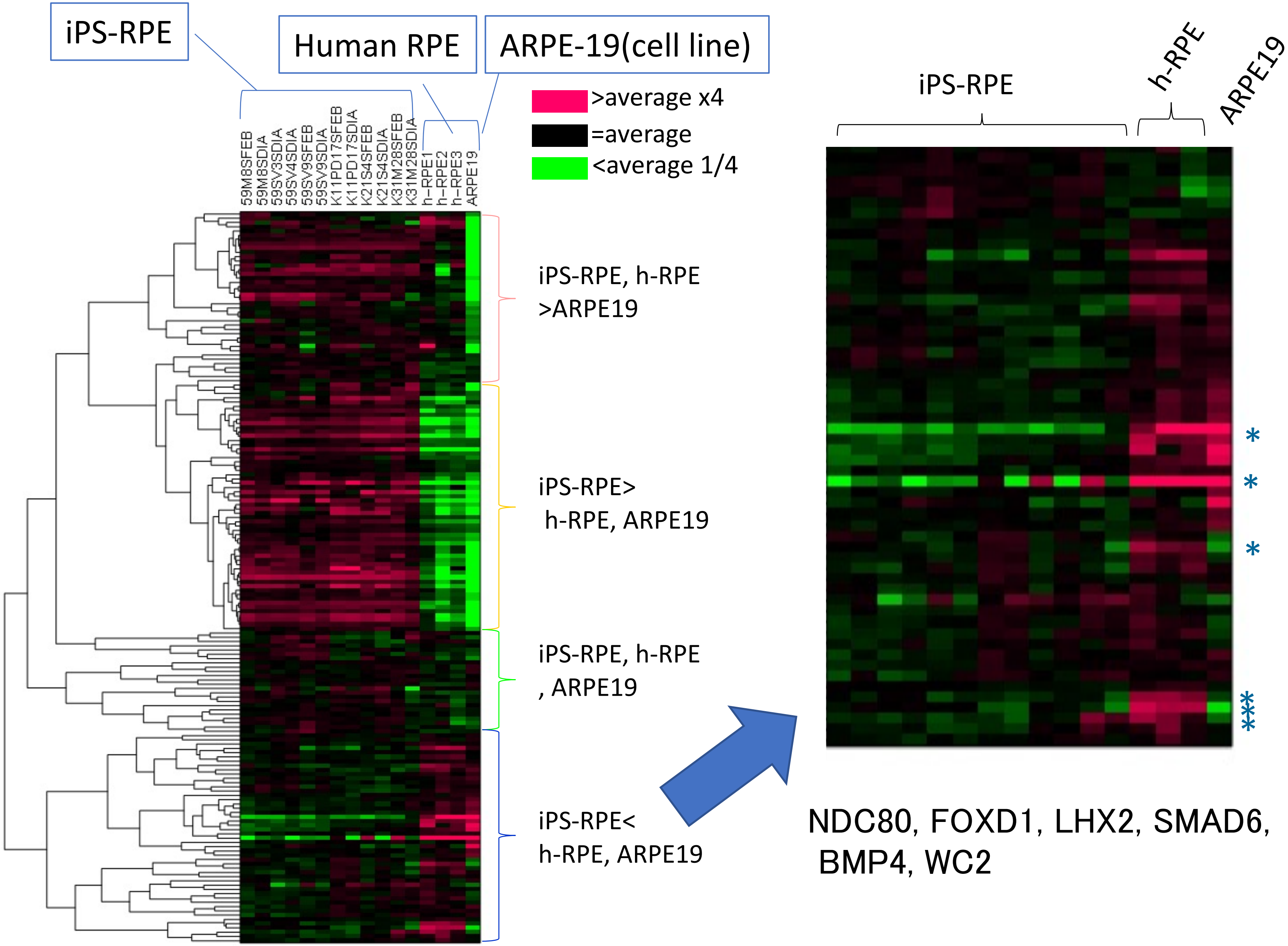
N.V. Strunnikova[1,4], A. Maminishkis[2,4], J.J. Barb5, F.Wang[2,4], C. Zhi[2,4], Y. Sergeev[1,4], W. Chen[6], A.O. Edwards[7], D. Stambolian[8], G. Abecasis[6], A. Swaroop[3,4], P.J. Munson[5] and S.S. Miller[2,4,*]





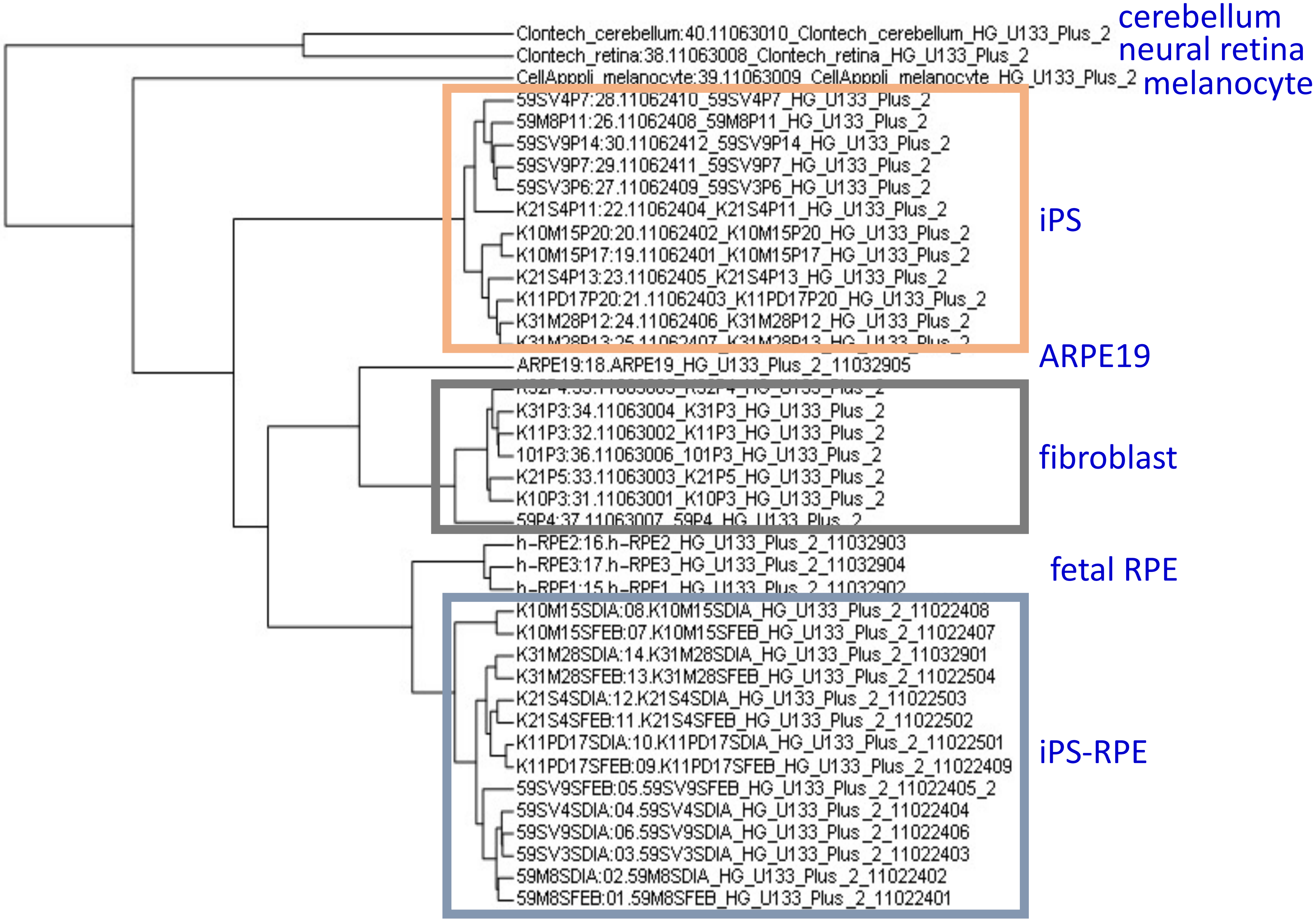
Common among Adult native, fetal cultured, fetal native= **154 genes**

Heatmaps by RPE signature genes



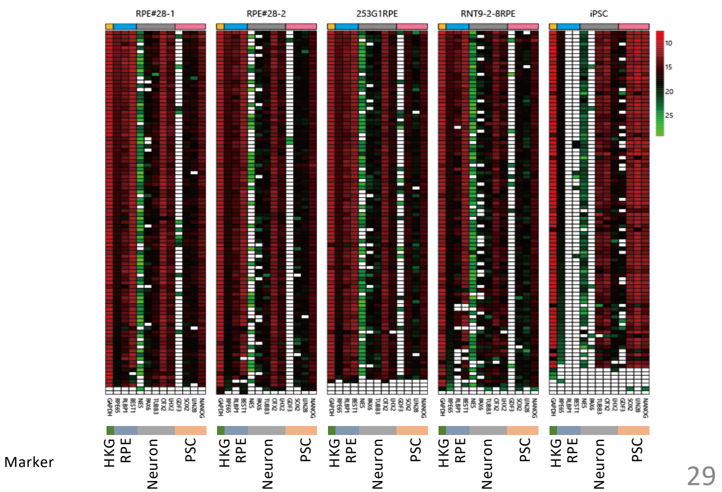
NDC80, FOXD1, LHX2, SMAD6, BMP4, WC2

Microarray cluster analysisc

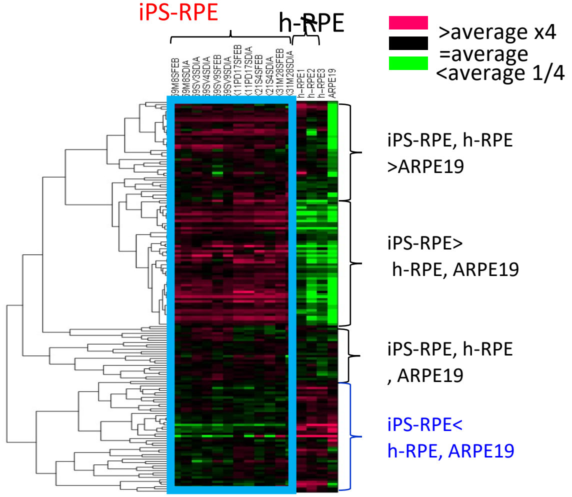


<Robust protocol > evaluation of purity and sameness

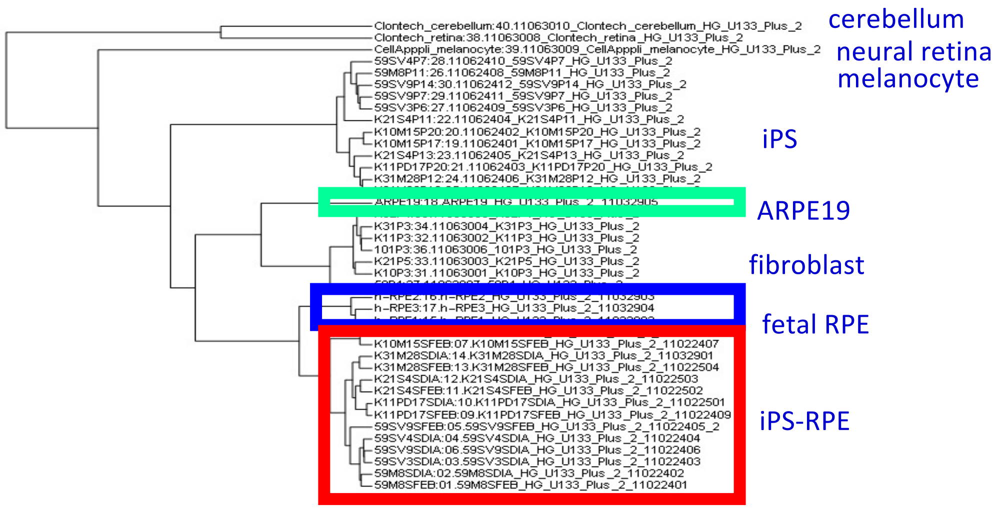
Single cell RT-PCR



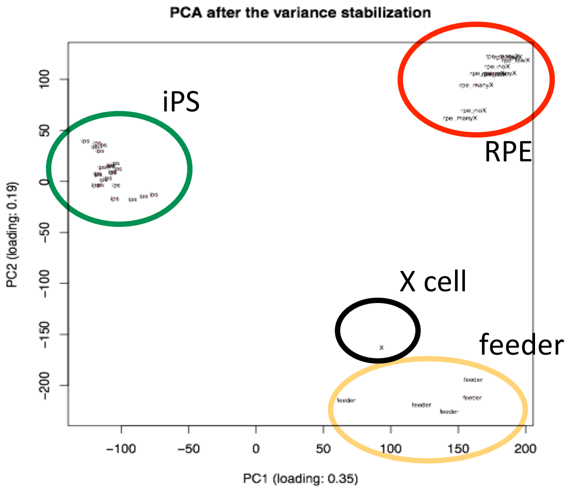
RPE signature genes



Microarray cluster analysis



Transcriptome analysis

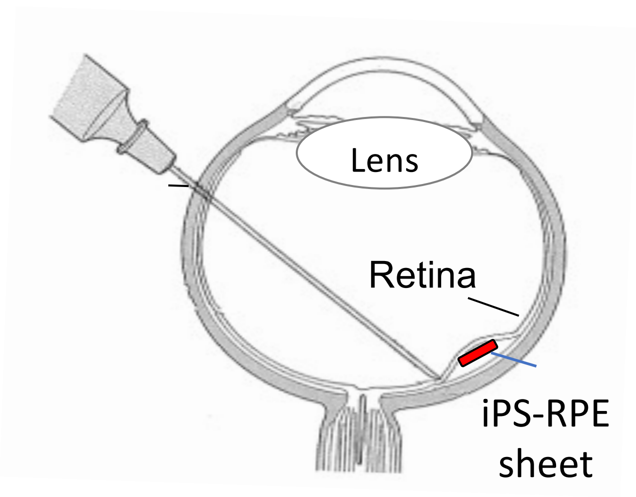


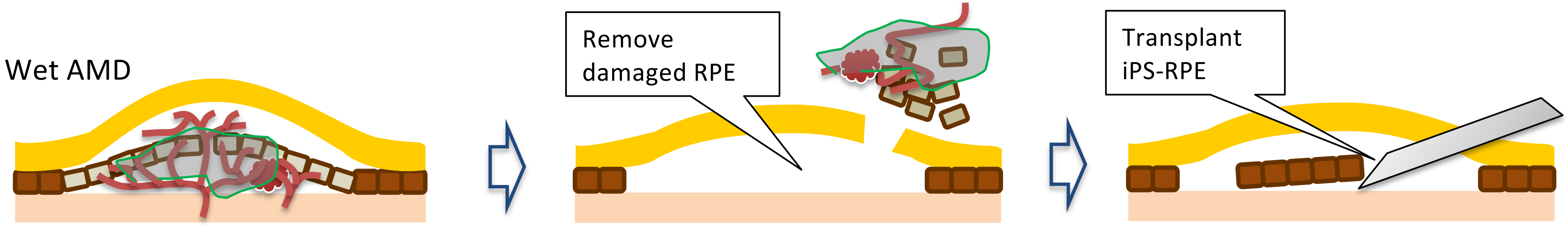
Genomic analysis

(@ CiRA, Prof. Yamanaka)

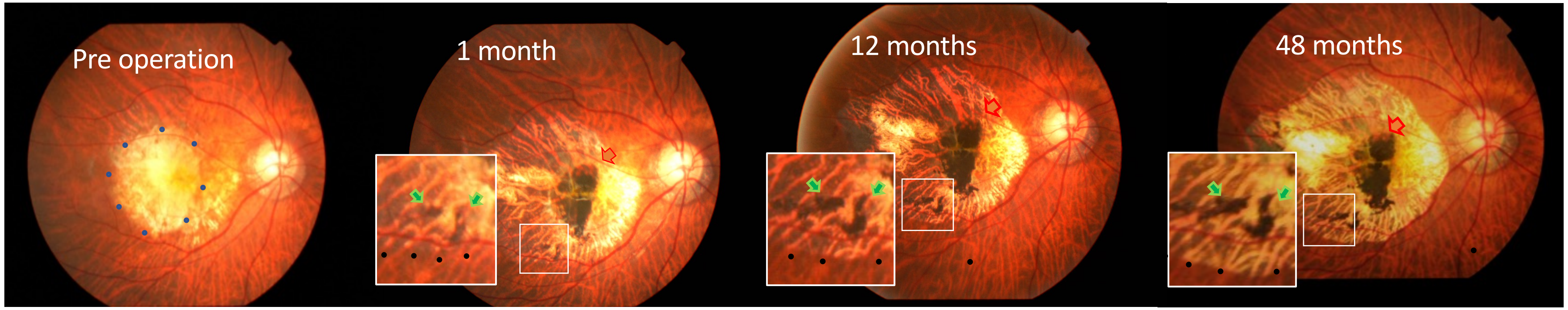
Points	methods
a) Plasmid fragment remnant check	WGS, qRT-PCR, capture Sequence
b) Copy number variation (CNV)	SNP array
c) Mutations in the driver genes	WGS
d) Epigenetic analysis	Methylome analysis
e) Purity	single cell RT-PCR

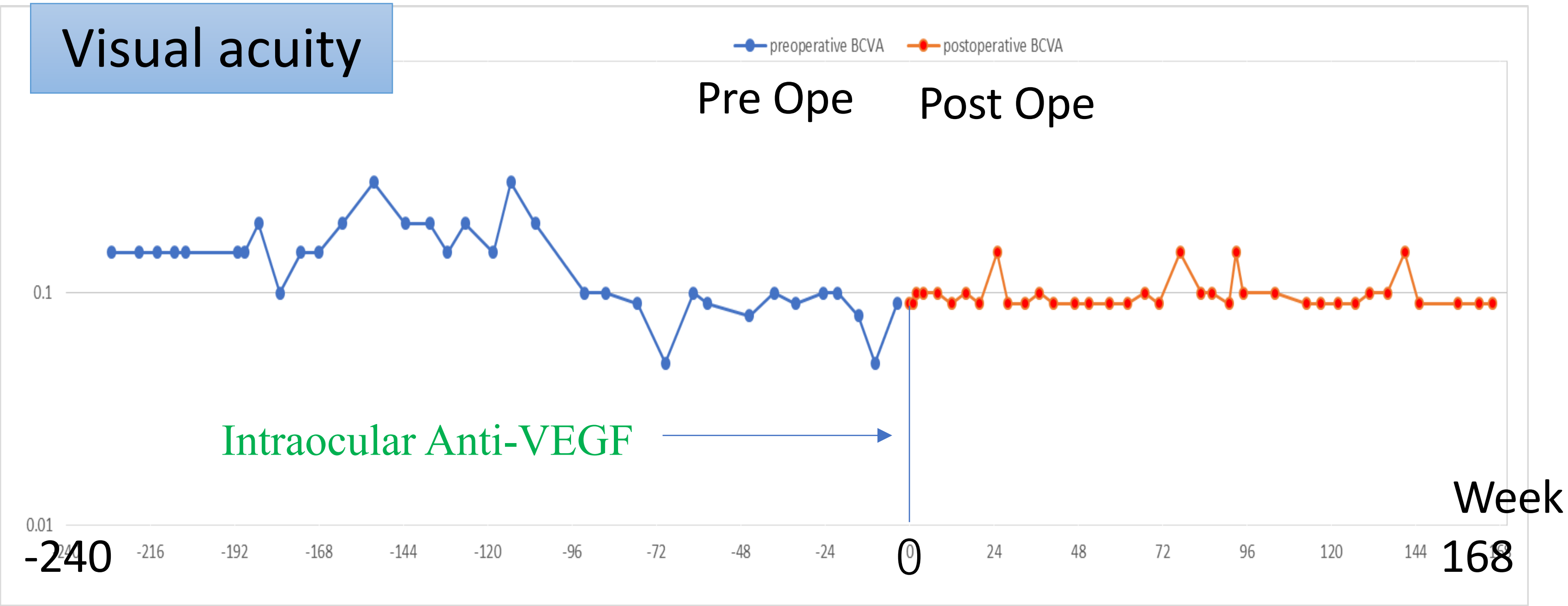
The first-in-man application of iPS-derived cells (2014)



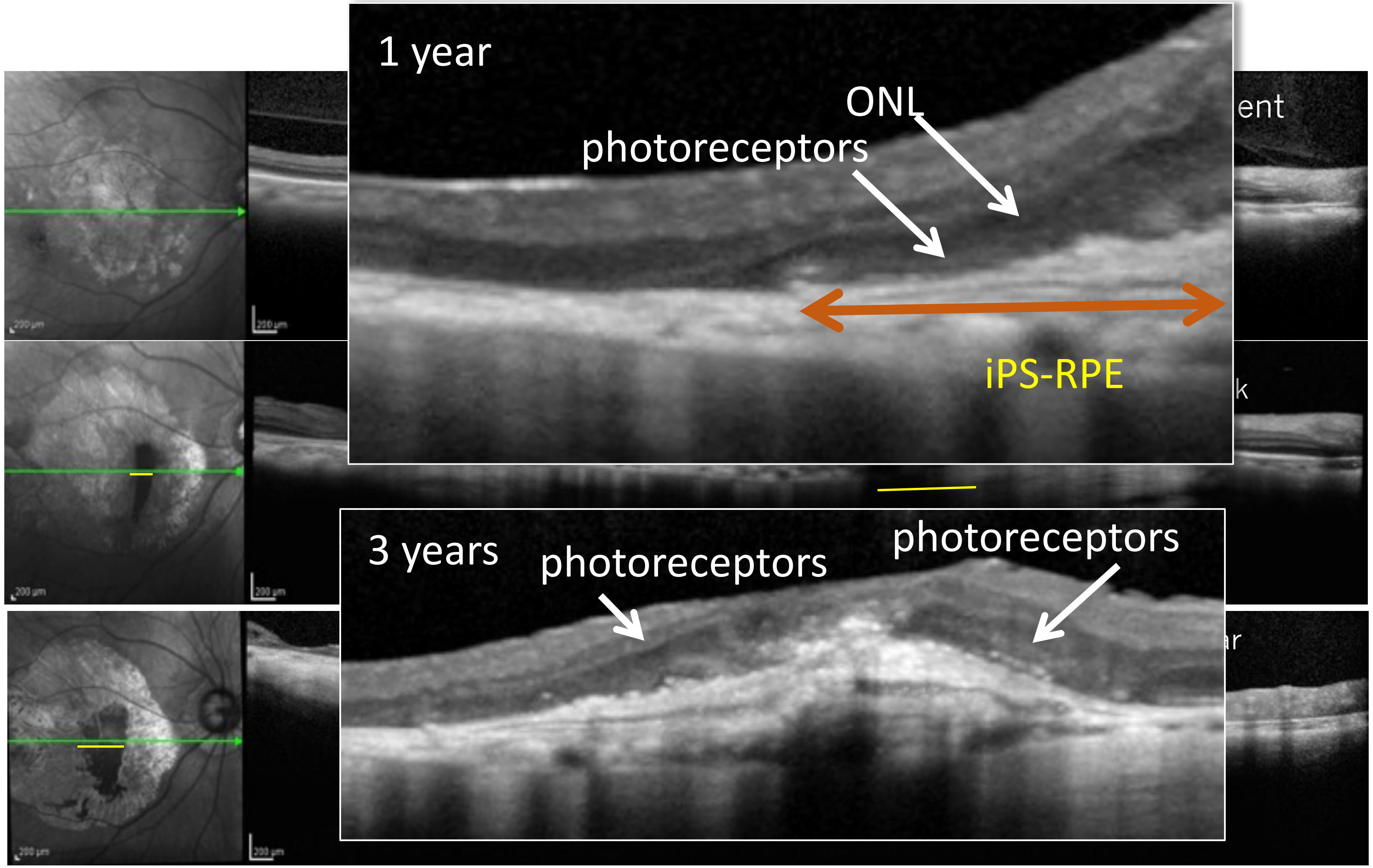


Fundus photos and visual acuity （4 years）

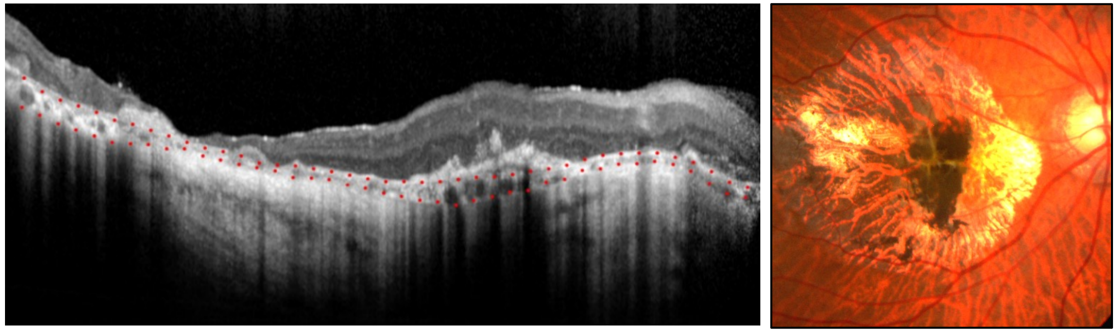


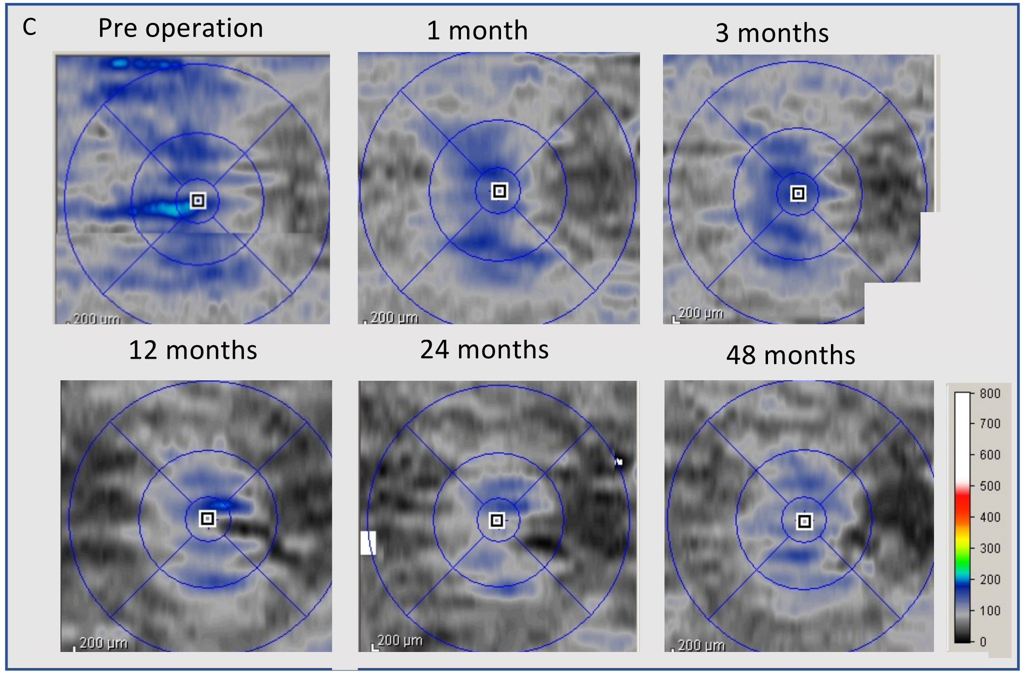


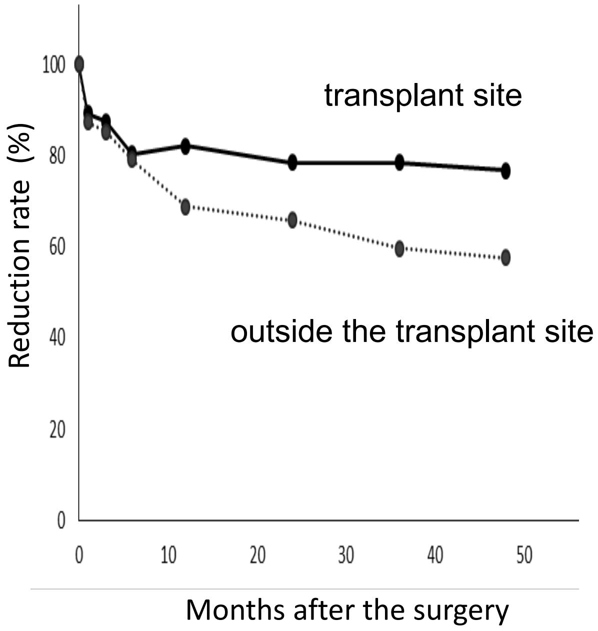
OCT (Transvers section of the retina)



Choroidal thickness





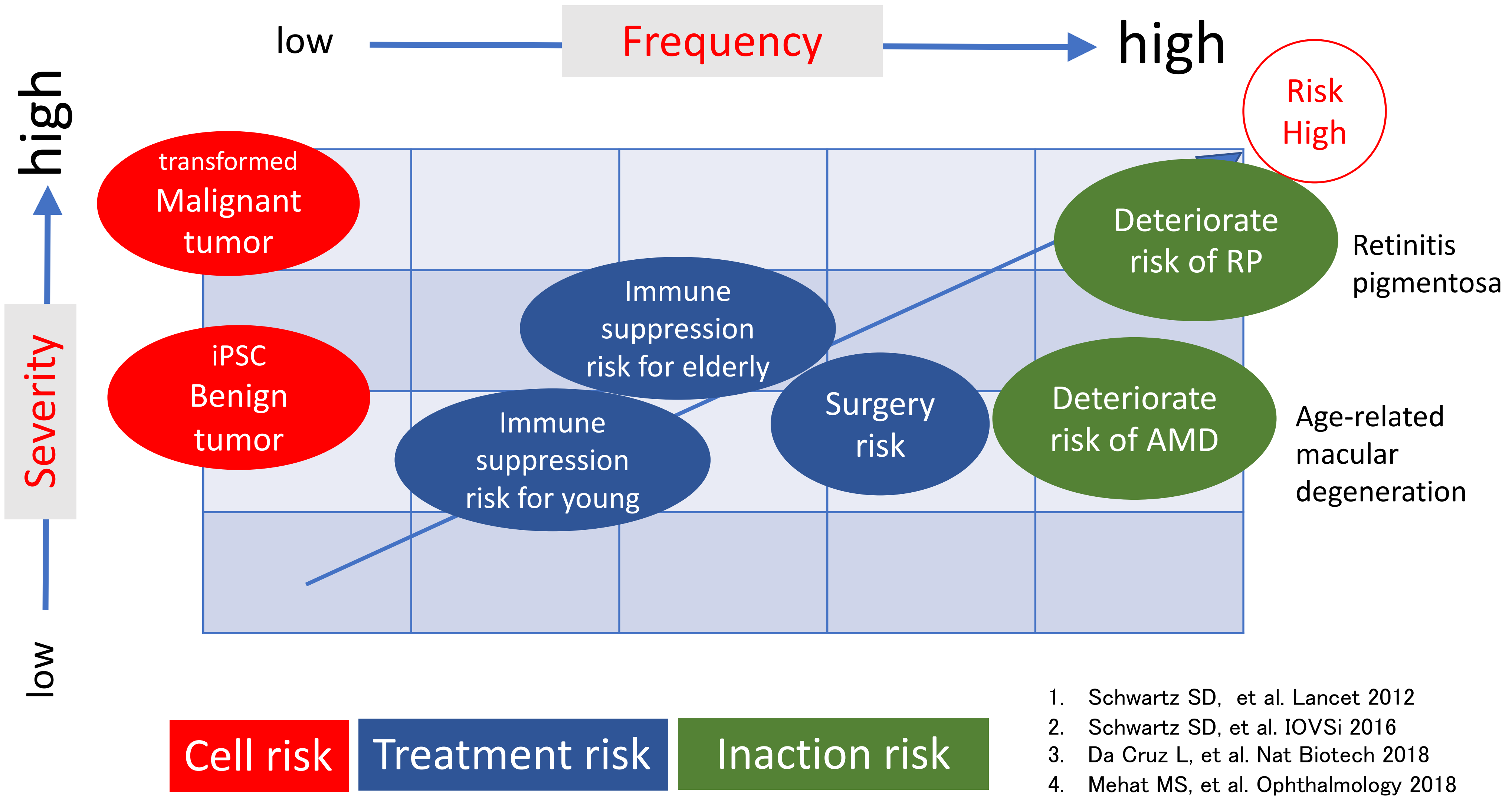


Risks of the cell therapy

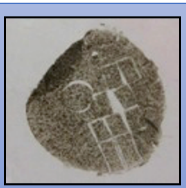
1. Cell risk - gene mutation etc.
2. Treatment risk - Immune suppression

   Surgery technique
3. Disease' risk (Risk of inaction) - deterioration risk

Risk Matrix for complication of iPSC-RPE (Image)



1. Schwartz SD, et al. Lancet 2012
2. Schwartz SD, et al. IOVSi 2016
3. Da Cruz L, et al. Nat Biotech 2018
4. Mehat MS, et al. Ophthalmology 2018
5. Kuriyan AE, et al. New Eng J Med 2017



RPE cells
(for RPE impaired diseases)

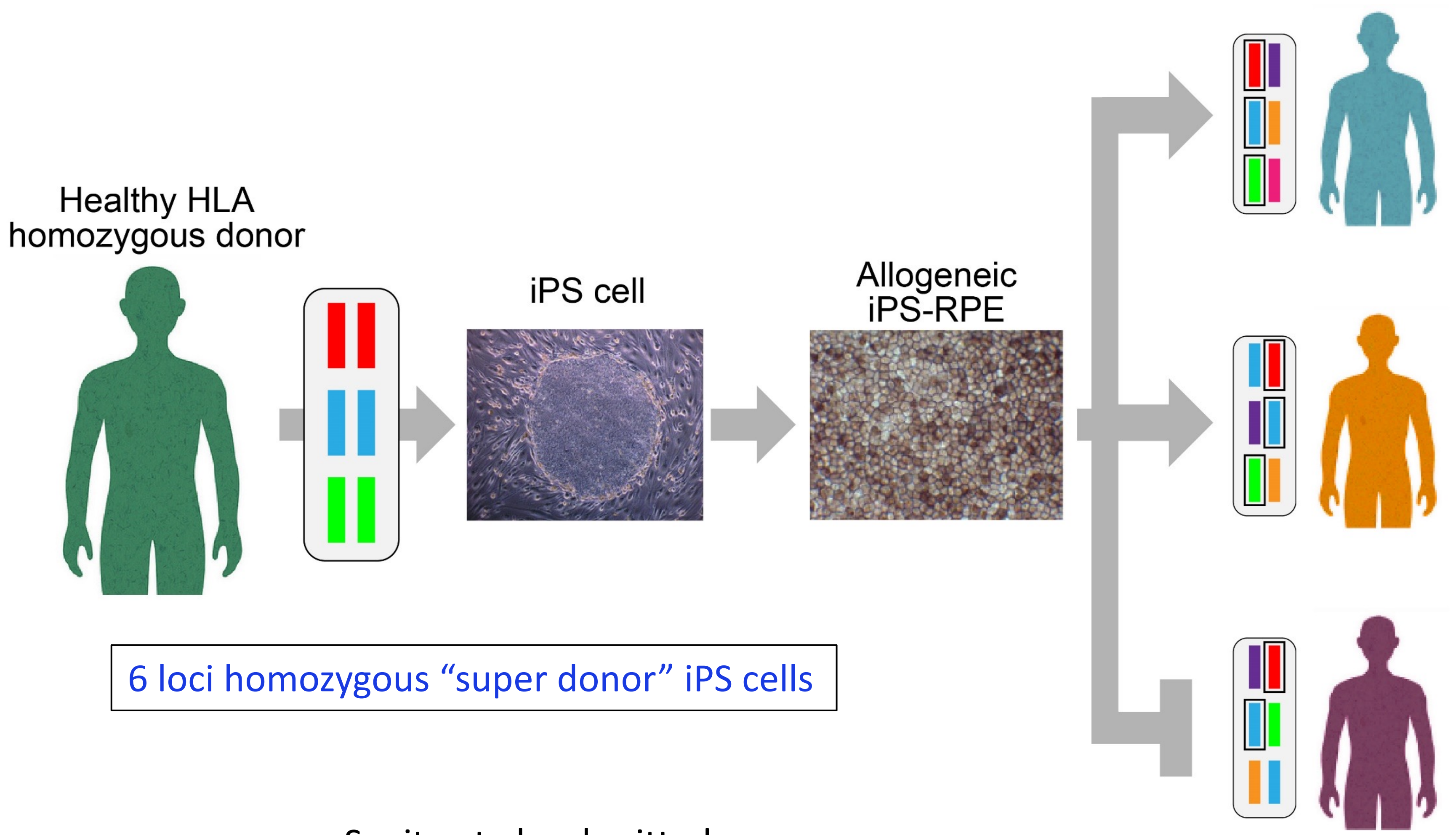
2nd clinical research

<u>HLA matched Allogeneic</u> iPSC-RPE
transplantation
(5 cases: 2017~2018)

To show the possibility of allogeneic transplantation
without systemic immune suppression
for elder patient

2nd clnical research

Allogeneic iPS-RPE transplantation (2017~)

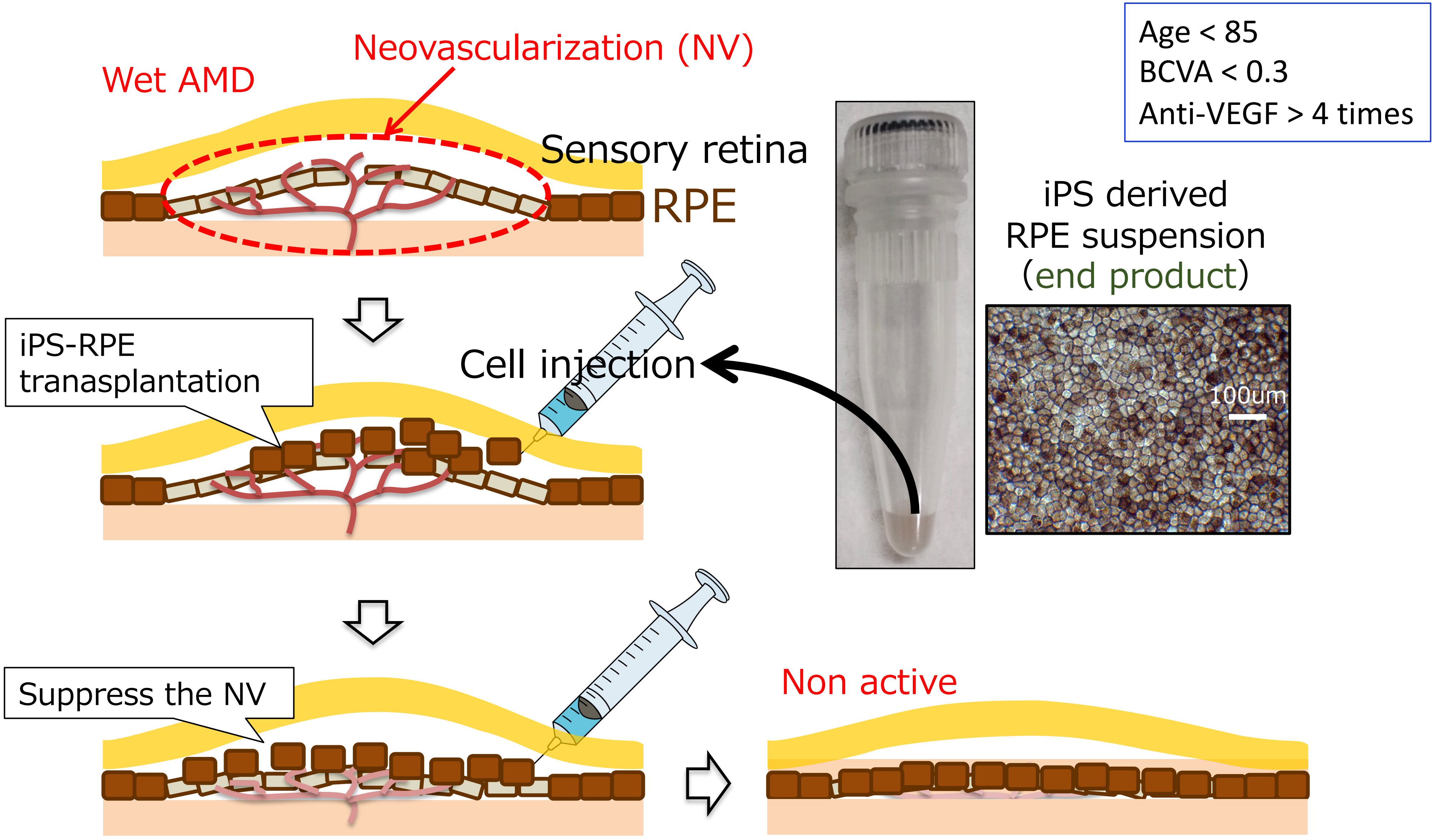


6 loci homozygous "super donor" iPS cells

Sugita et al. submitted

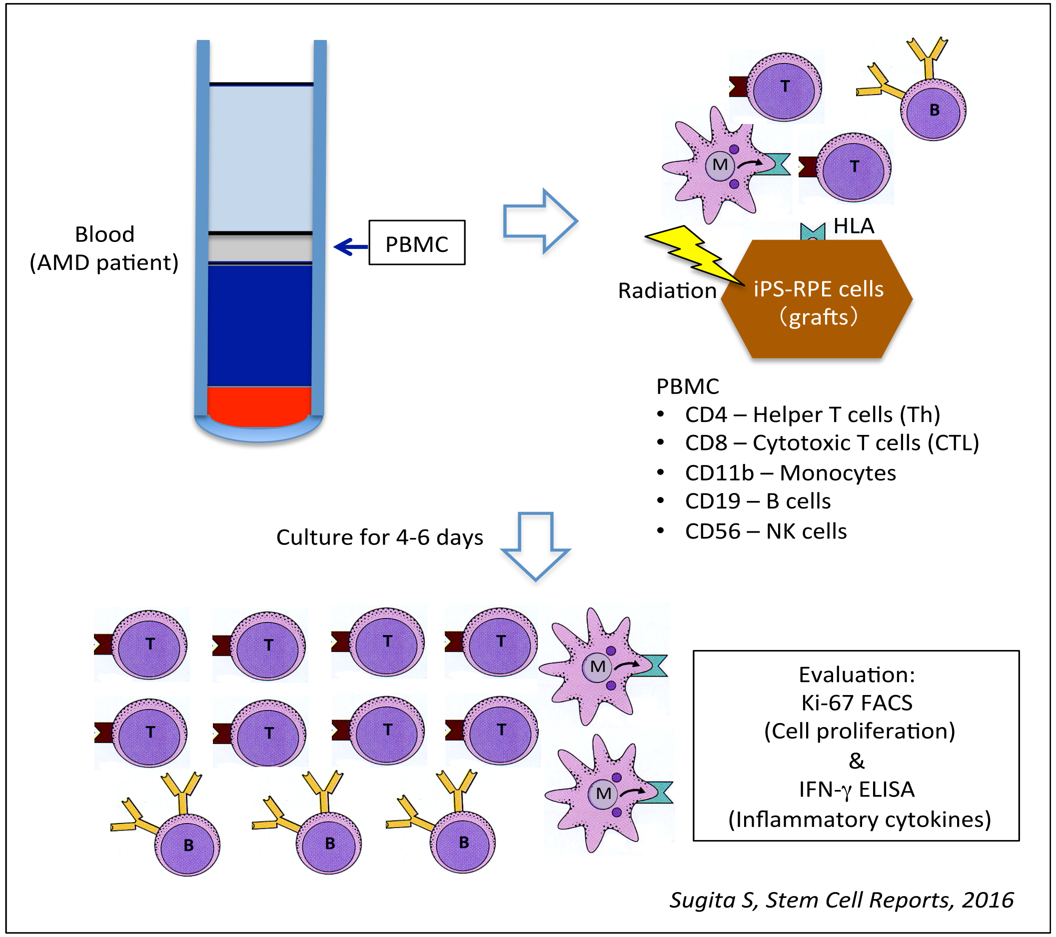
(Modified from JMA J. 2018;1(1):6-14)

RPE cell suspension transplant

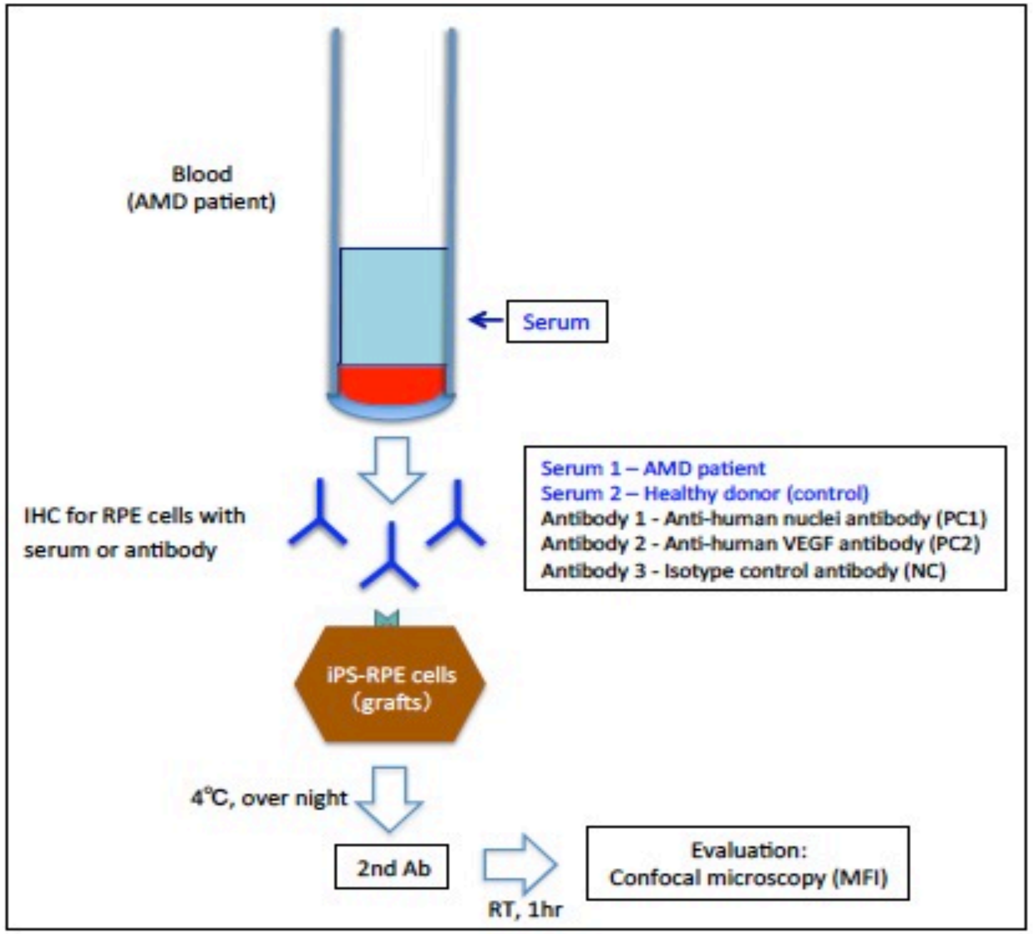


Real time immune reaction test

LGIR Test
(Lymphocytes-Grafts Immune Reaction test)

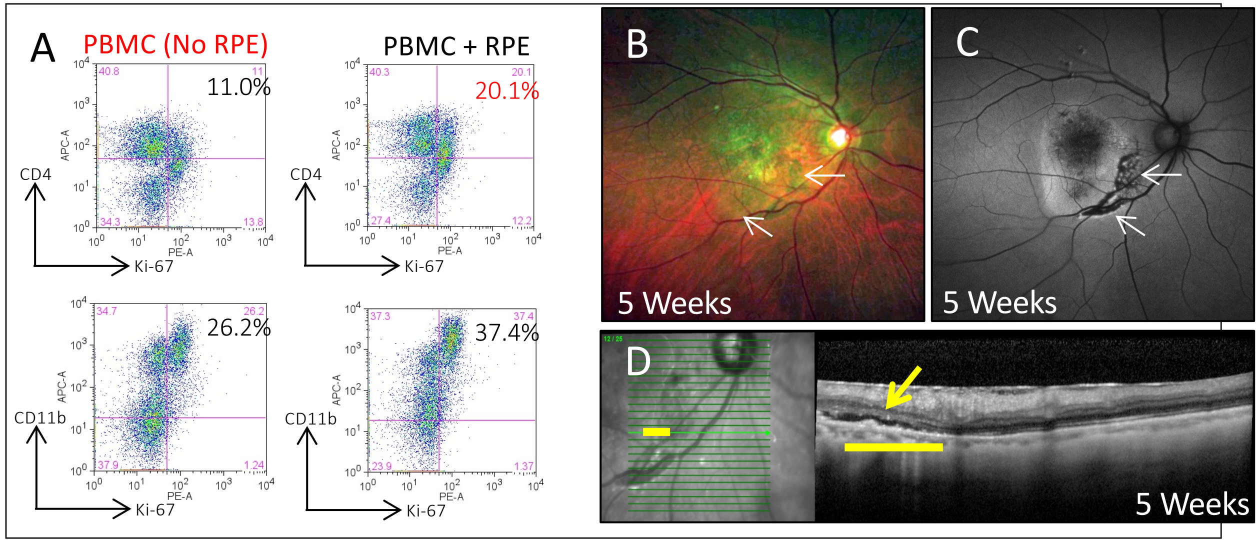


DSA Test
(donor specific antibody)

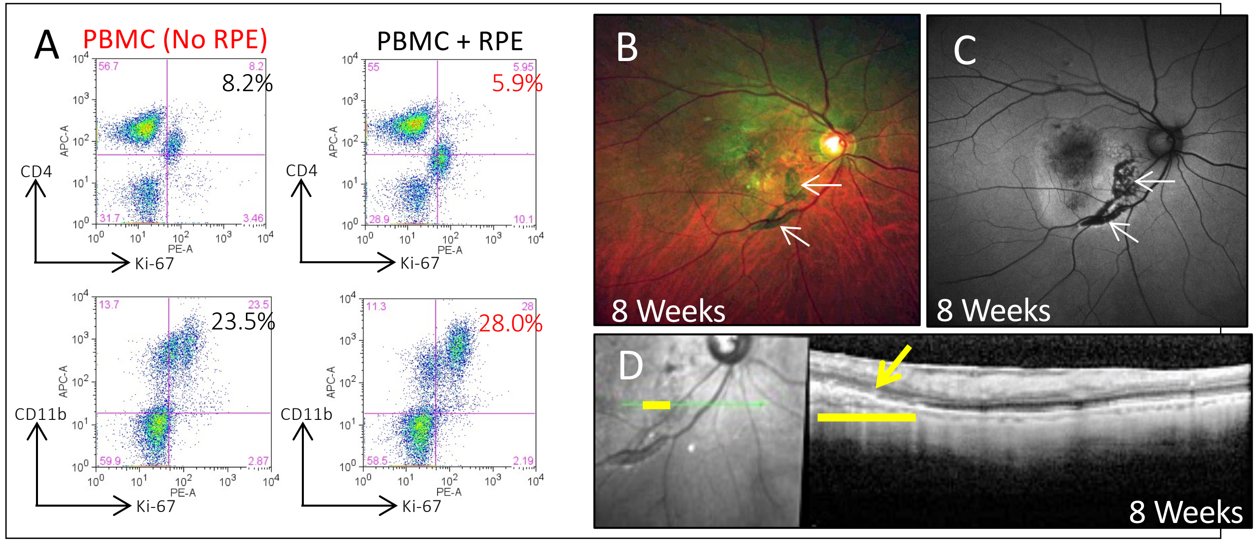


Efficacy of LGIR test Case 1

5W



8W



5W

Subtle subretinal fluid

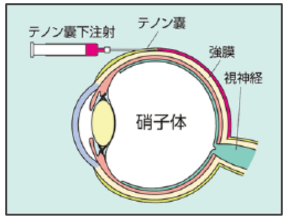
Immune rejection? or

Recurrence of disease ?

→ LGIR test

→ slightly positive

Local steroid injection



8W

→LGIR test negative

Graft survival !

Monitoring of immune reaction by LGIR & DSA

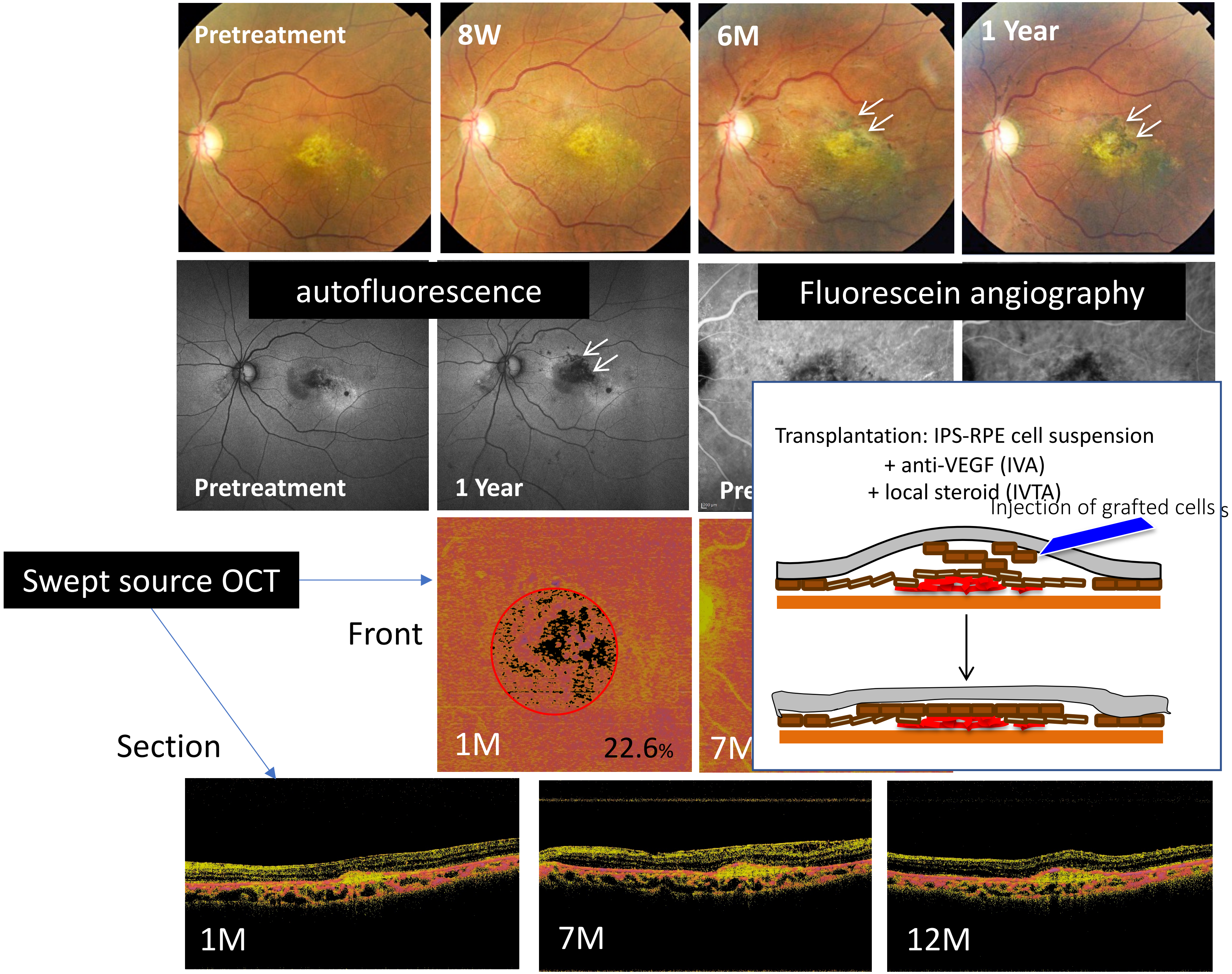
DSA (donor specific antibody); RPE specific antibody

Case #＼DSA	術前	術日	4W	8W	12W	24W	52W
#1	―	―	―	―	―	―	―
#2	―	―	―	nt	―	―	―
#3	―	―	―	nt	―	―	―
#4	―	―	―	nt	―	―	―
#5	―	―	―	nt	―	―	―

LGIR; Lymphocyte graft immune reaction test

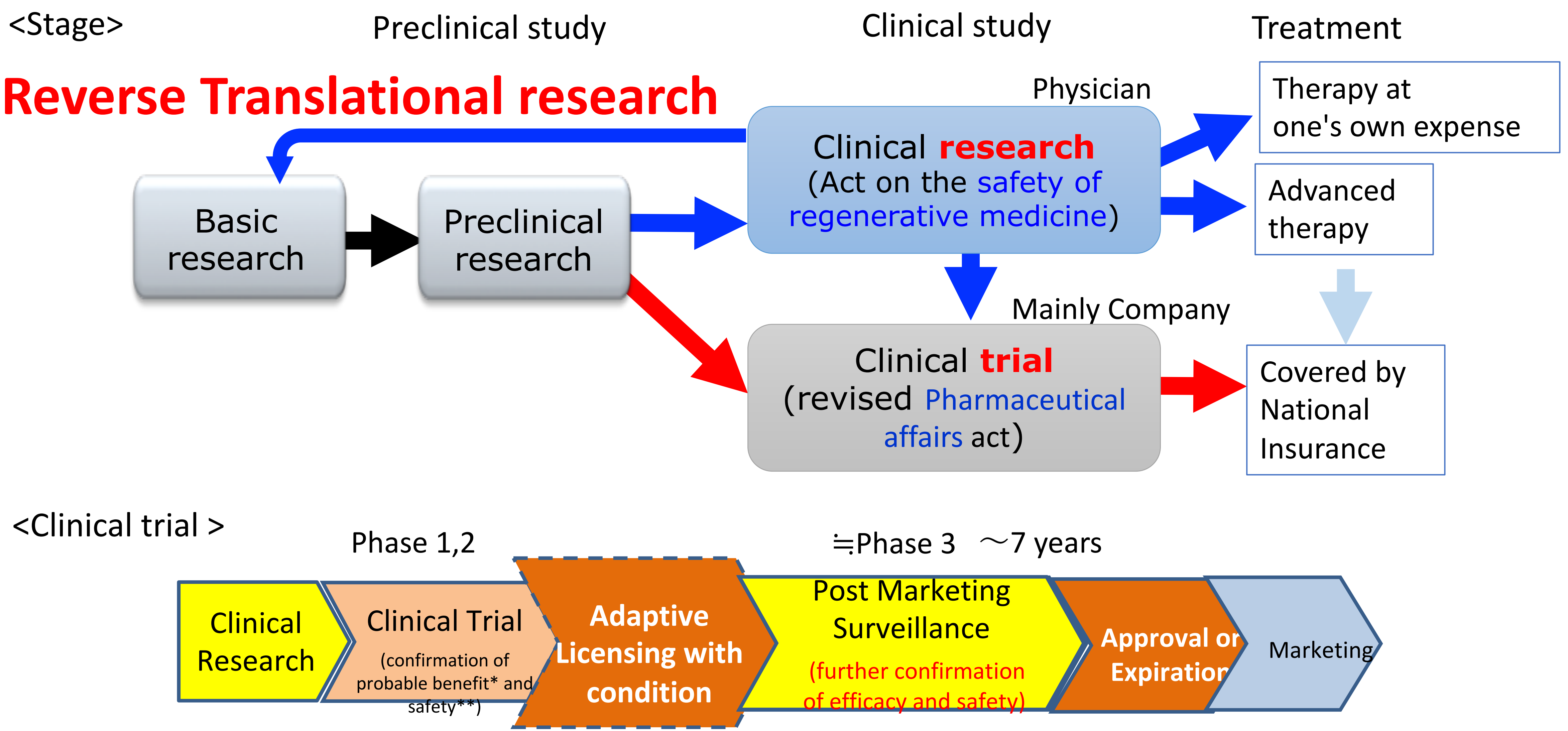
Case #＼LGIR	術前	術日	4W	8W	12W	24W	52W
#1	―	―	+	+	±	±	―
#2	―	―	―	nt	±	―	―
#3	―	―	―	nt	―	―	―
#4	―	―	―	nt	―	―	―
#5	―	―	―	nt	―	±	―

Case 2



Pretreatment
8W
6M
1 Year
autofluorescence
Pretreatment
1 Year
Fluorescein angiography
Pre
Transplantation: IPS-RPE cell suspension
+ anti-VEGF (IVA)
+ local steroid (IVTA)
Injection of grafted cells
Swept source OCT
Front
1M
22.6%
7M
Section
1M
7M
12M

Regulatory system in Japan



3rd clinical research (phase 2)

HLA matched & unmatched Allogeneic

iPSC-RPE transplantation

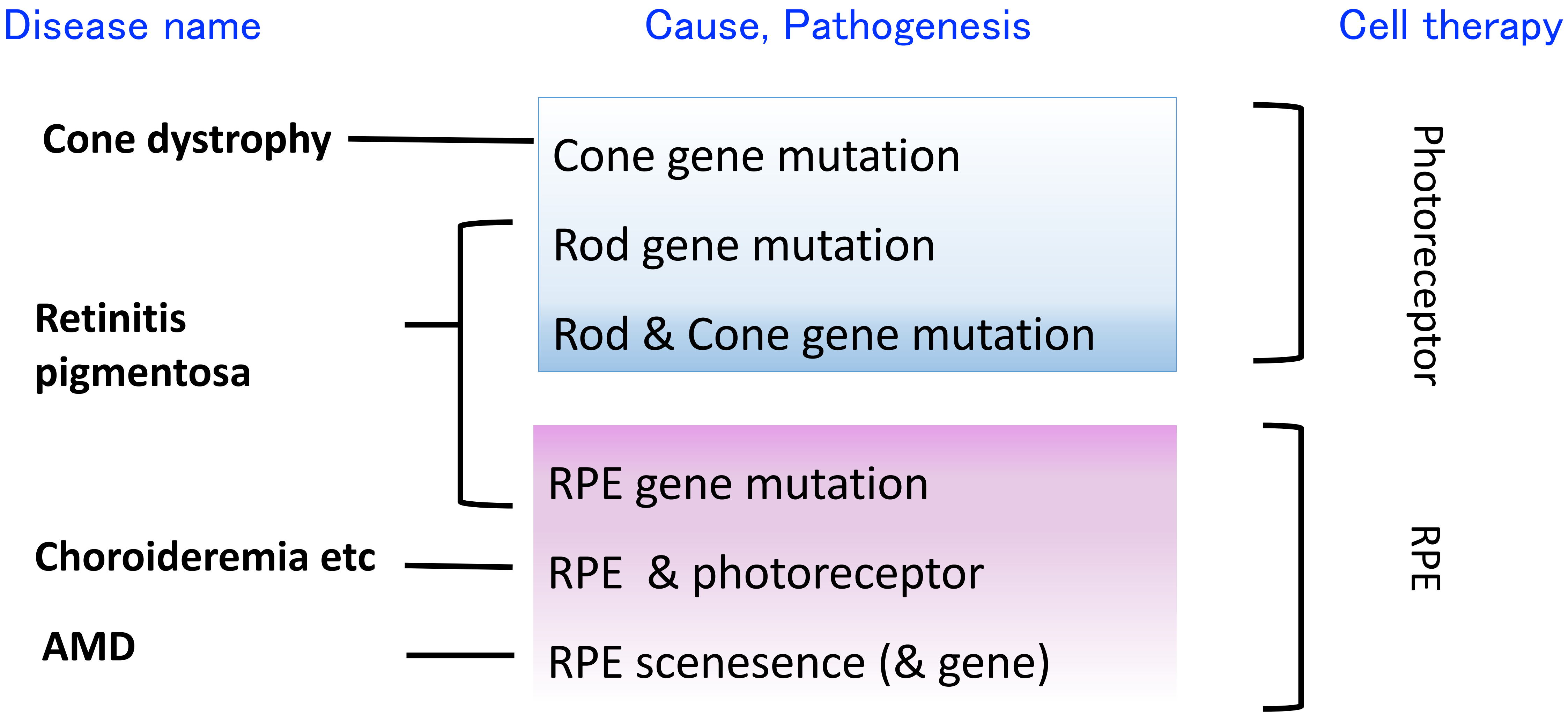
(50 cases: 2021.1~2016)

To evaluate the efficacy of iPS-RPE treatment

How to access the efficacy

What kind of cases are suitable

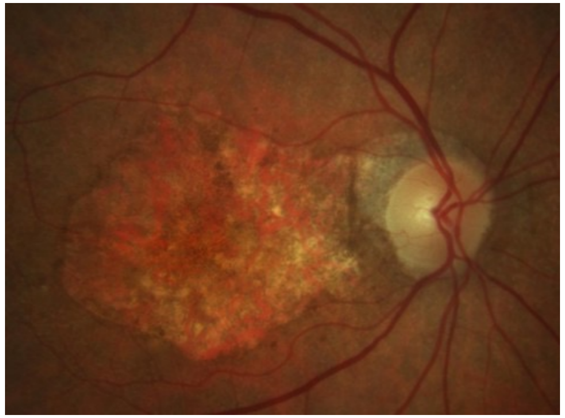
Retinal degenerative diseases



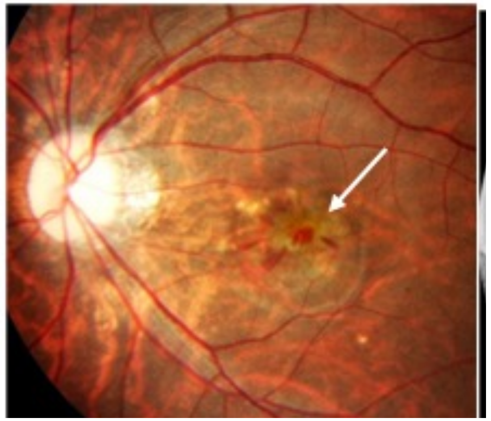
RPE impairment diseases

Crystalin retinopathy

Dry type AMD

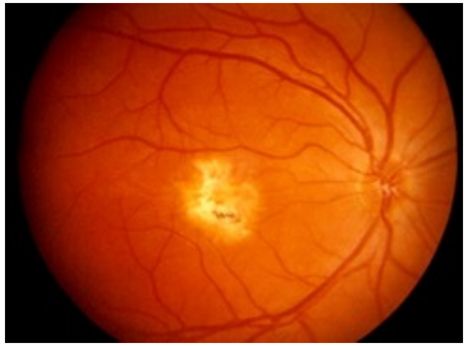


High Myopia

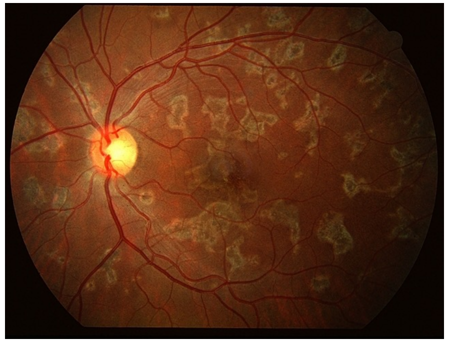


Stargardt disease

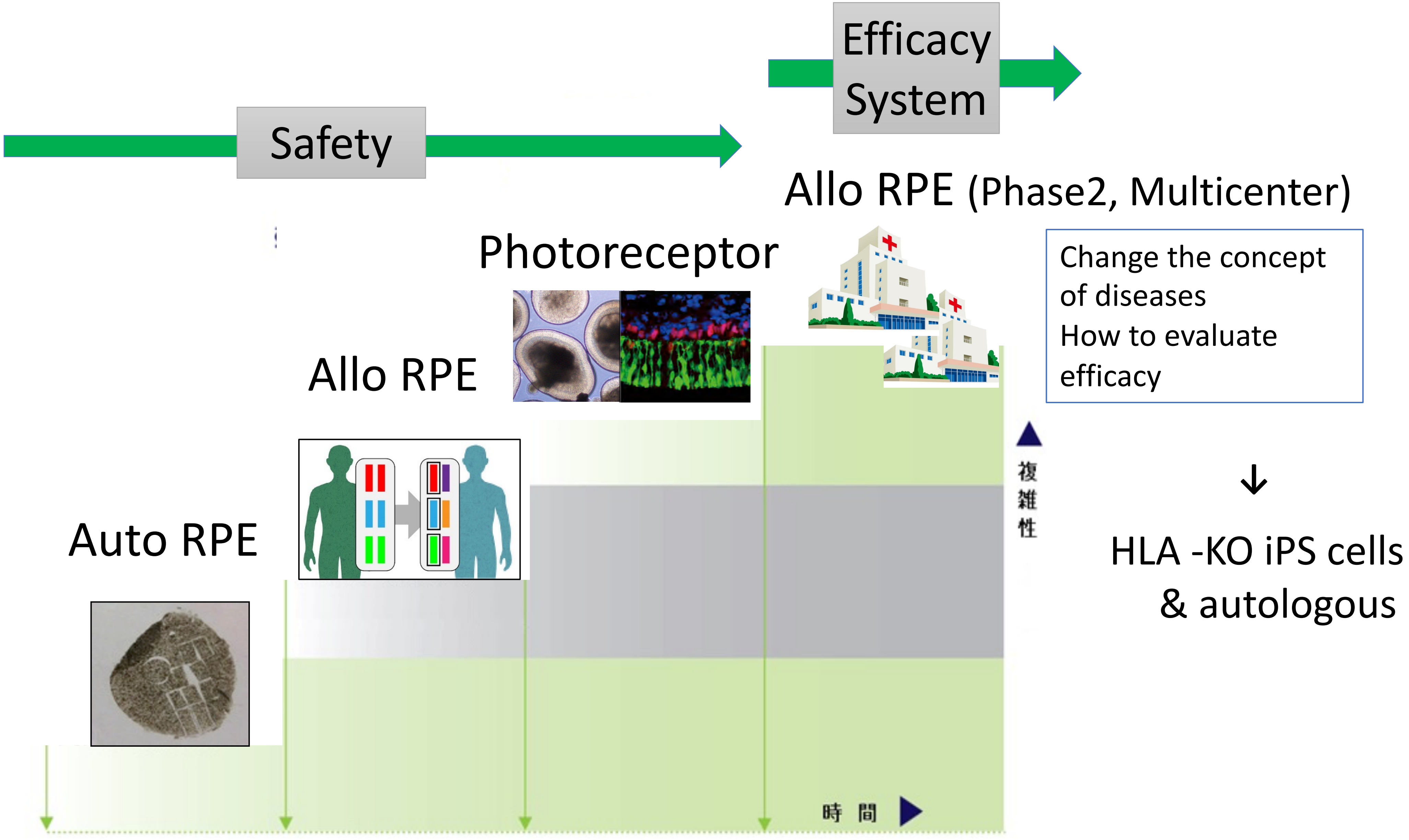
Best disease

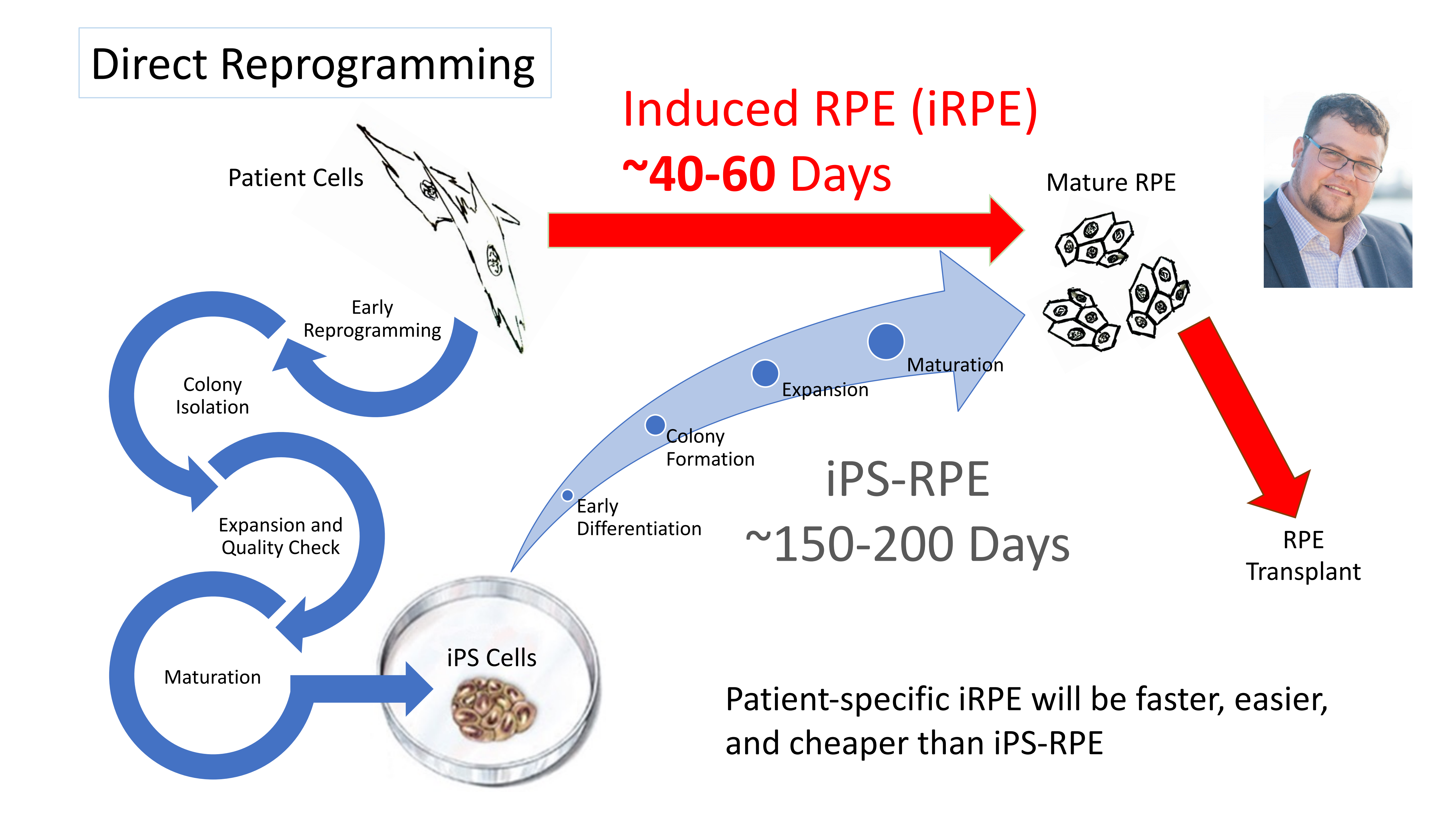


APMPPE

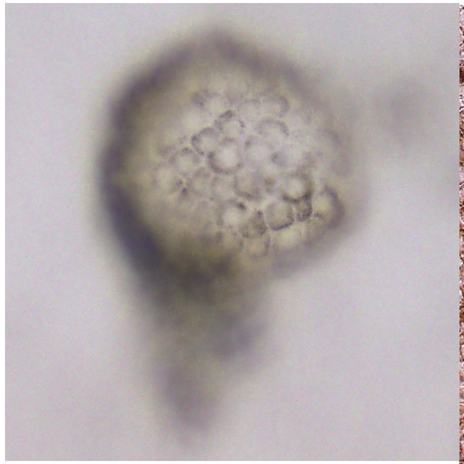


Progress of retinal cell therapy

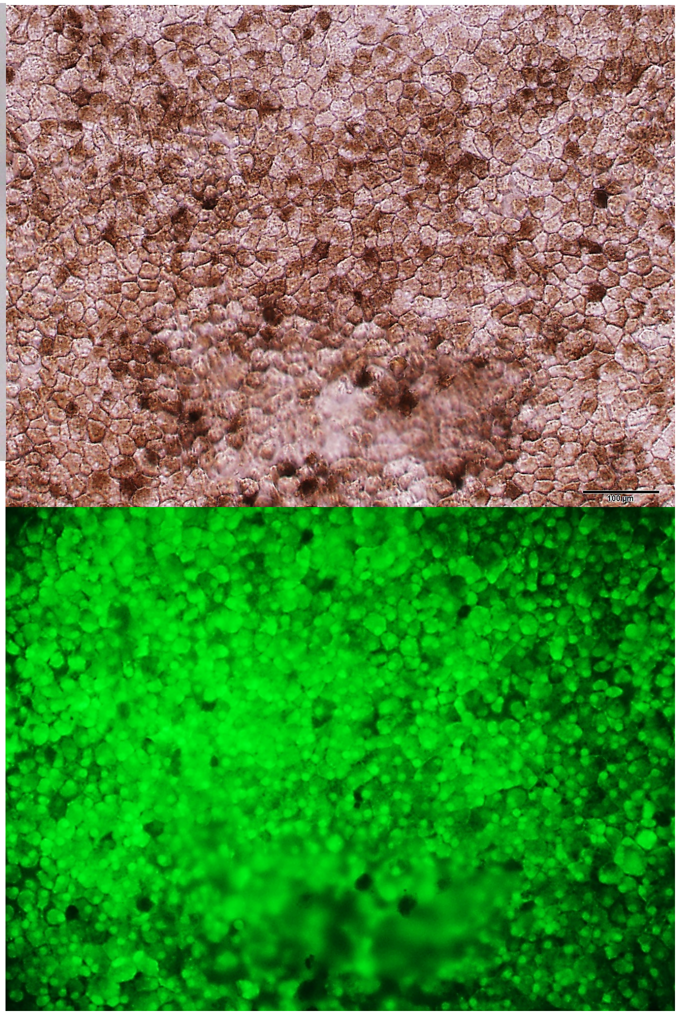



Direct Reprogramming
Induced RPE (iRPE)
~40-60 Days
Patient Cells
Mature RPE
Early
Reprogramming
Colony
Isolation
Expansion and
Quality Check
Maturation
iPS Cells
Early
Differentiation
Colony
Formation
Expansion
Maturation
iPS-RPE
~150-200 Days
RPE
Transplant
Patient-specific iRPE will be faster, easier,
and cheaper than iPS-RPE

iRPE From Human Skin

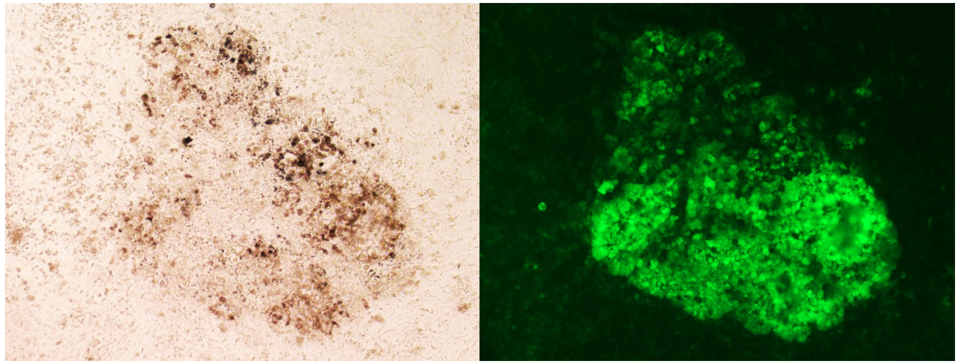


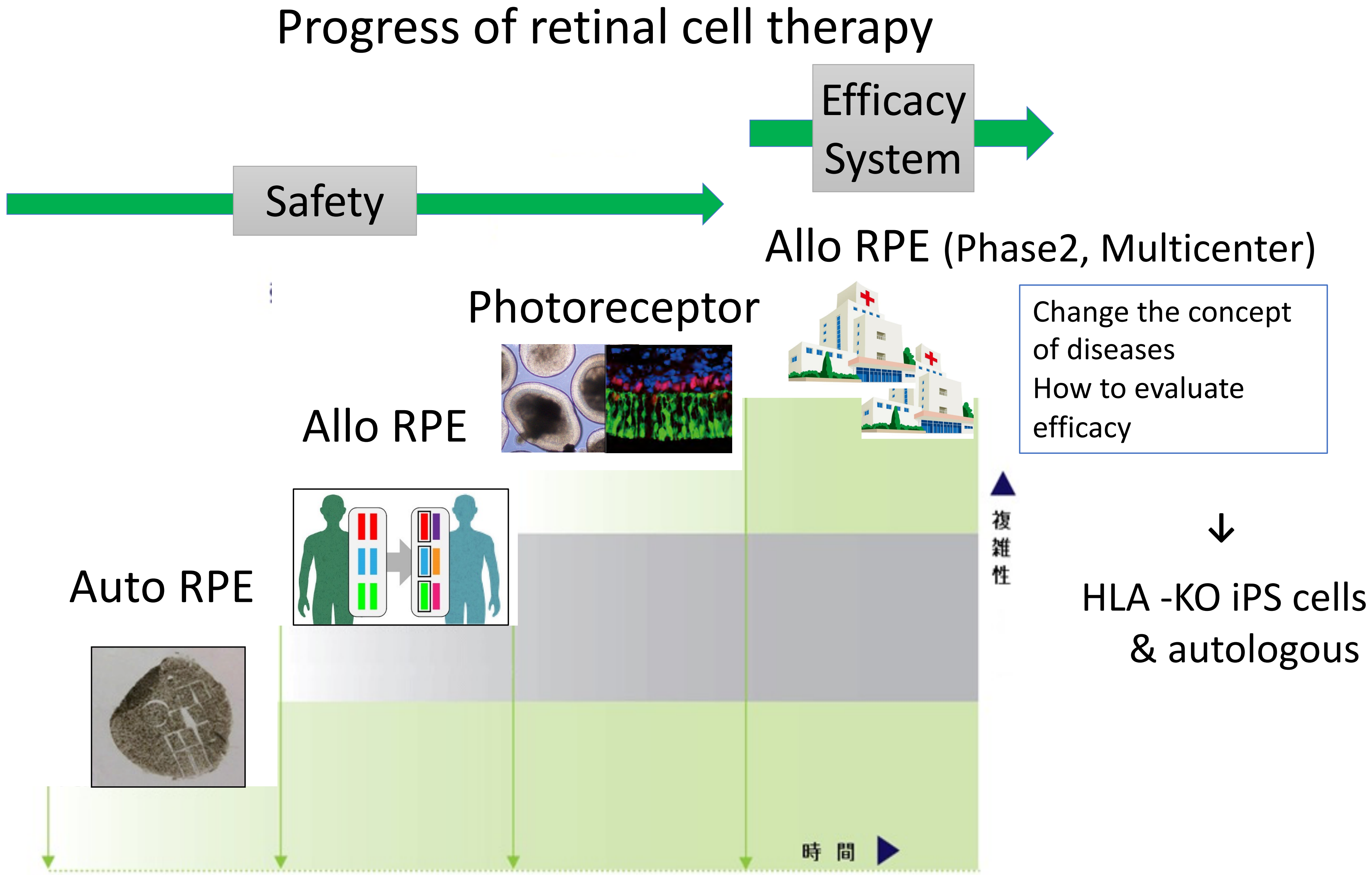
Expanded iRPE Colony



*Best1::*EGFP
Reporter Indicates RPE Maturation

Early Reprogramming iRPE Colony

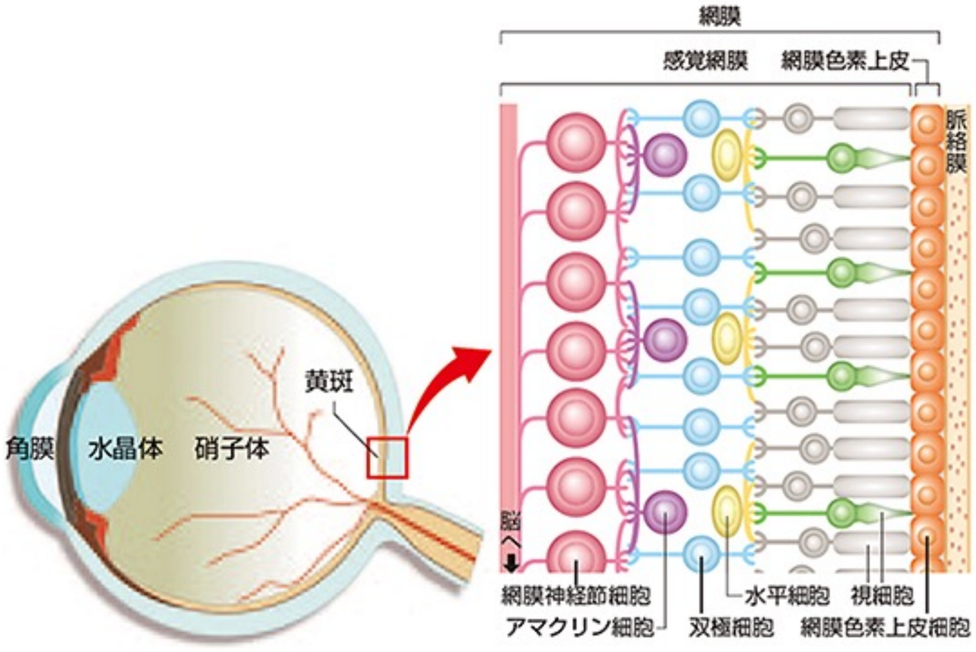




Progress of retinal cell therapy
Efficacy
System
Safety
Allo RPE (Phase2, Multicenter)
Photoreceptor
Change the concept of diseases
How to evaluate efficacy
Allo RPE
↓
Auto RPE
複雑性
HLA -KO iPS cells
& autologous
時 間

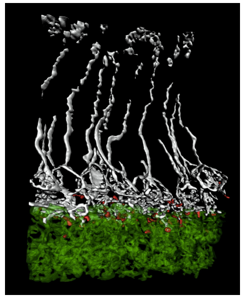
Retinal Cell Therapy

- iPSC-RPE (Phase 1,2)
  - RPE impairment diseases
- iPSC-photoreceptor cells (Phase 1)
  - Retinal degenerative diseases



RPE

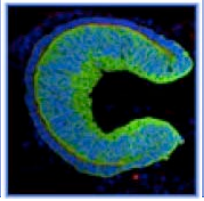
photoreceptor



Clinical study

RPE:
#1 2013-15 （auto）
#2 2017-18 （HLA matched）
#3 2020 - （Allo）

photoreceptor:
#1 2020 -



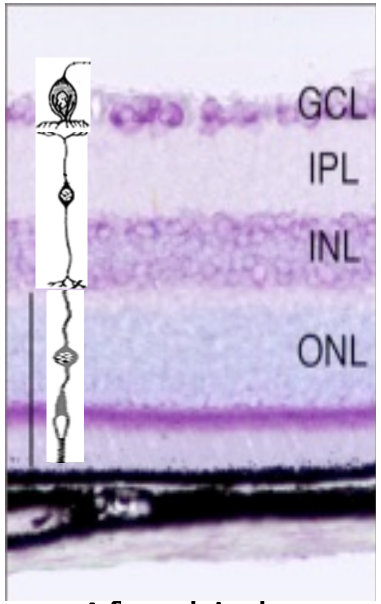
Photoreceptor
(for Retinitis pigmentosa)

Next project: 1st clinical research

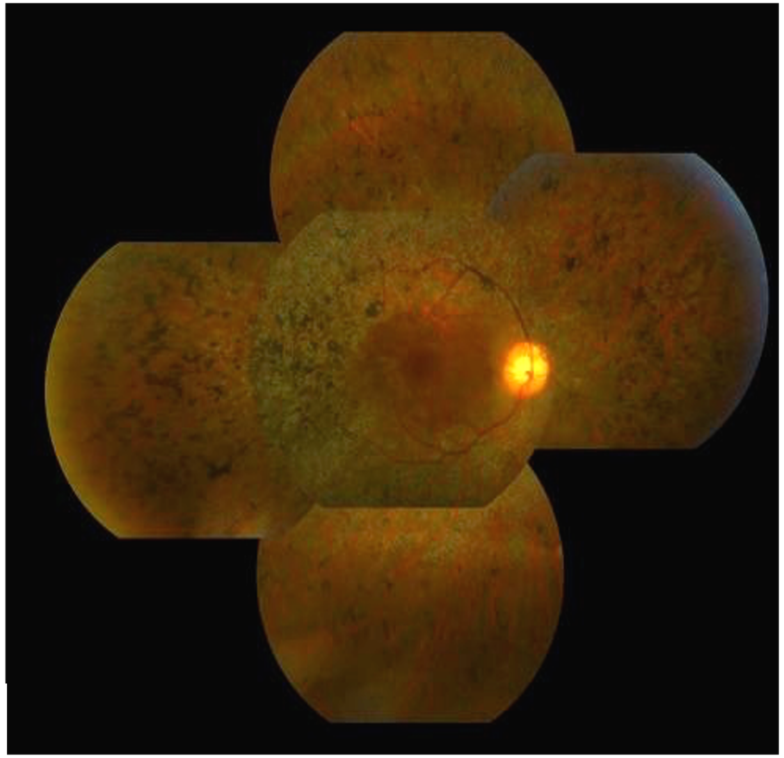
HLA unmatched Allogeneic
iPSC-photoreceptor transplantation
(2020~)

To show the organoid transplantation
To reconstruct neural network in the CNS

Retinitis pigmentosa
Photoreceptor degeneration



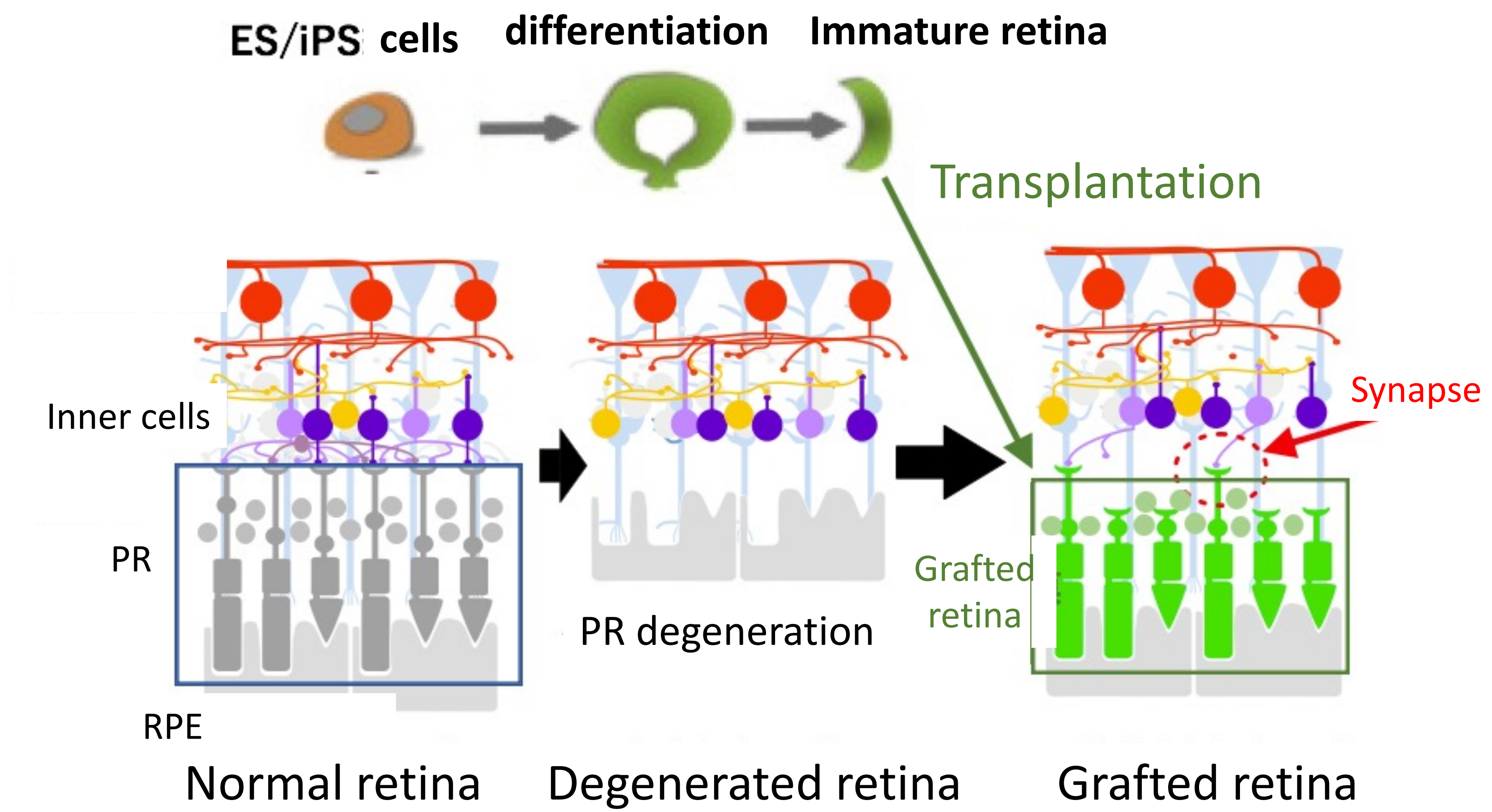
After birth



Photoreceptor degeneration

Visual field constriction

Photoreceptor transplantation



Start of Organoid Research

Self-organizing optic-cup morphogenesis in three-dimensional culture

April 7, 2011

Mototsugu Eiraku,
Nozomu Takata,
Hiroki Ishibashi,
Masako Kawada,
Eriko Sakakura,
Satoru Okuda,
Kiyotoshi Sekiguchi,
Taiji Adachi,
Yoshiki Sasai
(CDB, RIKEN)

Prof. Sasai

Prof. Eiraku

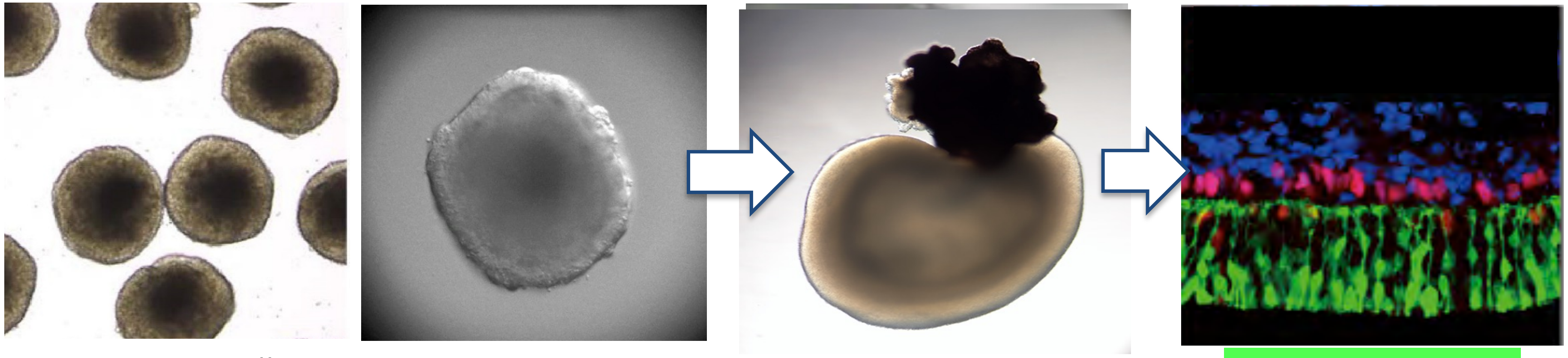
iPS / ES cell-derived retinal organoids(Embryonic retina)

(Eiraku et al. Nature 2011)

iPS / ES cell aggregates
(embryoid bodies)

Retinal organoid

Fully layered retina



12,000 iPS cells

Self-organizing
retinal morphogenesis

Photoreceptors

Cell biology

Eiraku MD, PhD

Animal experiment

Mandai MD, PhD

Organizer
clinical study)

Takahashi MD, PhD

QA, QC
Clinical trial

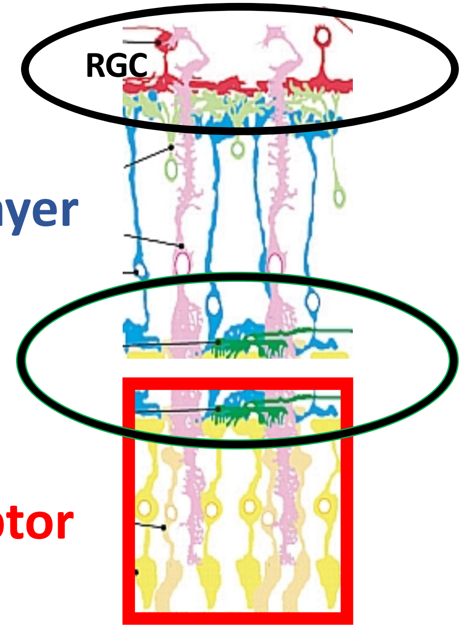
Sumitomo Dainippon
Pharma

Eiraku et al. *Nature* 2011
Nakano et al. *Cell Stem Cell* 2012
Kuwahara et al. *Nature Communications* 2015
Kuwahara,Yamasaki et al. *Scientific Reports* in press

iPS-photoreceptor transplantation preclinical study

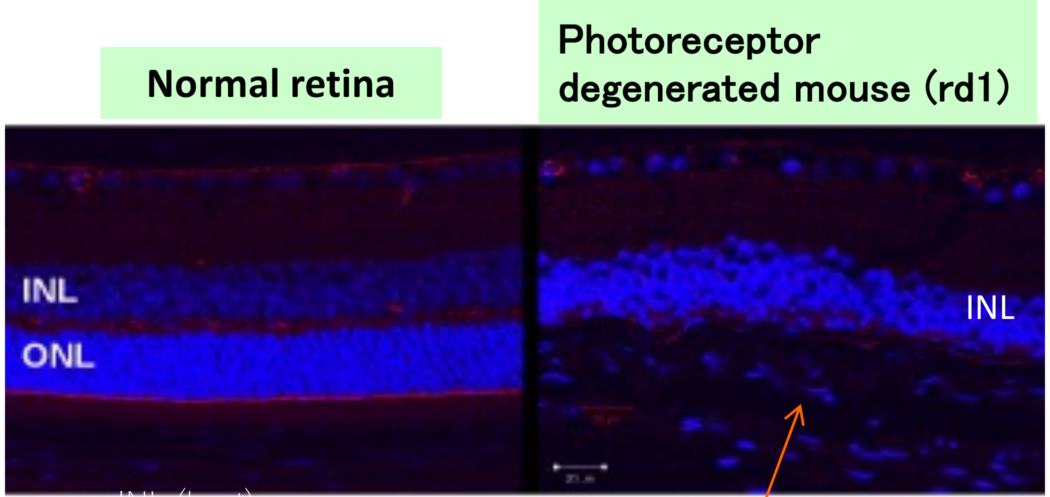
4 steps of Proof of Concept

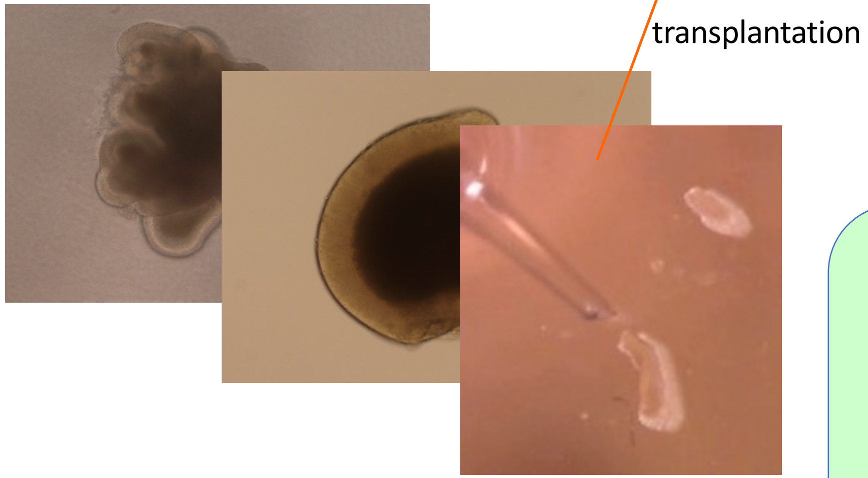
Mouse

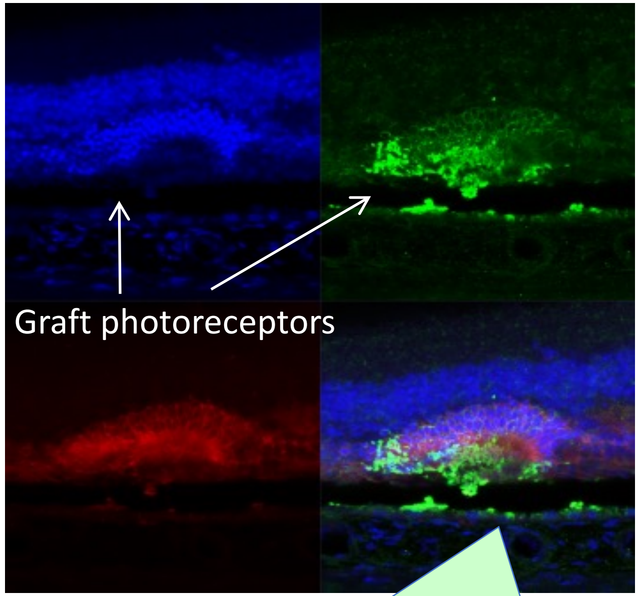


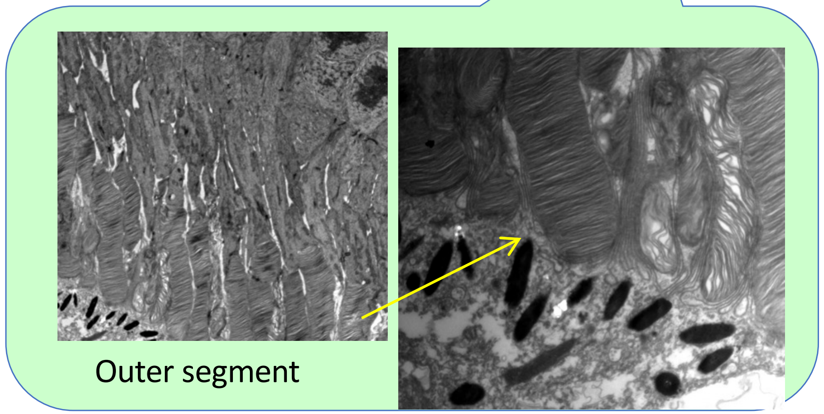
1. Survival of grafted cells

Assawachananont J et al
Stem Cell Reports. 2014



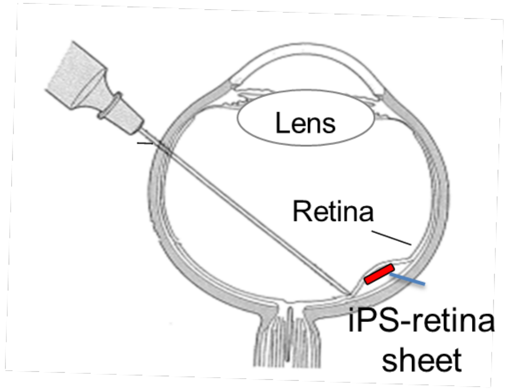


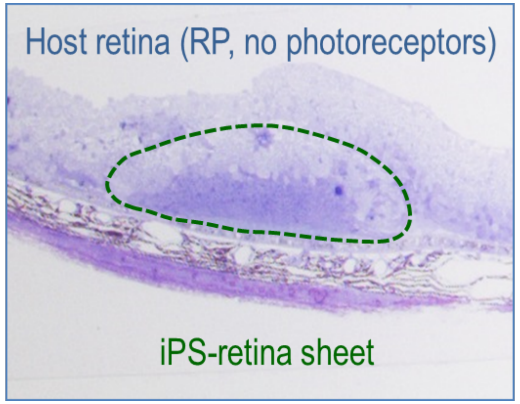


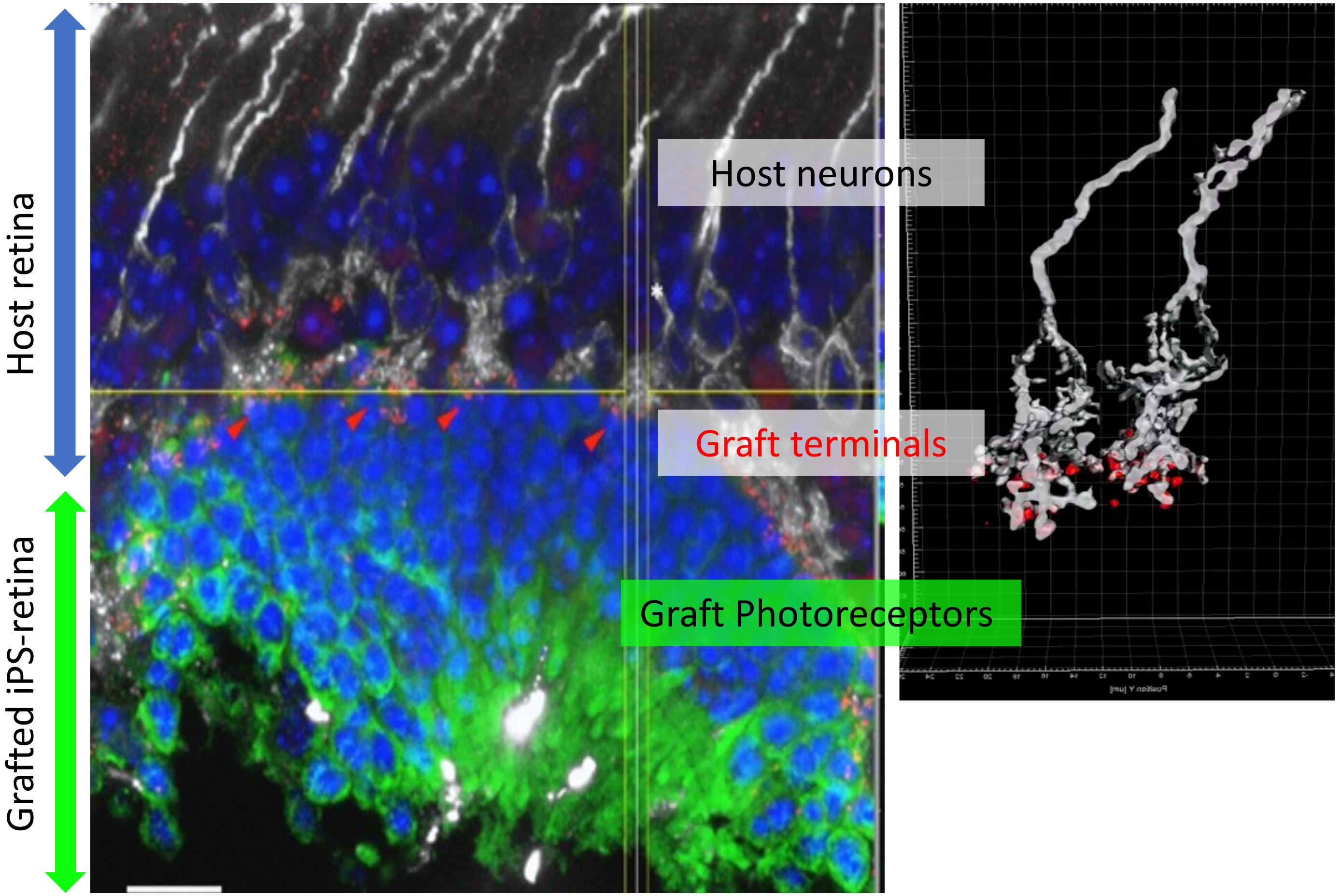


Assawachananont et al *Stem Cell Reports,2014*

2. Synapse formation between the host and graft



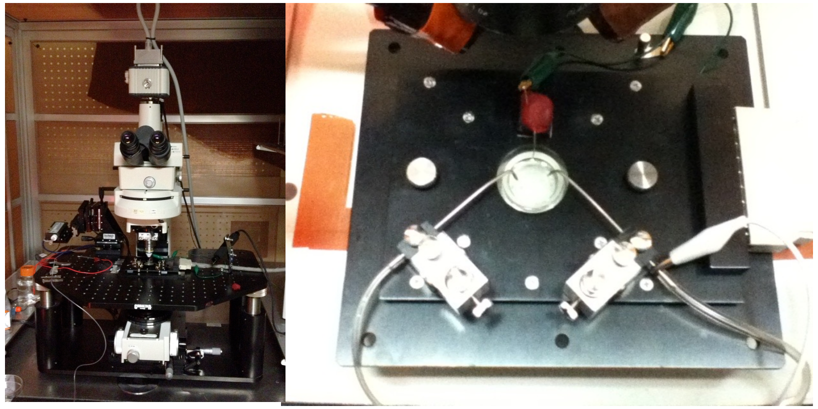




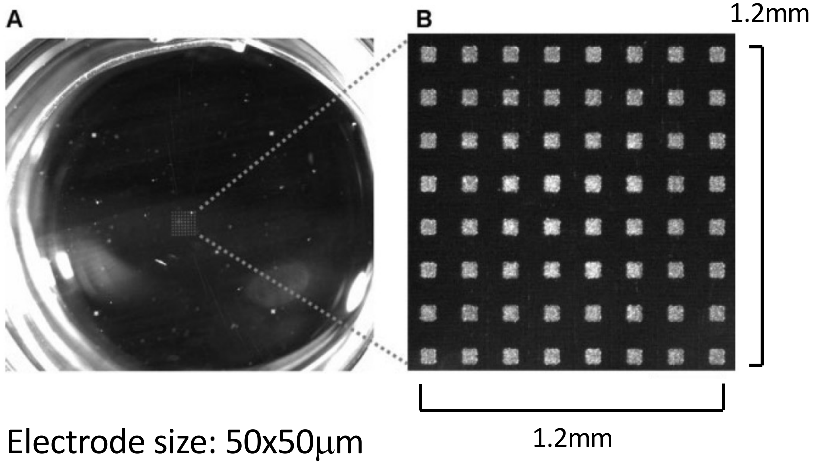
(Assawachananont et al. Stem Cell Report 2014)

3. Electrophysiological study

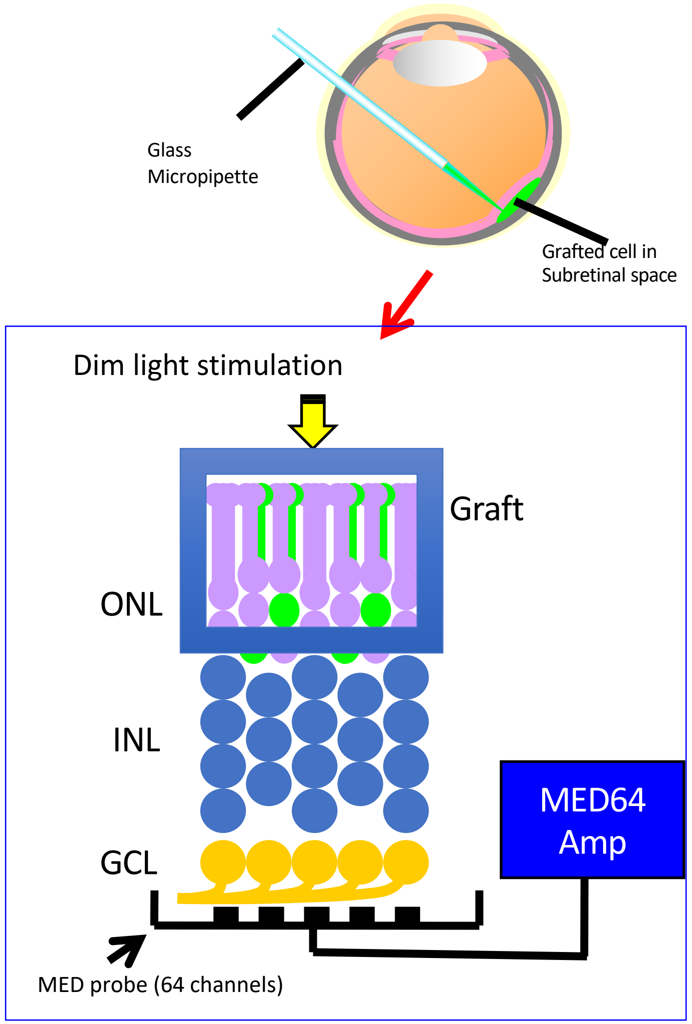
MED-64



MED probe (MED-P5155)

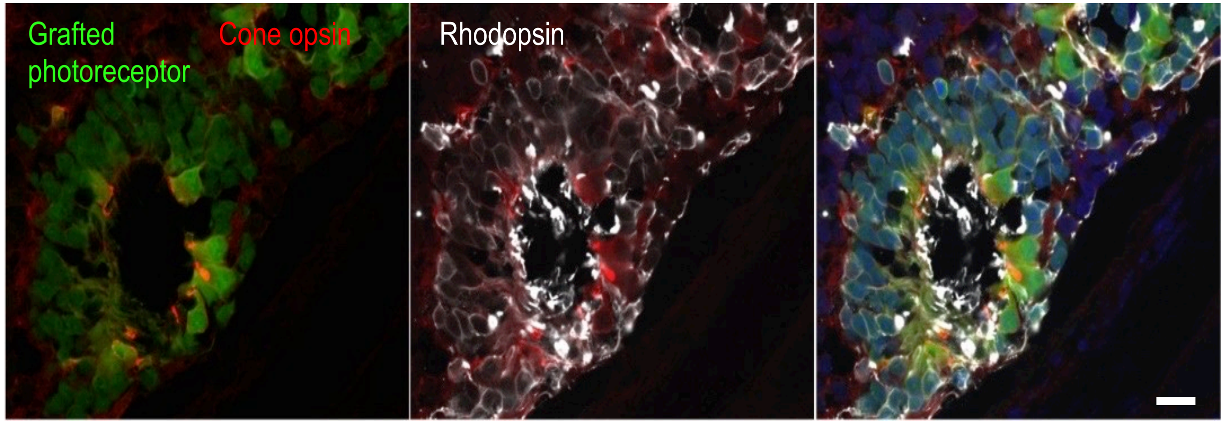


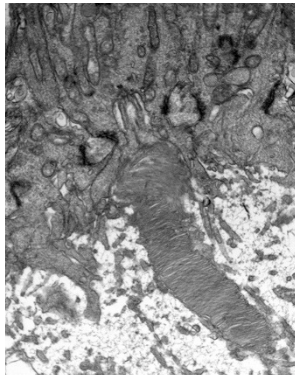
Electrode size: 50x50μm

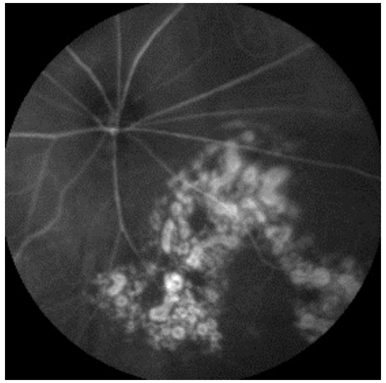


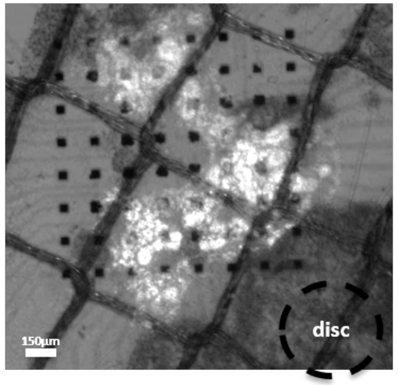
Post-grafting maturation and response to light in a mouse model of end-stage retinal degeneration

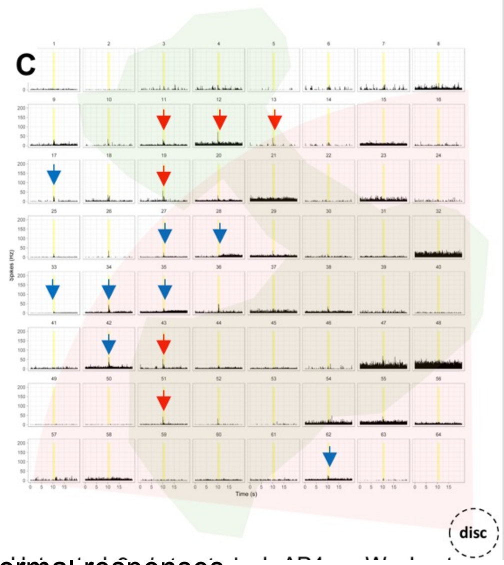
(Iraha and Tu et al Stem Cell reports 2018)

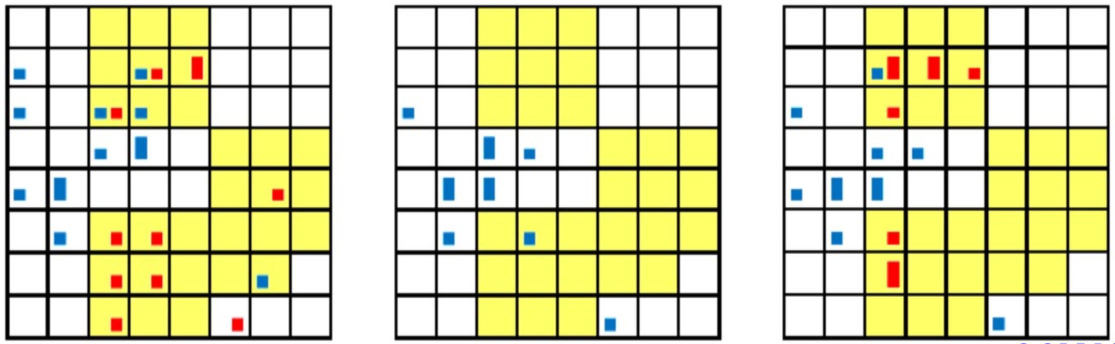












Normal responses

Hyperactive responses

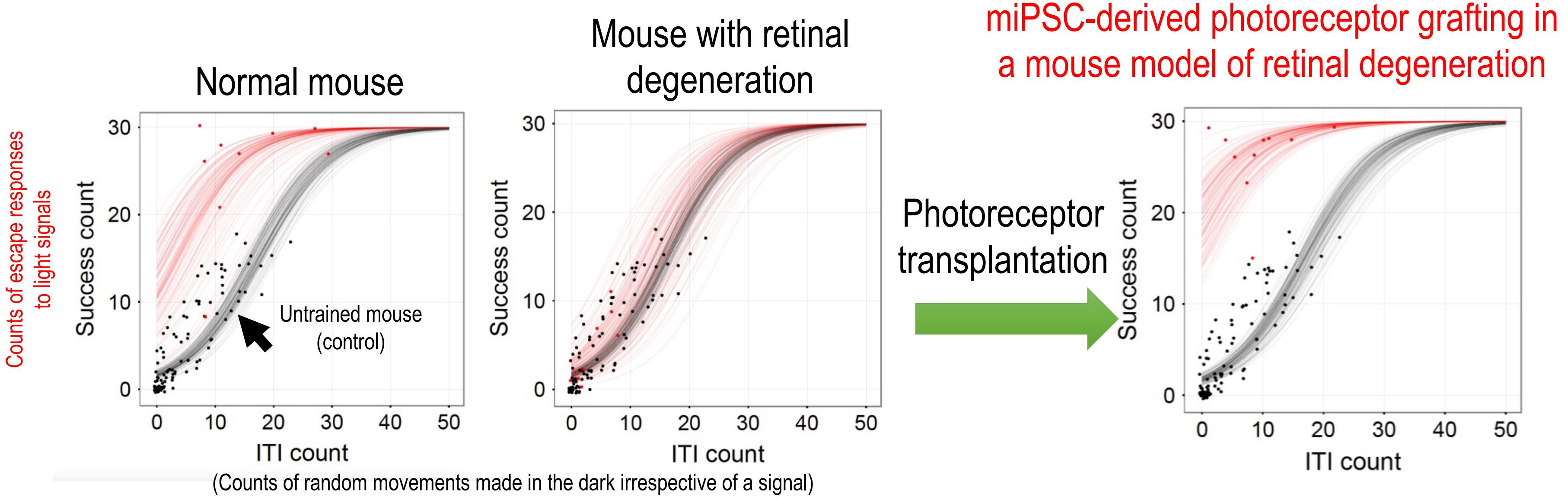
on graft

off graft

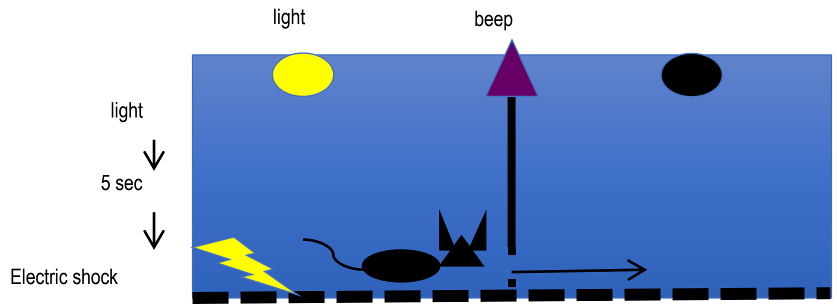
28W1D (19W6D after transplantation)

4. Behavior test : shuttle avoidance test

Effect of transplantation confirmed by behavioral analysis of the mouse model



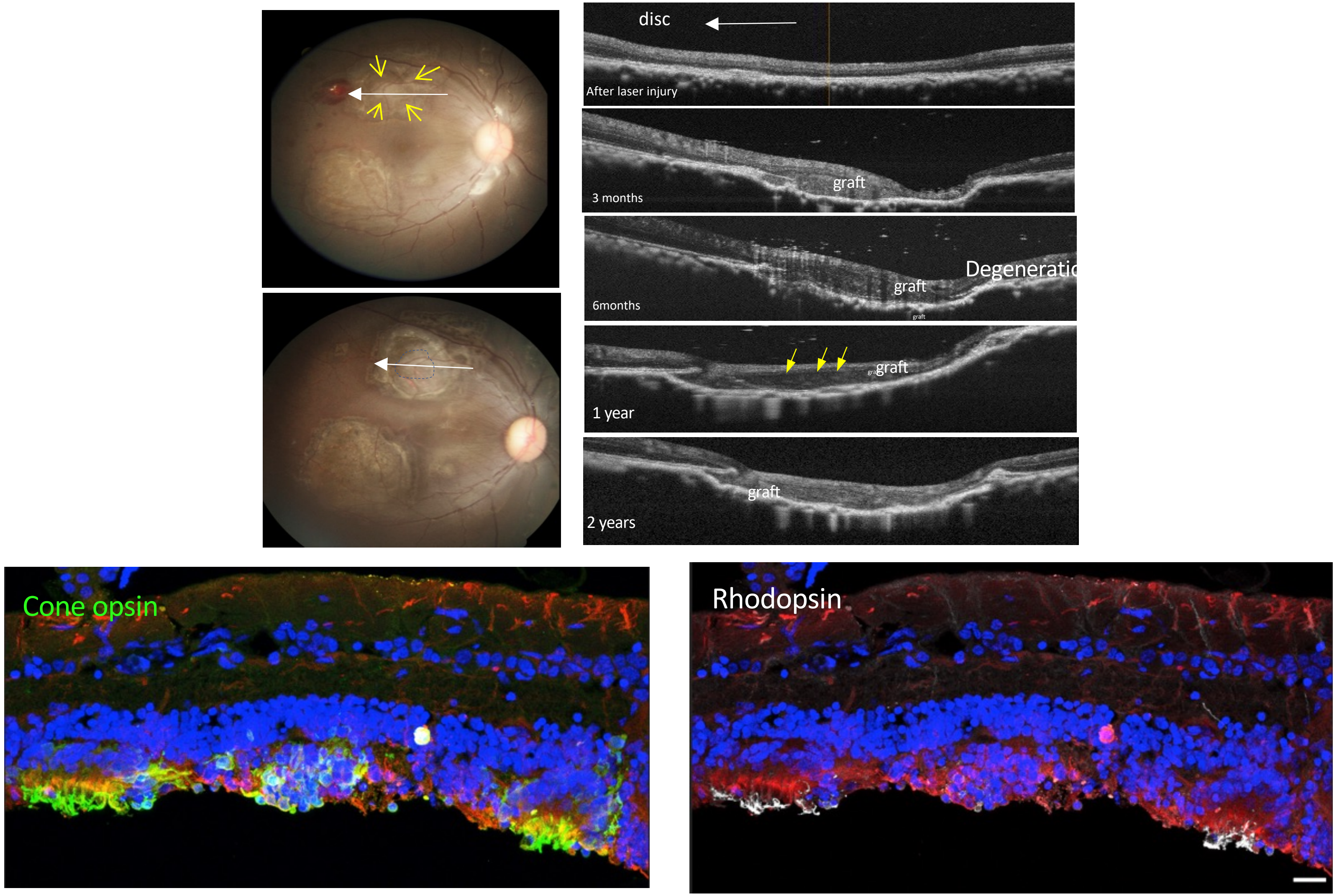
Behavioral pattern similar to that of normal mice (escape responses to light signals) shown immediately after grafting



- Mice were trained to learn that an electric shock would follow a light stimulus

Long-term survival of hiPSC-retina was also confirmed (2 years)

Monkey model eye

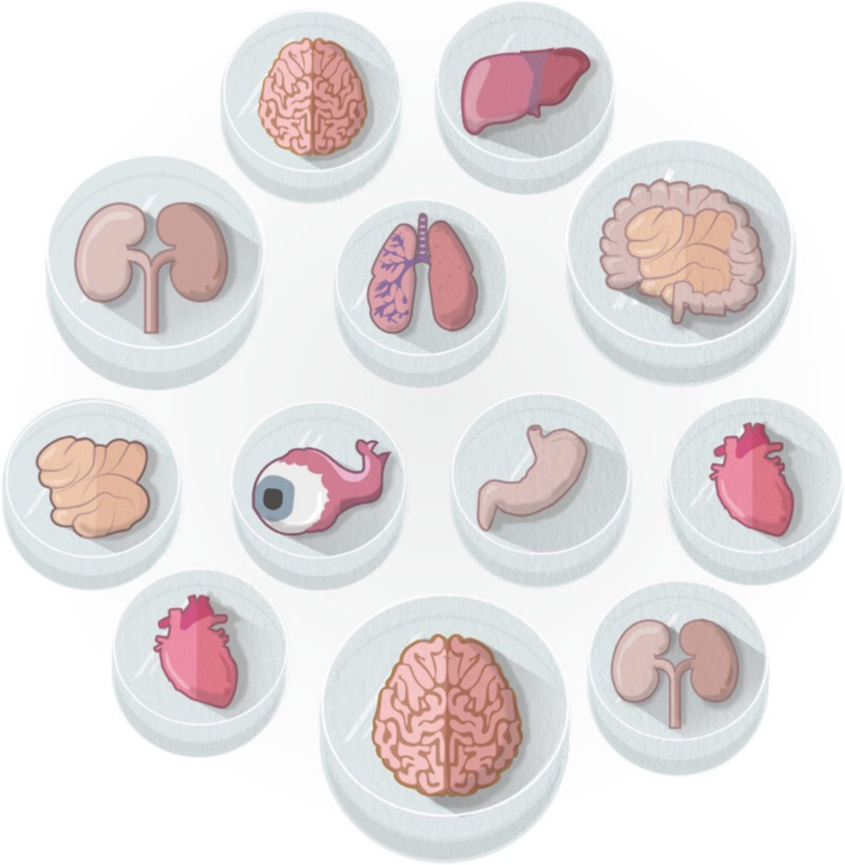


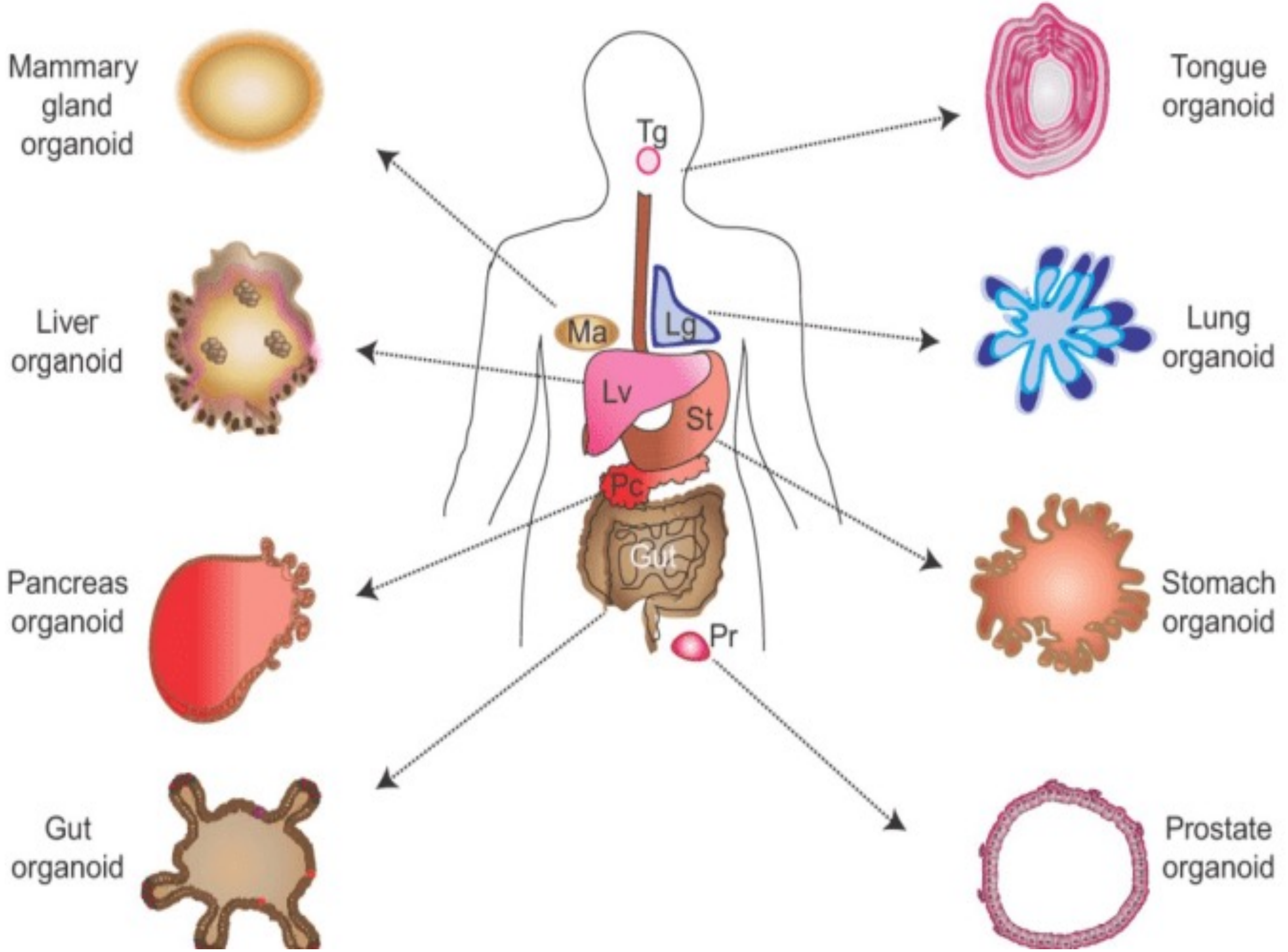
Clinical study of

allogeneic iPS cell-derived retinal sheet transplantation for retinitis pigmentosa

- To evaluate the safety and efficacy
- Target number of subjects: 2 cases
- Eligible criteria:
  - retinitis pigmentosa, age 20 years and older
  - corrected visual acuity less than 0.2
  - MD less than -30dB on Humphrey's visual field test (10-2)
- 6 months registration and 1 year observation period

Organoid research





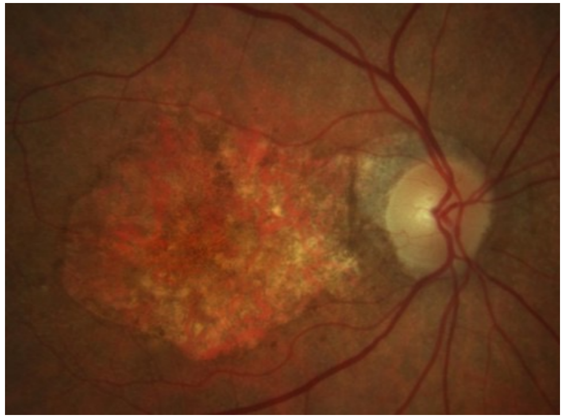
The ideal state of regenerative medicine from the patients' & doctors' perspective

• Optimal treatment for each case	- Reconsideration of disease names - Various forms (suspension & sheet)
• Reduce treatment costs	- Regulation, CPF
• Sustainable treatment as a medical system	- Consider hospitals profits (Japan)
• From cell products to therapy Around the treatment	- Surgery - Evaluation tests - QA of genetic diagnosis

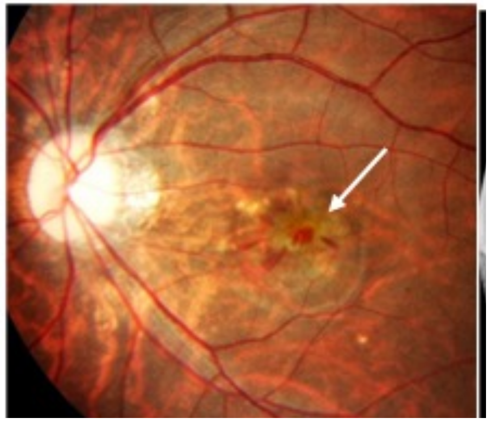
RPE impairment diseases

Crystalin retinopathy

Dry type AMD

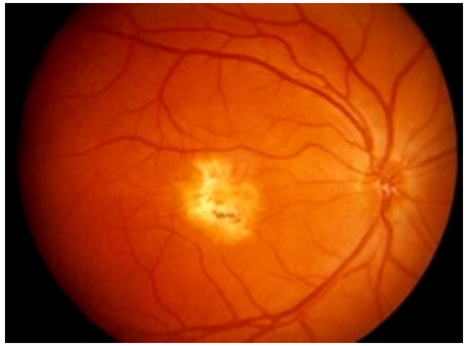


High Myopia

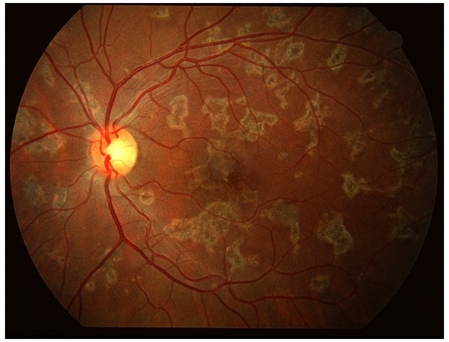


Stargardt disease

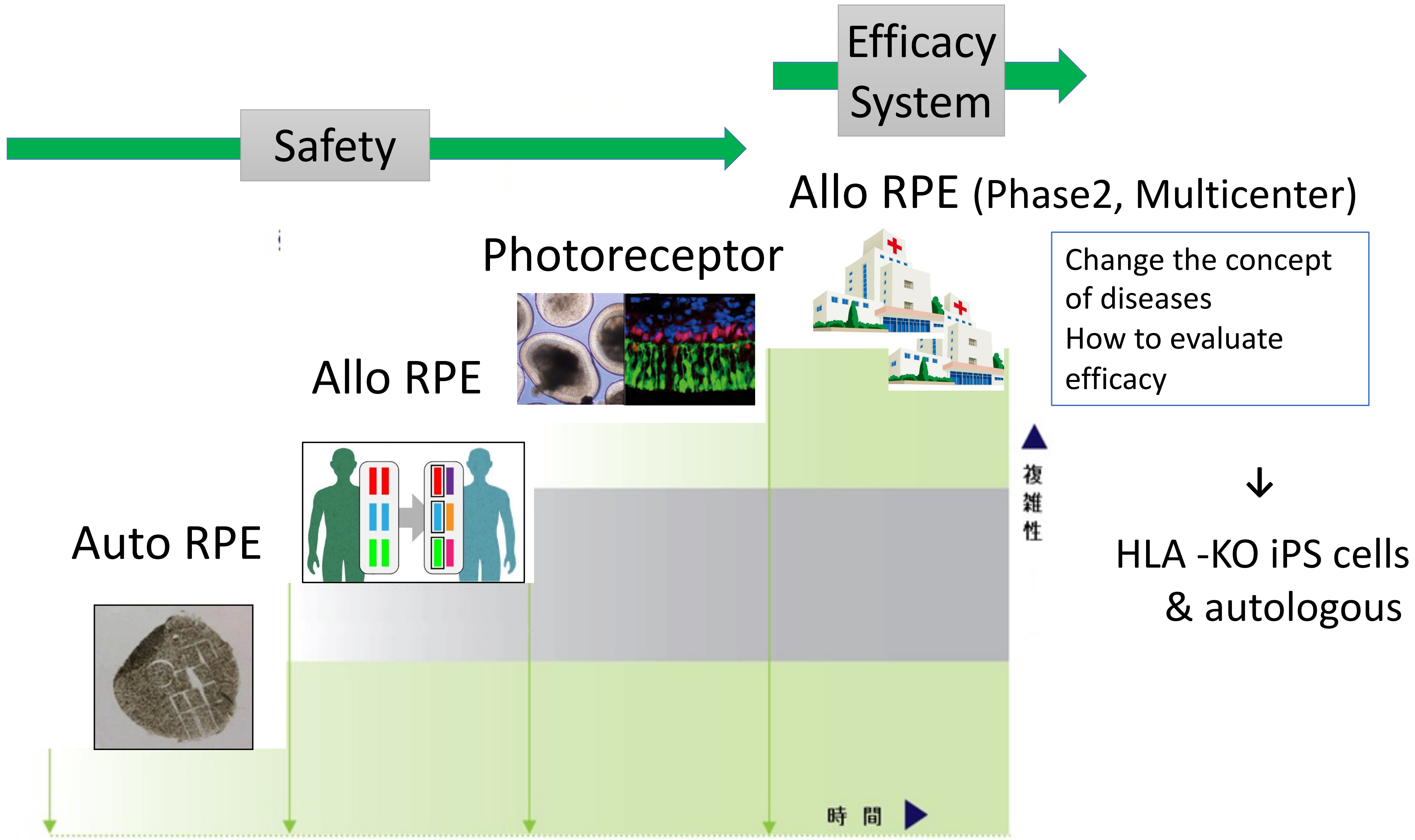
Best disease



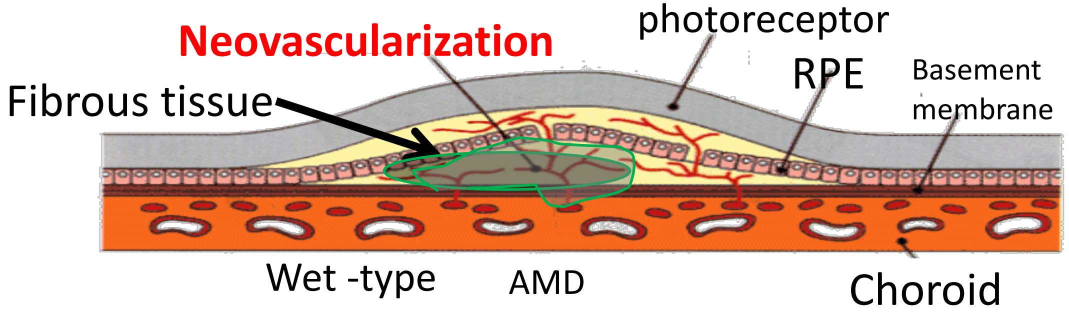
APMPPE



Progress of retinal cell therapy



Stages of AMD & suitable treatment



Neovascularization — **Existing treatment** PDT, anti-VEGF drugs

Fibrous membrane — CNV removal

RPE damage — RPE transplantation

Photoreceptor damage — RPE + photoreceptor transplantation

Choroidal damage — RPE + photoreceptor + Choroid transplantation

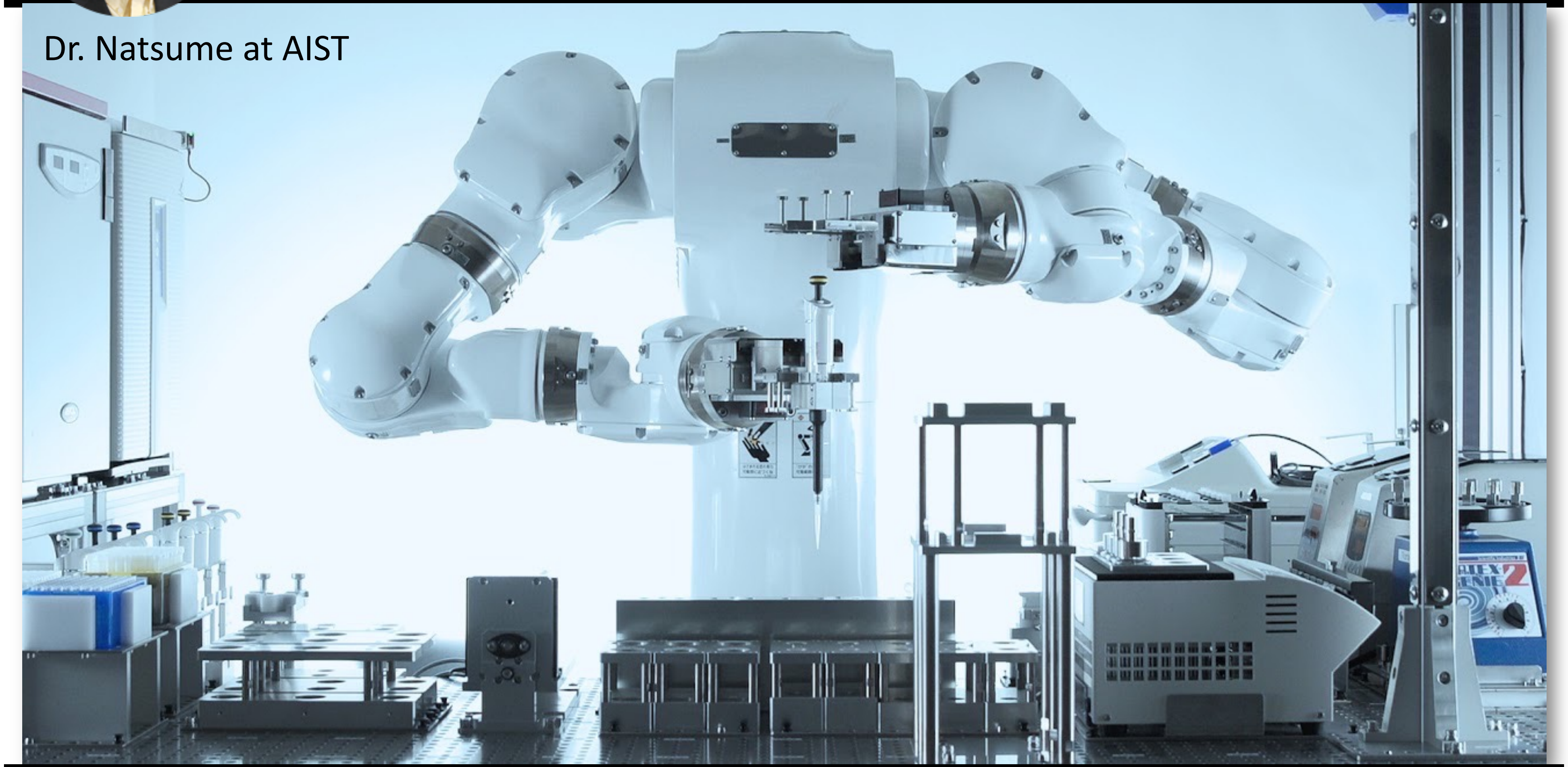
Wet type AMD

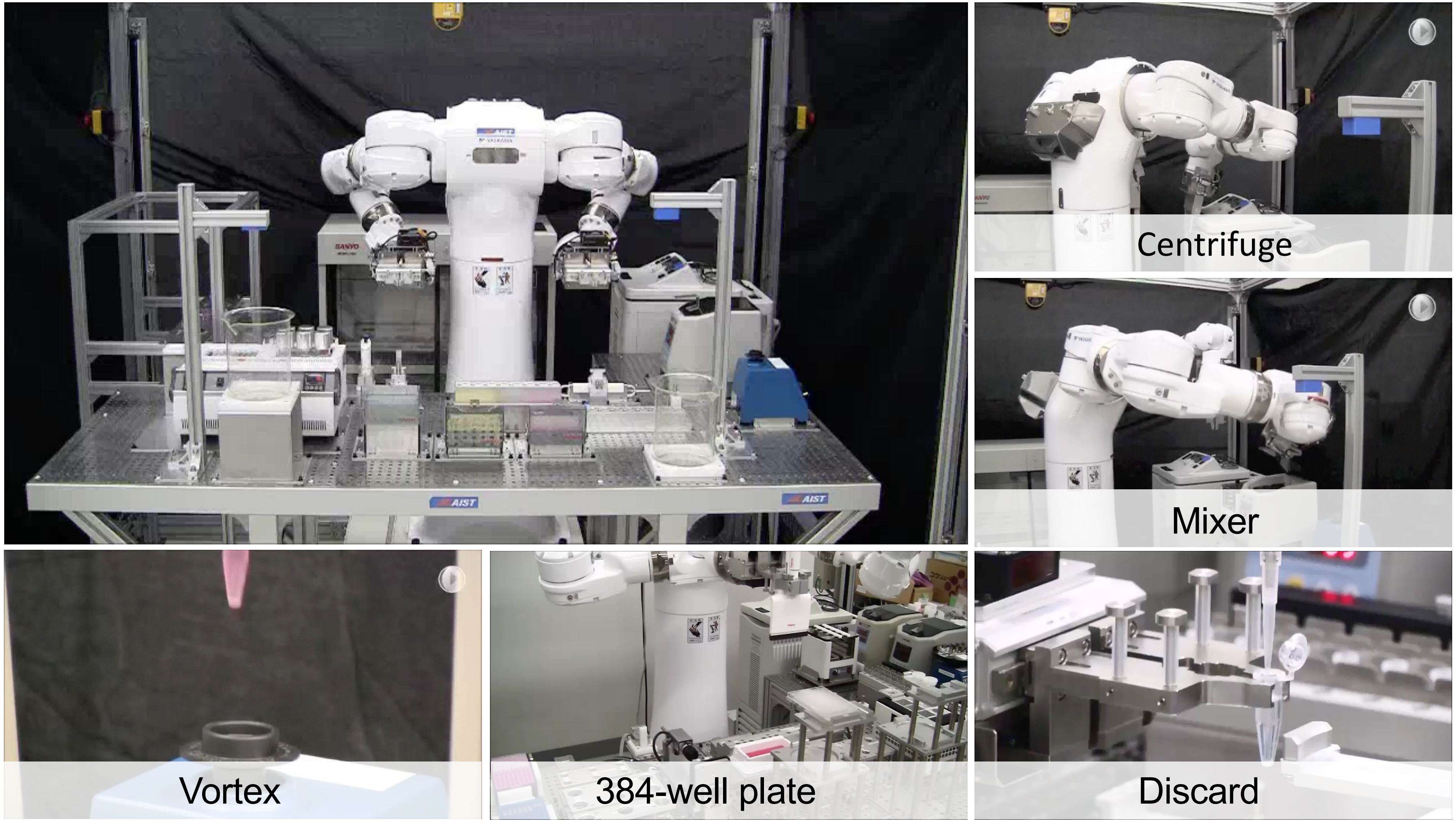
Dry type AMD

Regenerative medicine

ic Biology
oid - Mahoro

RBI

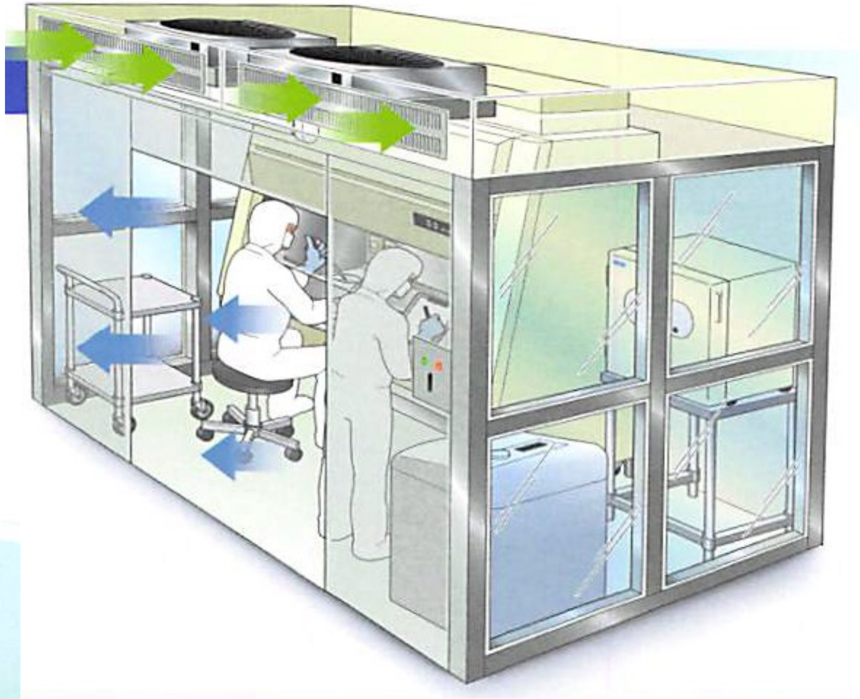



Centrifuge
Mixer
Vortex
384-well plate
Discard

Half open booth for CPF

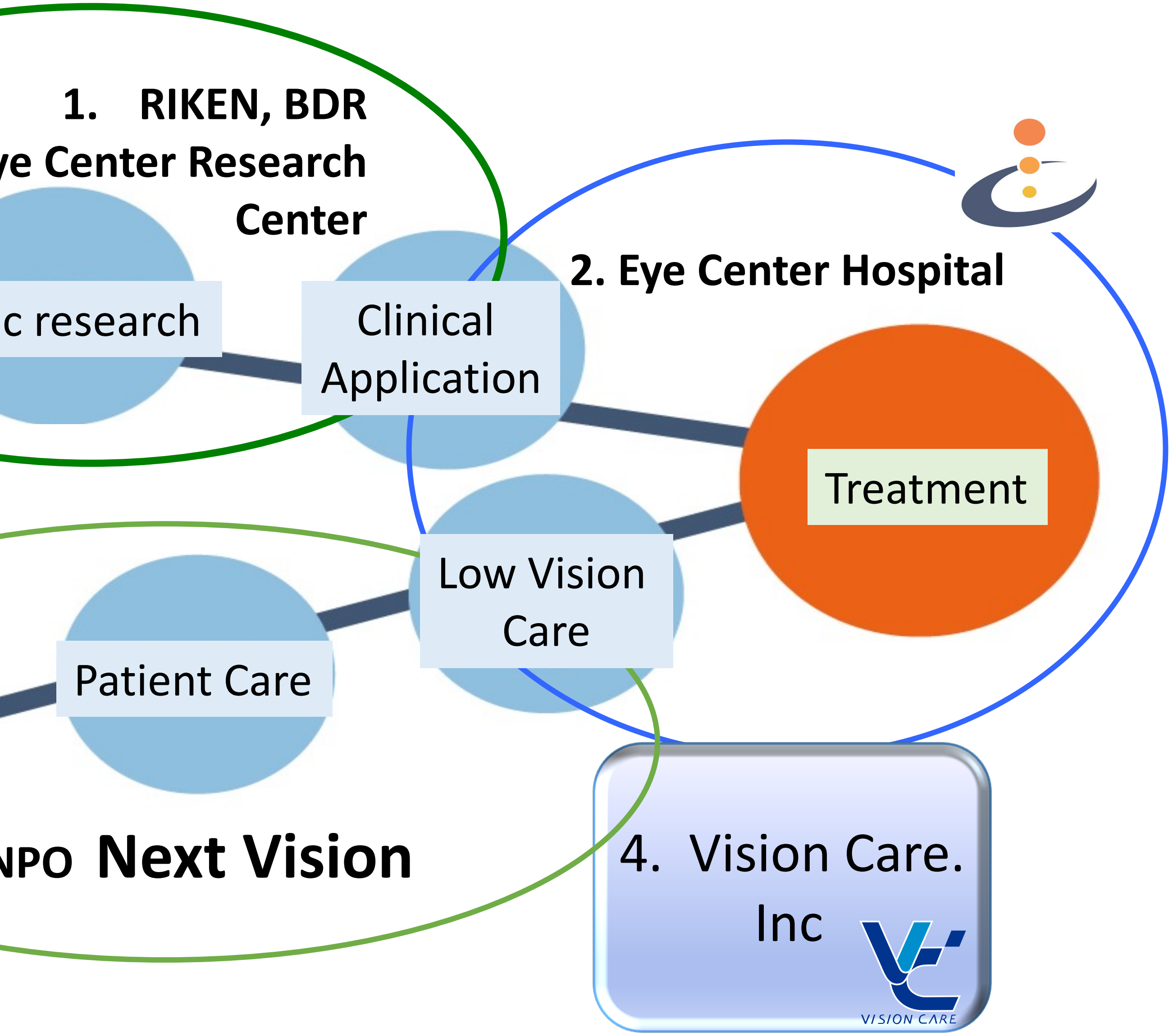
光と空気と水を生かす
ダイダン

DAIDAN



semiconductor

with Kobe Eye Center Hospital



With the hospital

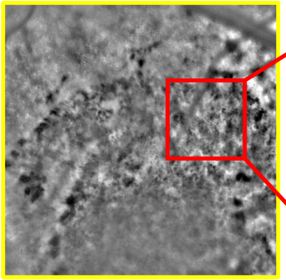
- Cell manipulation room (Thawing)

- Genetic diagnosis, counseling
- Immunological test (LGIR, DSA)
- Surgical technique, devise
- Ophthalmology test
  - Adaptive Optics camera
  - Polarized light OCT
- Low Vision Care (mental care)

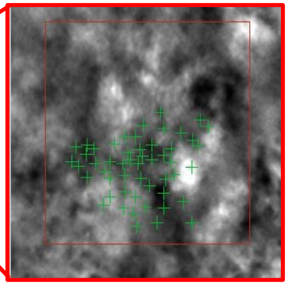
Clinical imaging of iPS-RPE using adaptive optics

rtx1 AO camera
(Imagine Eyes, FR)

Adaptive
optics image

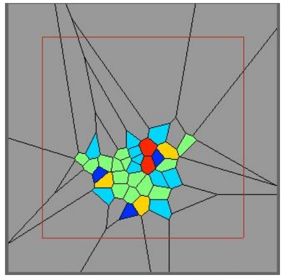


ROI - cell
detection

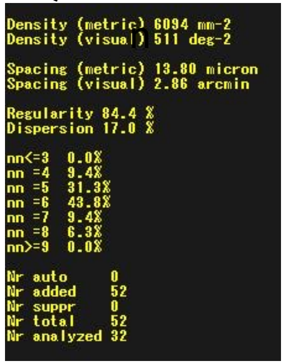


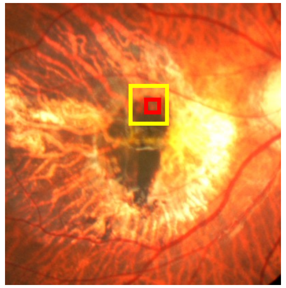
Detection by 5
evaluators

Voronoi
analysis

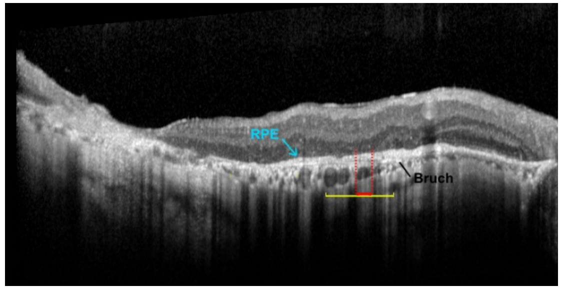


Quantification





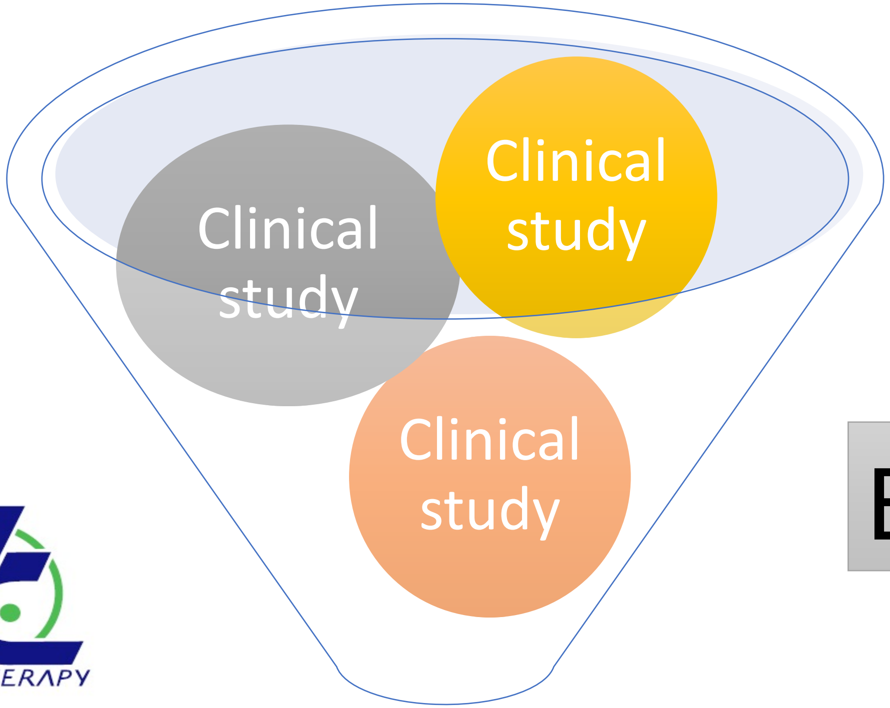
Co-localization
with color fundus
image



Co-localization
with OCT image

Regenerative medicine

Start up



B to B

RPE suspension

RPE sheet

Pharmaceutical company

Clinical trial

Approval

B to C

Parkinson's disease

20 years of history

RCT

Masayo Takahashi Lab. → Vision Care Inc.

Laboratory for Retinal Regeneration,

RIKEN Center for Biosystems Dynamics Research

“Any approaches to restore lost vision”