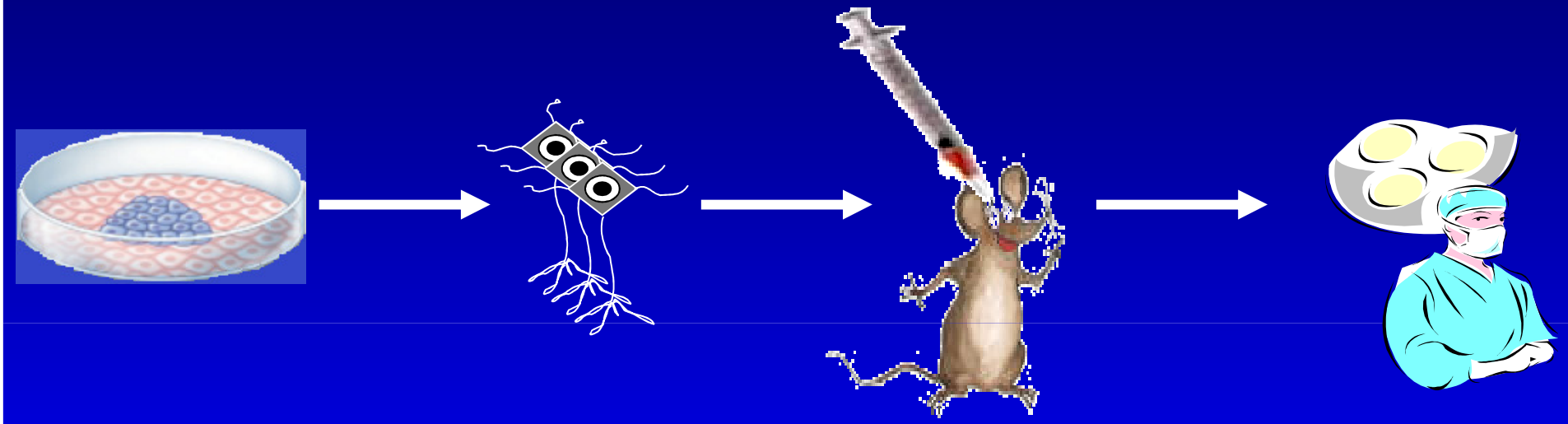
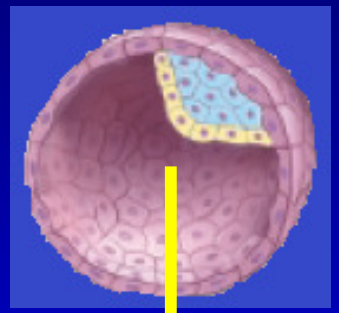
Human Embryonic Stem Cells – From Bench to Patients

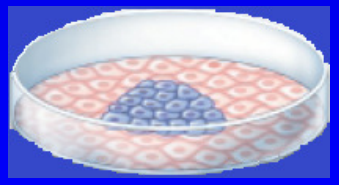


Benjamin Reubinoff M.D. PhD

The Hadassah Human Embryonic Stem Cell Research Center
The Goldyne Savad Institute of Gene Therapy &
The Obstetrics & Gynecology Department
Hadassah University Hospital

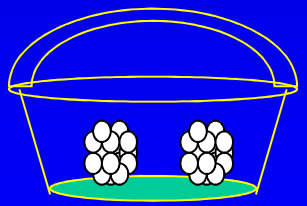
Road map for preclinical development of hESCs for transplantation in neurological and retinal disorders



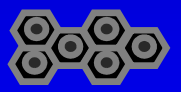


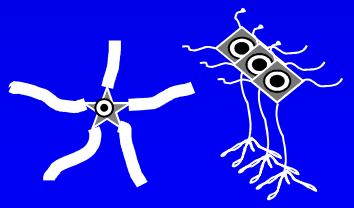
Clinical grade hES cells

Controlled defined process

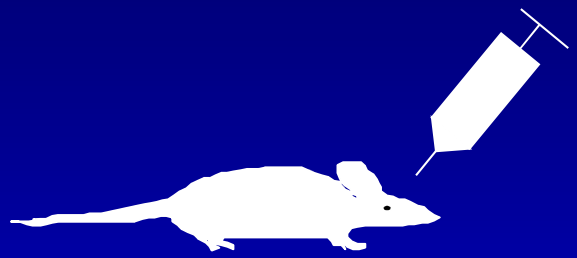


Neural Precursors

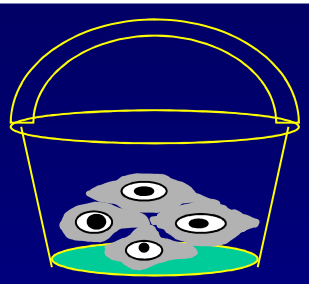




Neural Progenitors / Cells



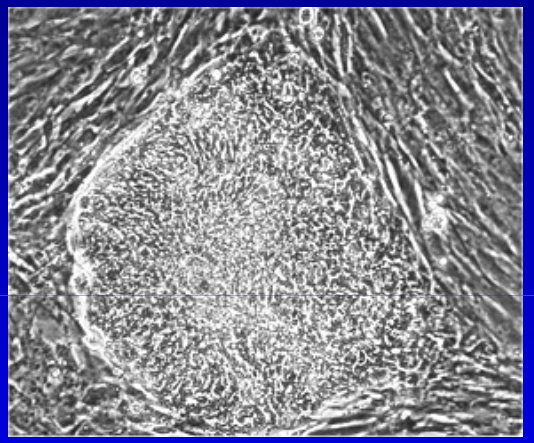
Animal models



Clinical grade human ES cell lines

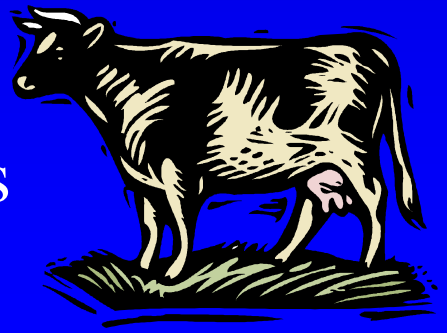
Current cell lines are not ideal for clinical use

1. Mouse feeders –
   xenotransplantation



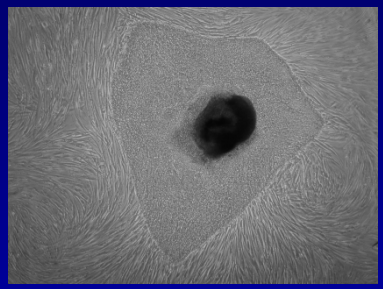
2. Inappropriate documentation of derivation processes

3. Inappropriate culture system-
   with animal products



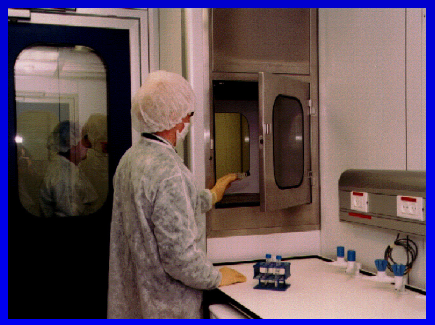
New Clinical Grade Human ES Cell Lines

1. Human feeders / feeder free

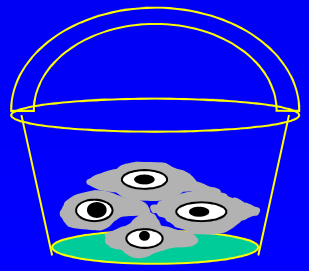


2. Defined animal-free culture systems

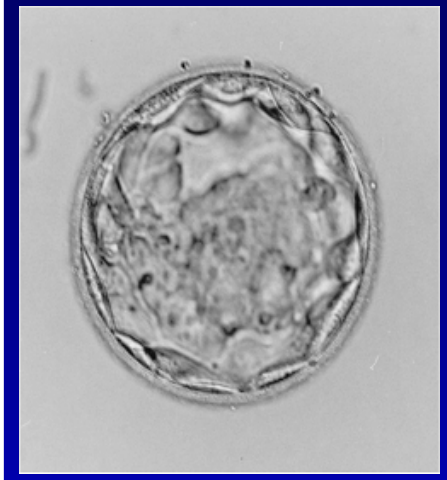
3. GMP facility

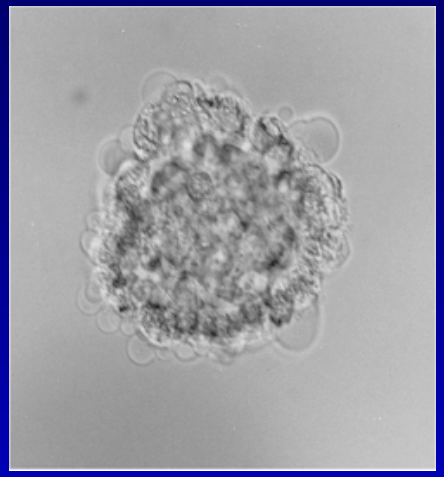


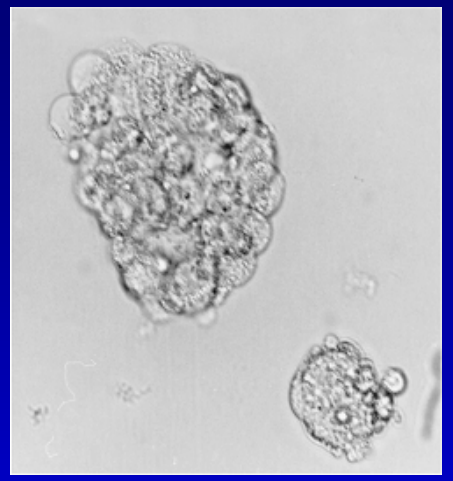
4. Bulk cultures

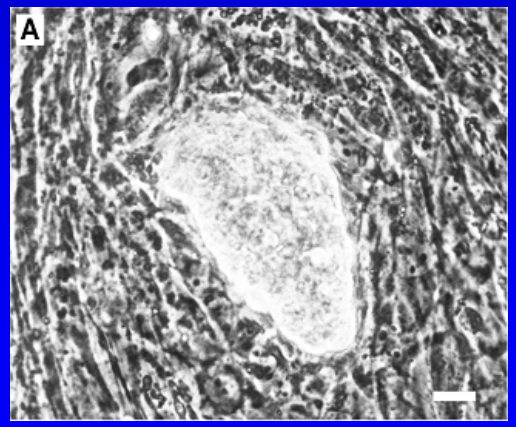


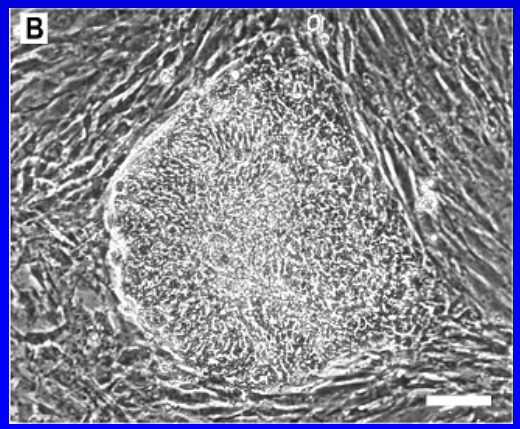
Derivation of human ES cell lines

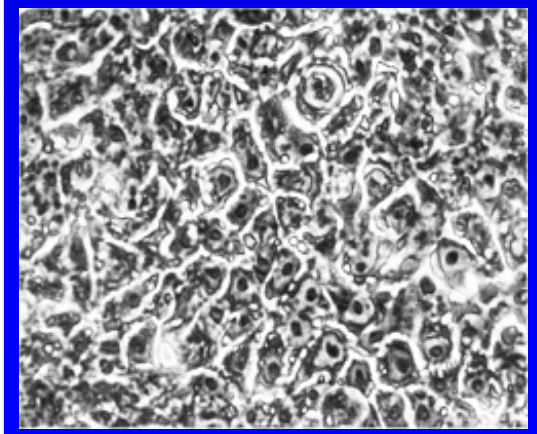








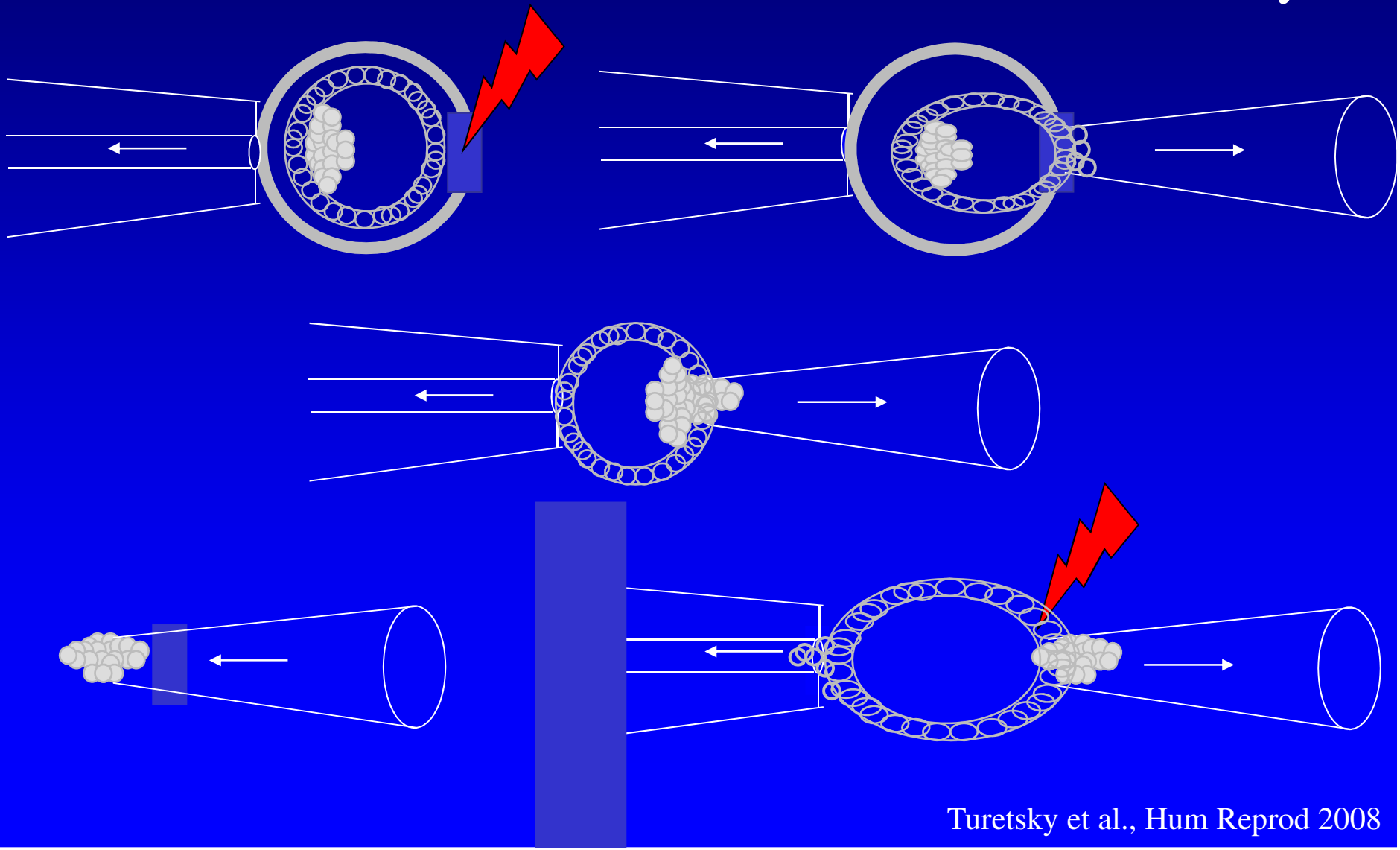




Reubinoff et al., Nature Biot. 2000

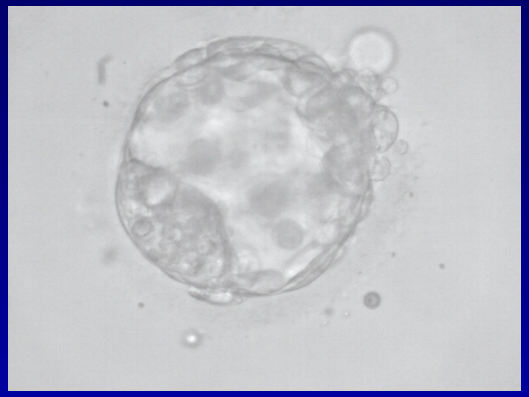
Clinical Grade Human ES Cell Lines

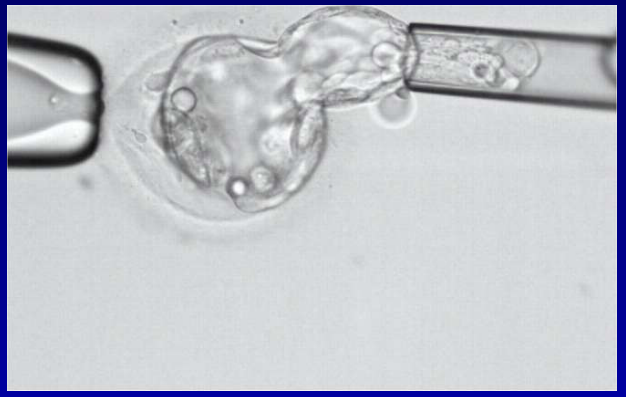
Lazer assisted isolation of ICM cells from the embryo

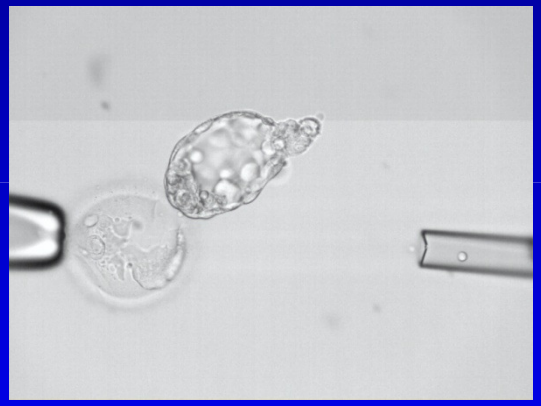


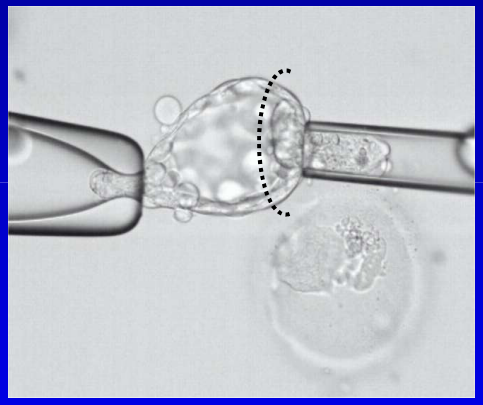
Turetsky et al., Hum Reprod 2008

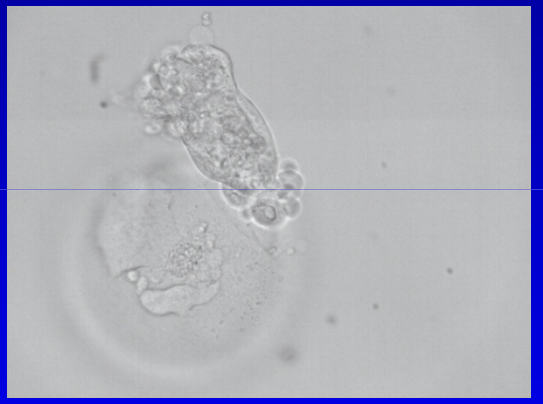
Lazer assisted isolation of stem cells from the embryo

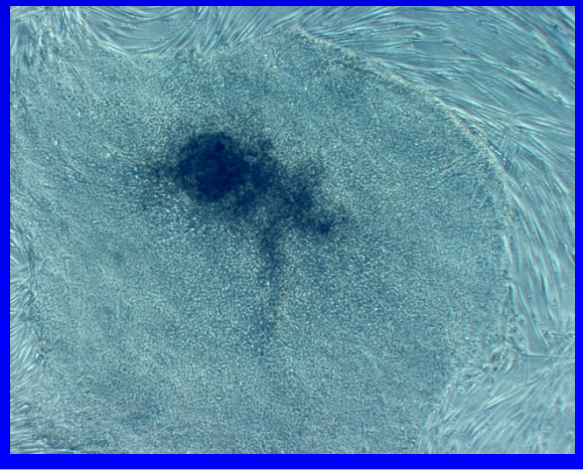






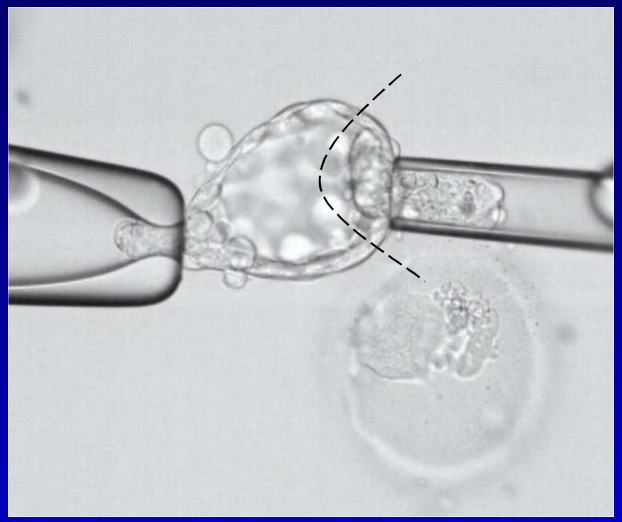




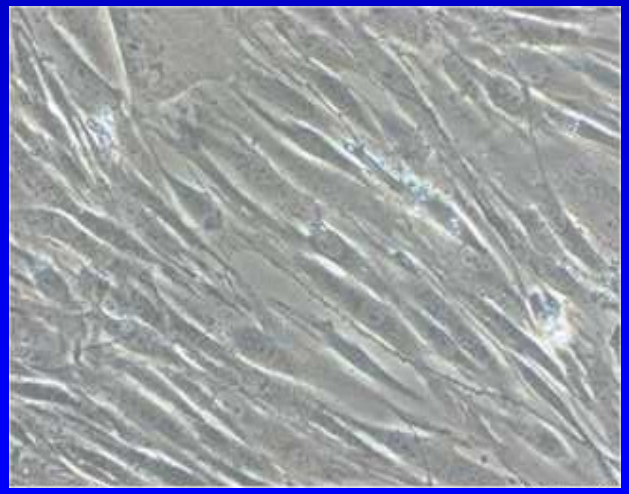


Turetsky et al., Hum Reprod 2008

Development of clinical grade hESCs



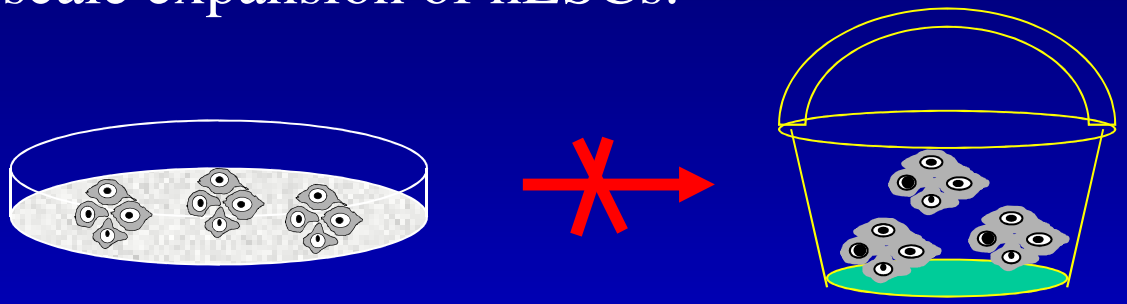
GLP-grade new hESC lines



Clinical grade
human feeders

Limitations of the culture system.

- Adherent feeder-dependent culture is a major limitation for large-scale expansion of hESCs.

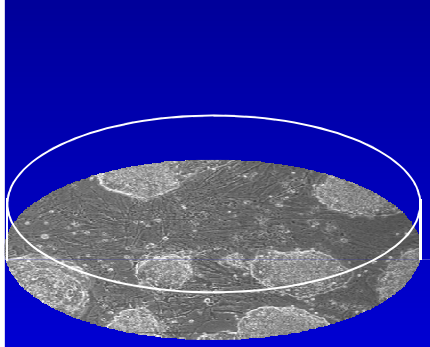


- The current notion is that detachment into free-floating clusters induces differentiation (EBs).

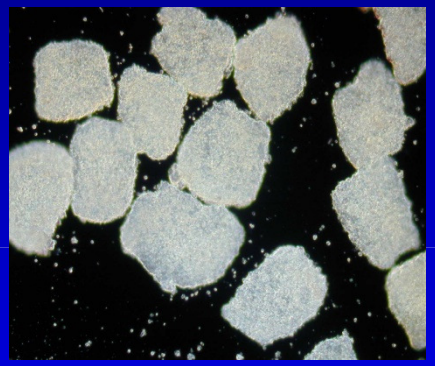
Monolayer colonies

Differentiation (EB's)

Upon neural differentiation, the obtained neural spheres were mixed with small clusters of undifferentiated hESCs.



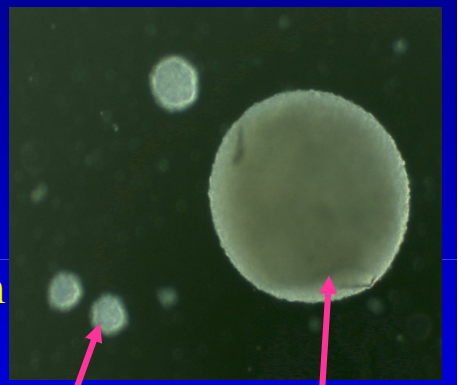
hESC colonies



hESC floating clusters

Neural differentiation

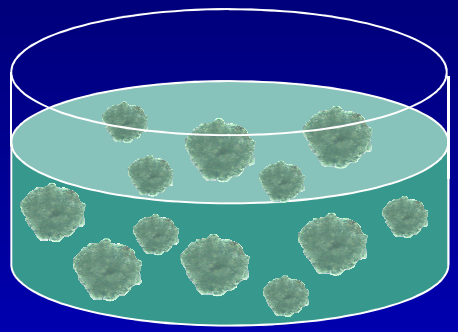
Neurobasal Medium



Neural spheres

Clusters of undifferentiated hESC

The key components of the suspension culture system



Neurobasal medium

Serum replacement
KO-SR + Nutridoma-CS*

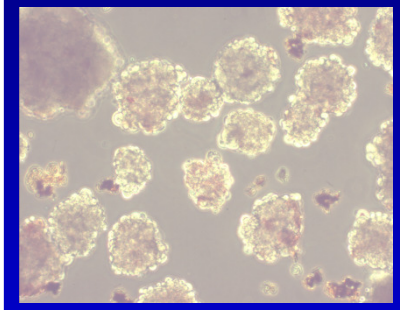
Extra cellular matrix prot .
laminin + fibronectin + gelatin

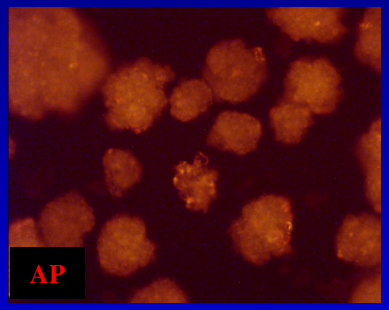
Factors for pluripotency
FGF2 + Activin A

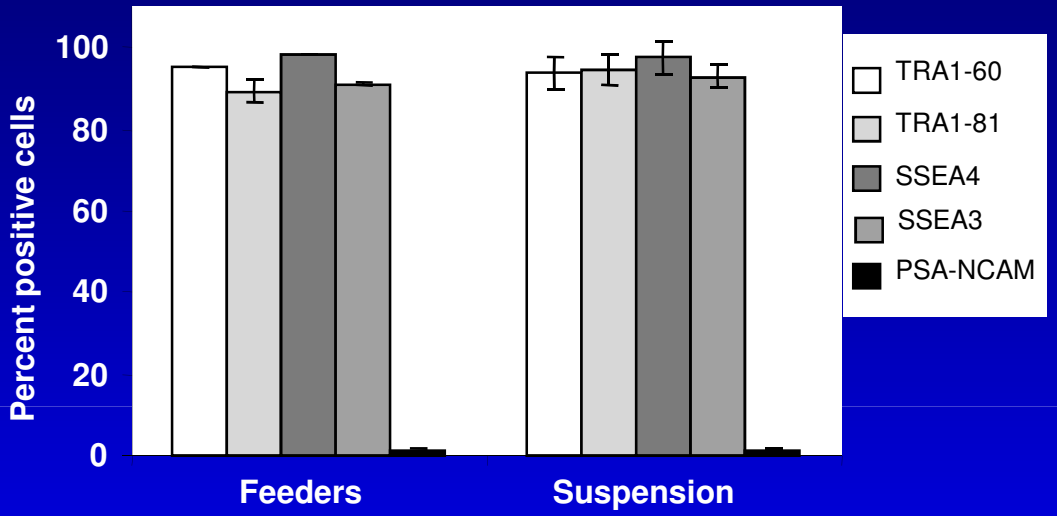
* Beta-d xylopyranose, a ring-shaped sugar,
allows the culture of cells in suspension without serum.

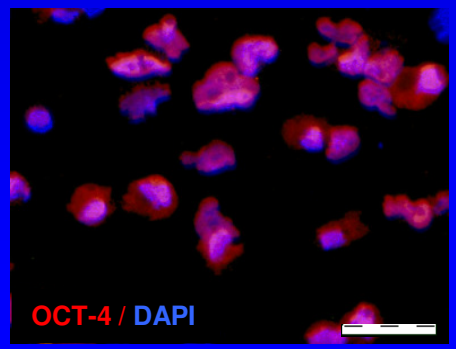
Steiner *et al.*, Nature Biotech. 2010.

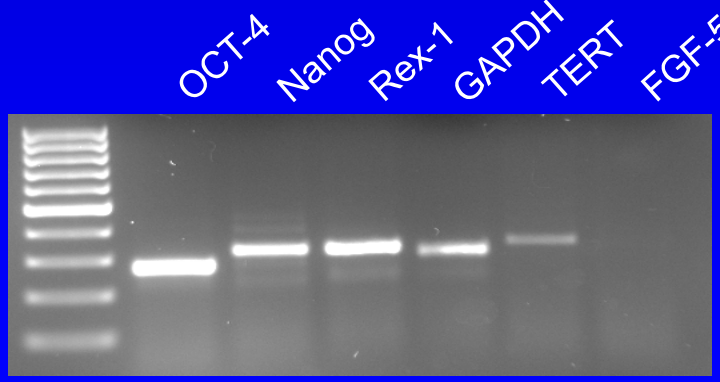
hESCs cultivated in suspension for 7-10 weeks maintain their pluripotency











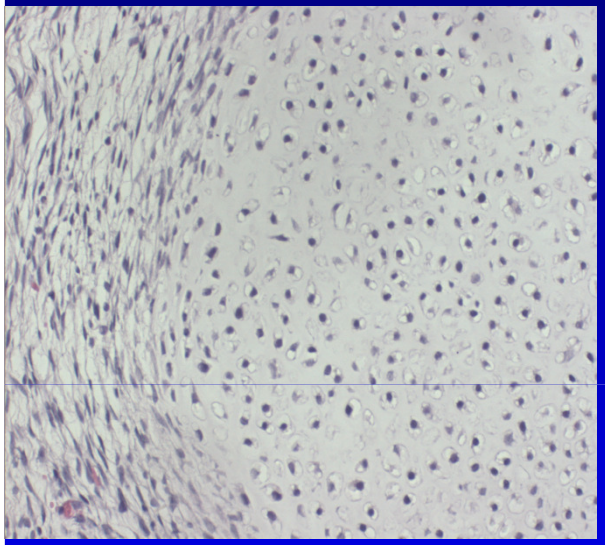
Steiner *et al.*, Nature Biotech. 2010

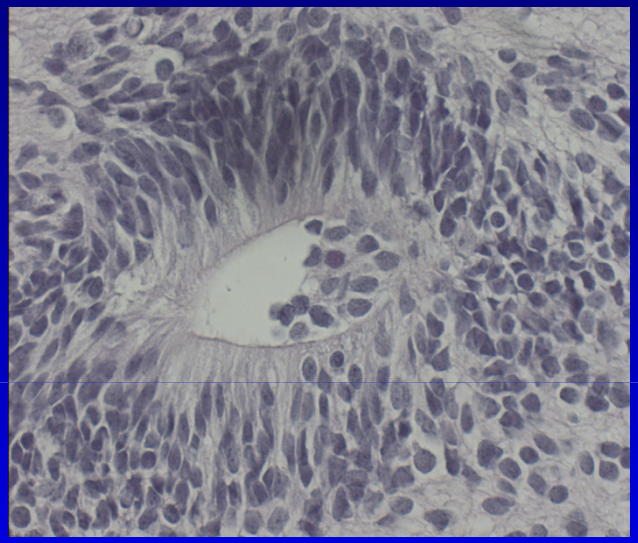
hESCs cultivated in suspension for 7-10 weeks differentiate into the three germ layers *in vitro* and *in vivo*.

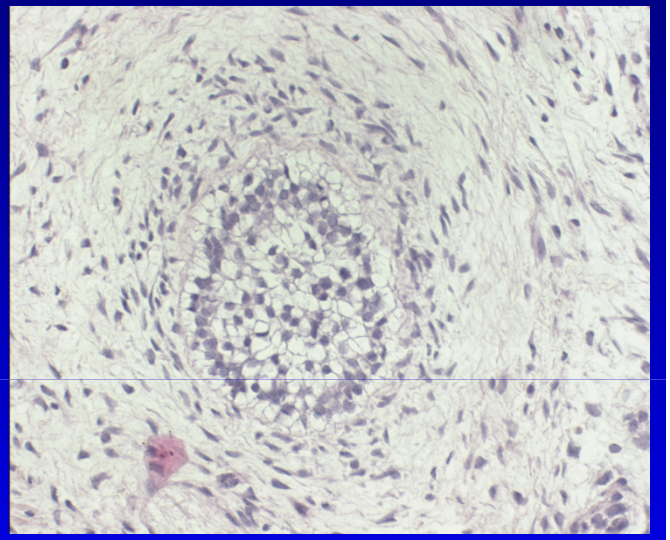
mesoderm

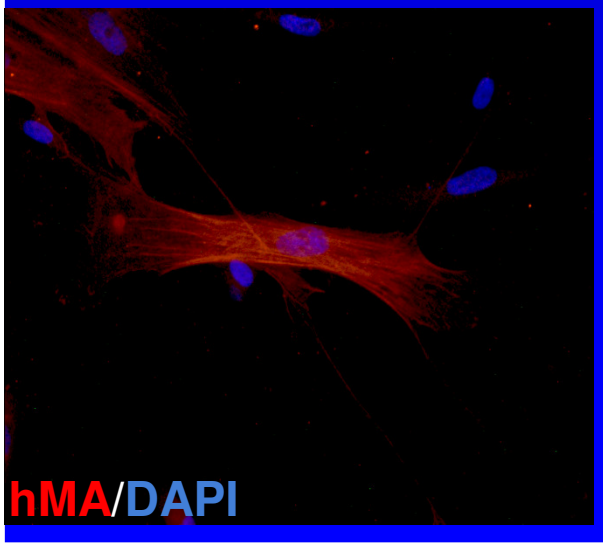
ectoderm

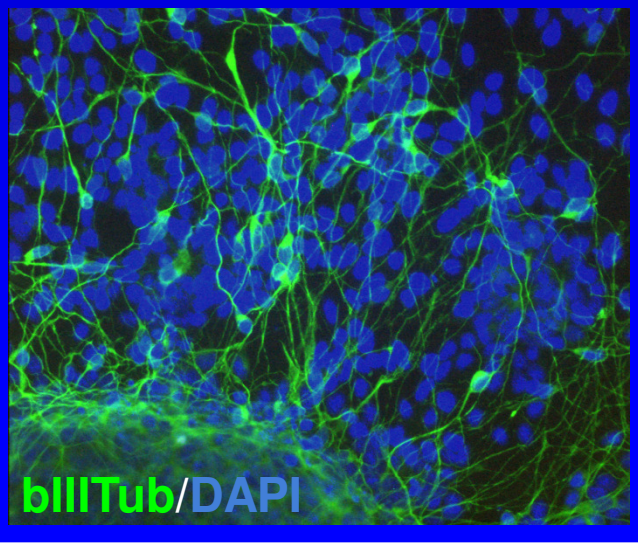
endoderm

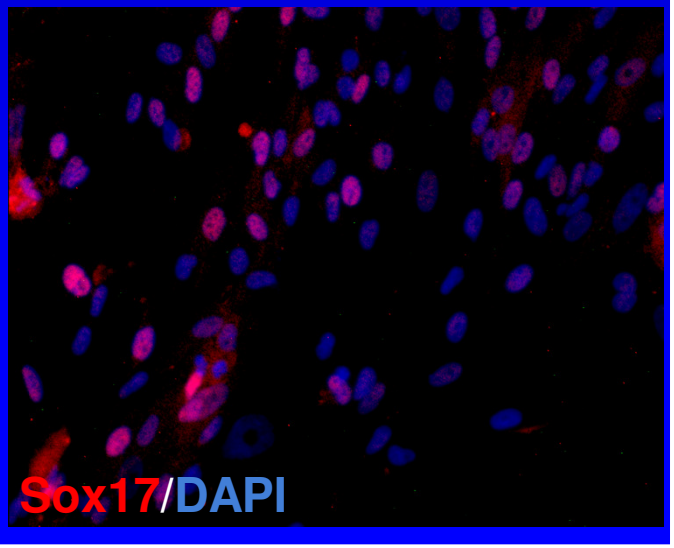




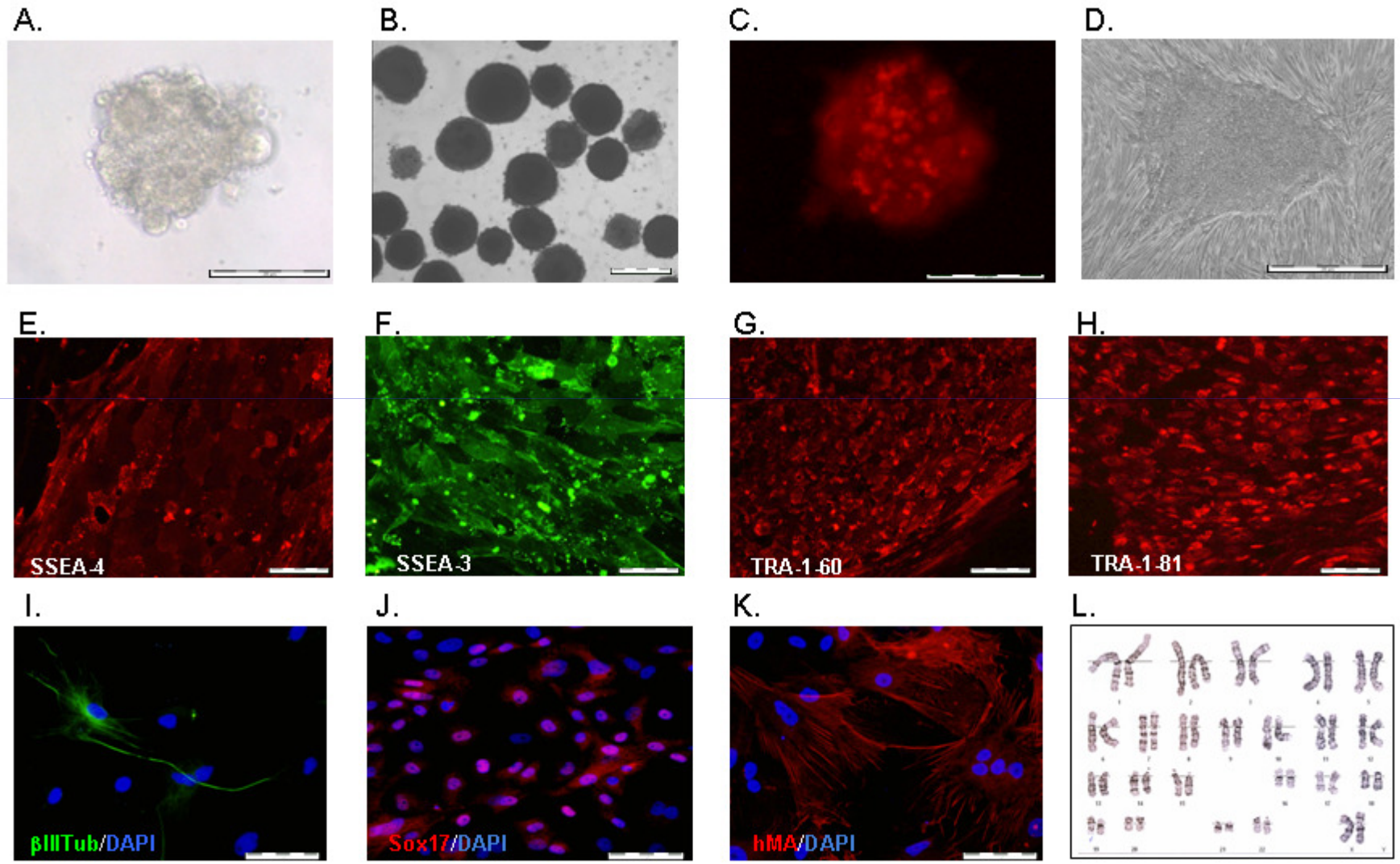








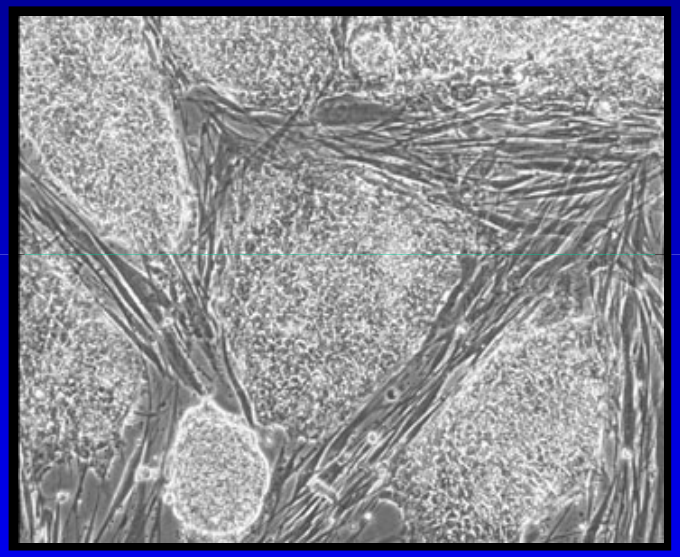
Derivation of three new hESC lines in suspension



15 ICMs; 1 intact embryo

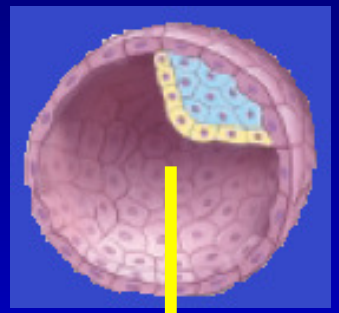
Steiner *et al.*, Nature Biotech. 2010

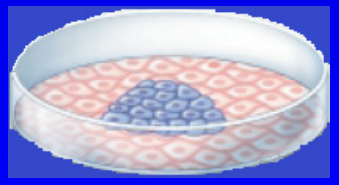
Bulk cultures of cells suitable for clinical trials.



RCCS-4 (Rotary Cell Culture System)

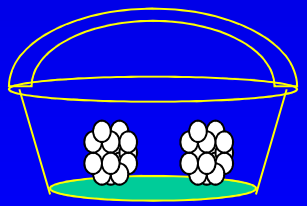
Road map for preclinical development of hESCs for transplantation in neurological and retinal disorders



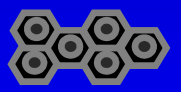


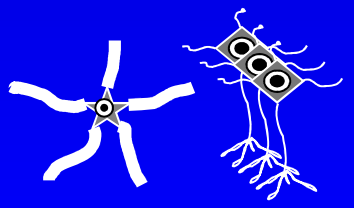
Clinical grade hES cells

Controlled defined process

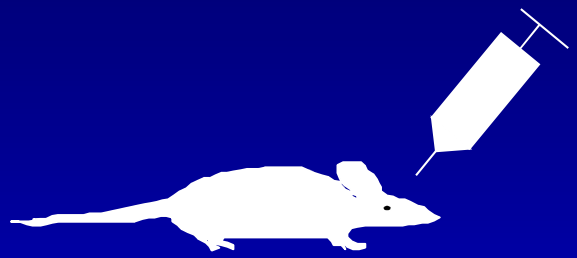


Neural Precursors



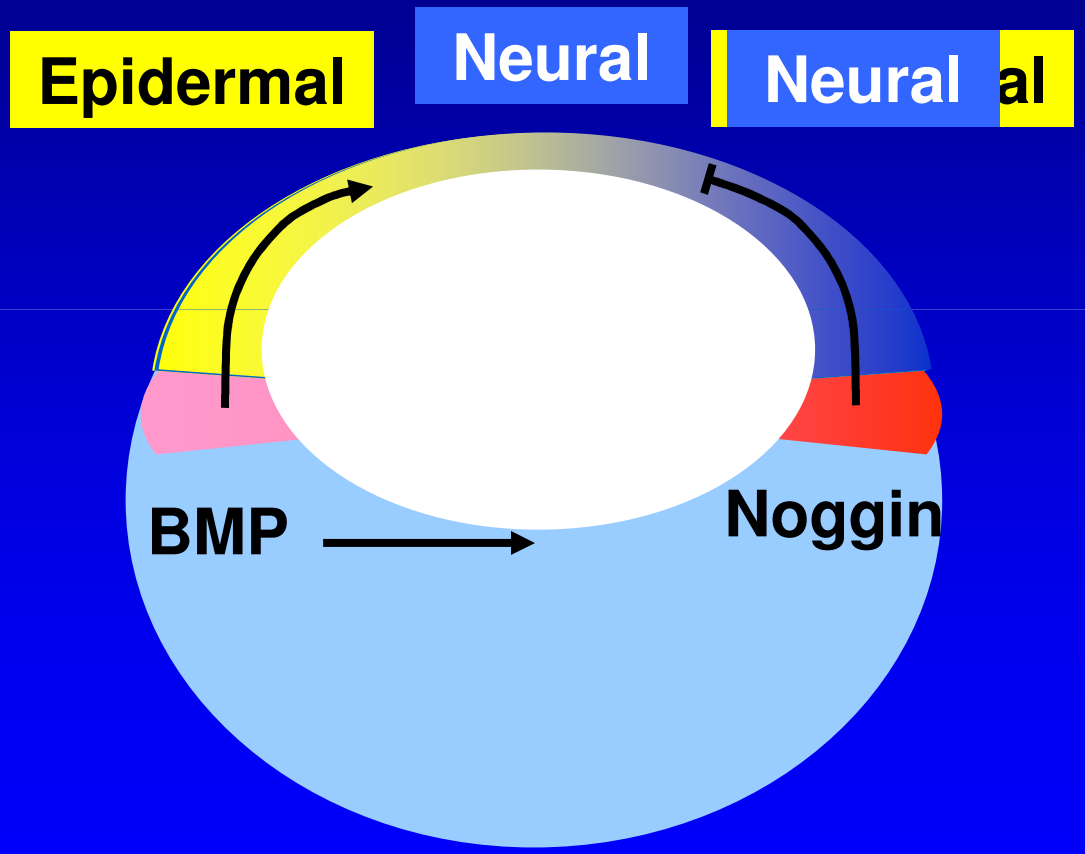


Neural Progenitors / Cells



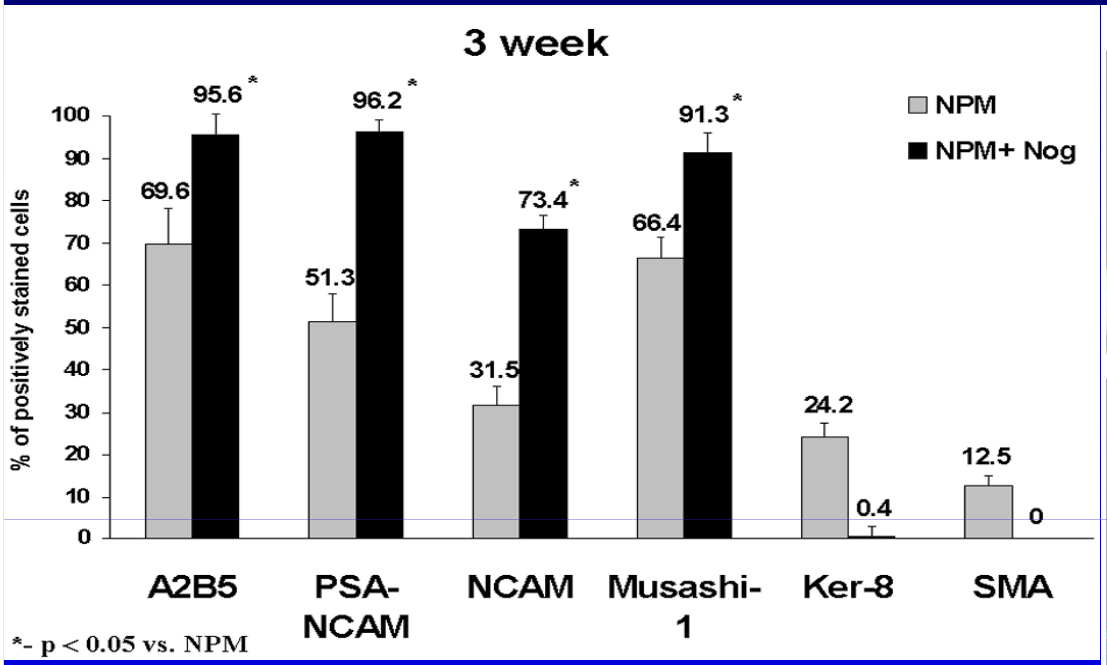
Animal models

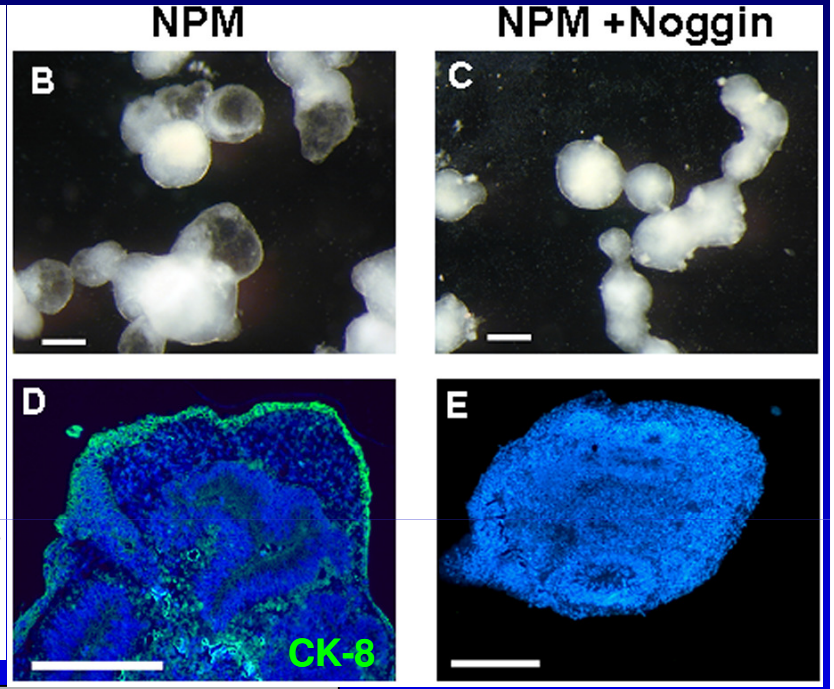
The default model in *Xenopus* blastula

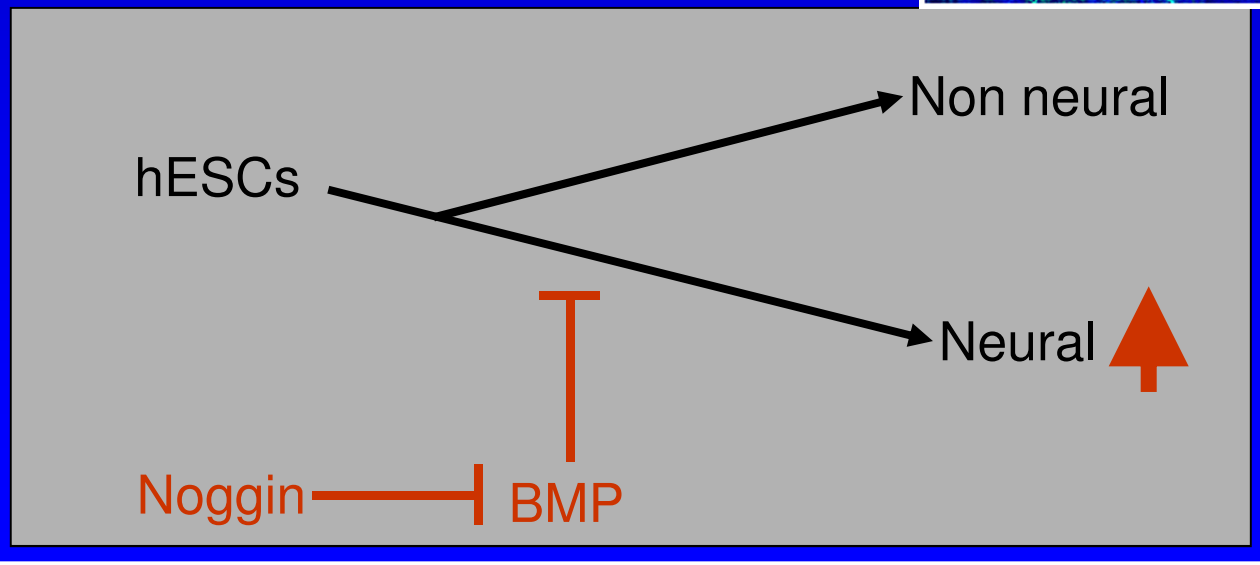


Hemmati-Brivanlou ,1994

BMP-signaling Inhibition and Neural Induction of hESCs

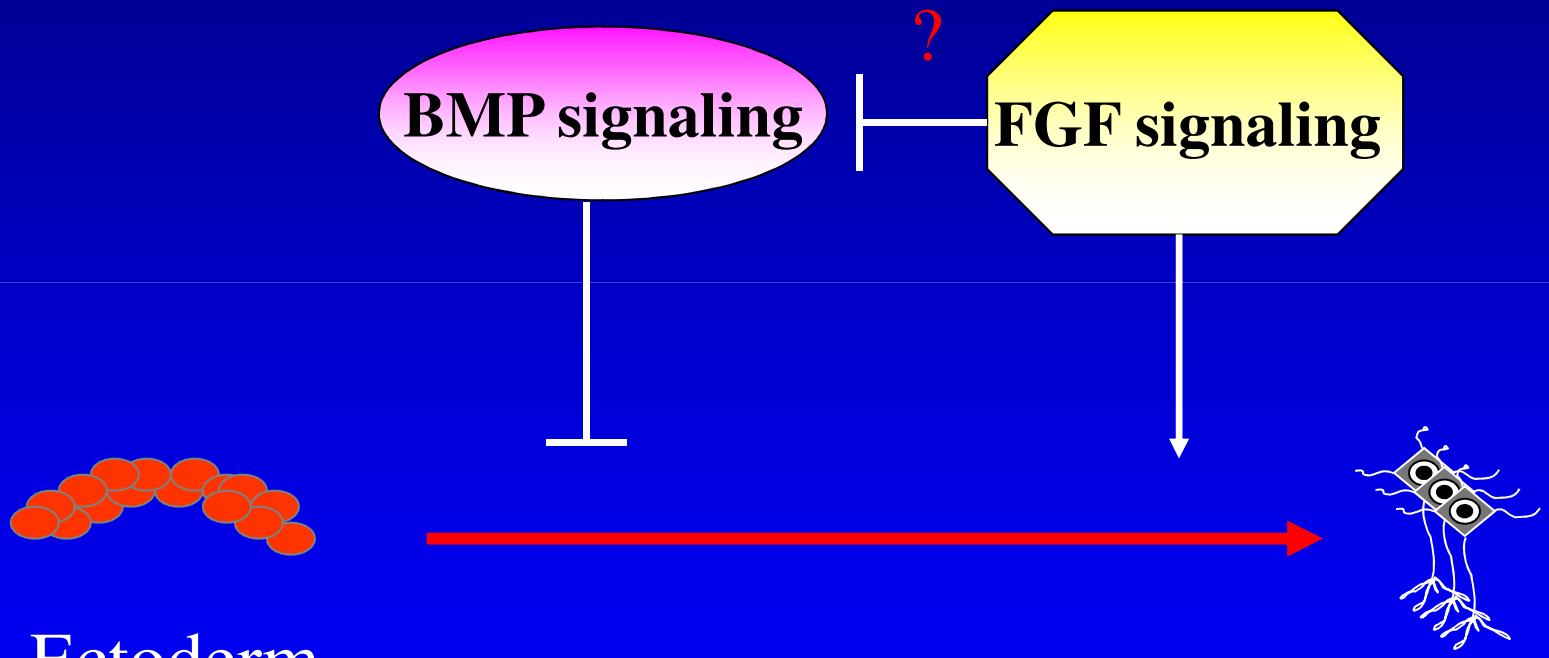






Itsykson et al. MCN (2005)

FGF signaling initiates and is required for neural induction in the chick and xenopus

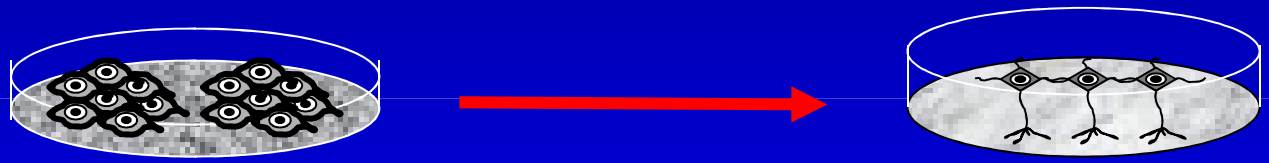


FGF signaling probably also repress BMP signaling

The role of FGF-signaling in mammals neuralization?

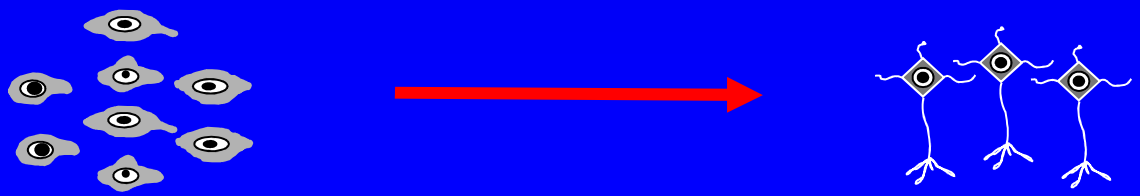
- Neural differentiation in mouse ESC cultures is not a simple default pathway but **depends on autocrine FGF** induced Erk1/2 signaling.

(Ying et al., Nat. Biotechnol. 2003)
(Stavridis et al., Development. 2007)

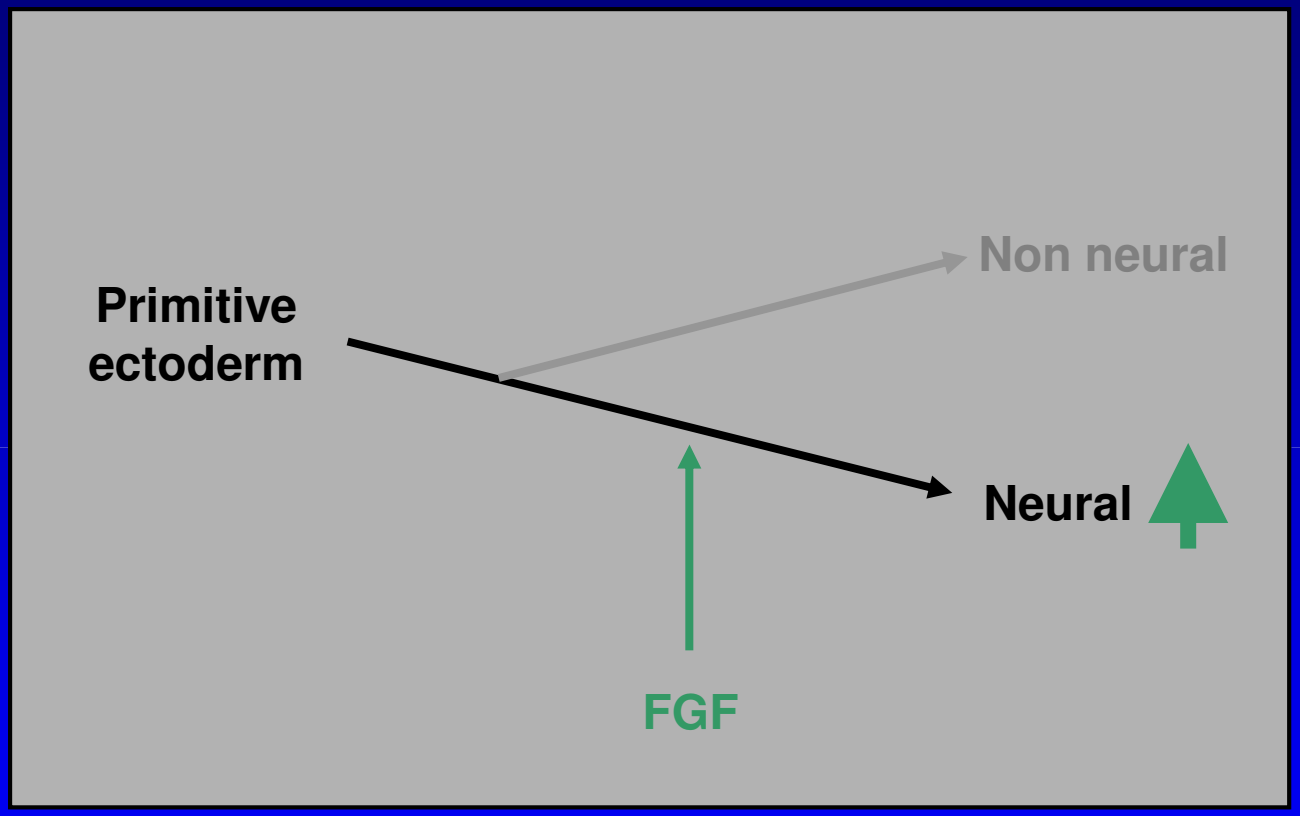


- Neuralization of mouse single ESCs is **independent of FGF** signaling

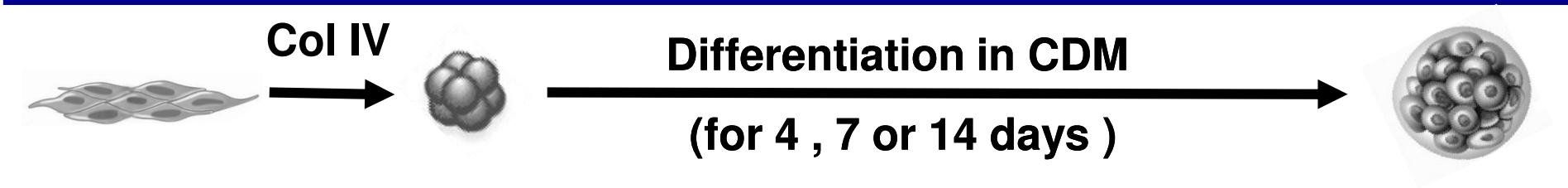
(Smukler et al., J Cell Biol. 2006)



FGF-signaling and Neural Induction of hESCs



The role of FGF in neural induction of hESC
The experimental model



hESC

hESC clusters

+/- bFGF

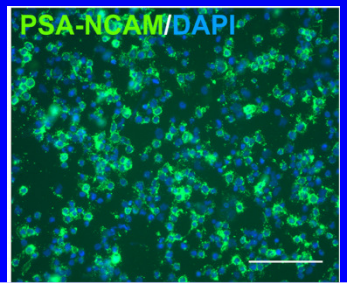
+/- FGF/Erk signaling inhibitors
SU5402 / U0126

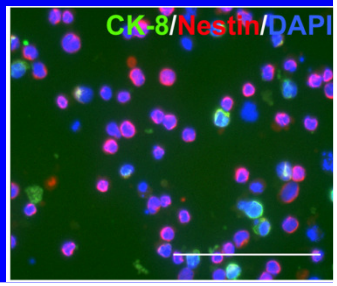
+/- Noggin

Characterization

Neural

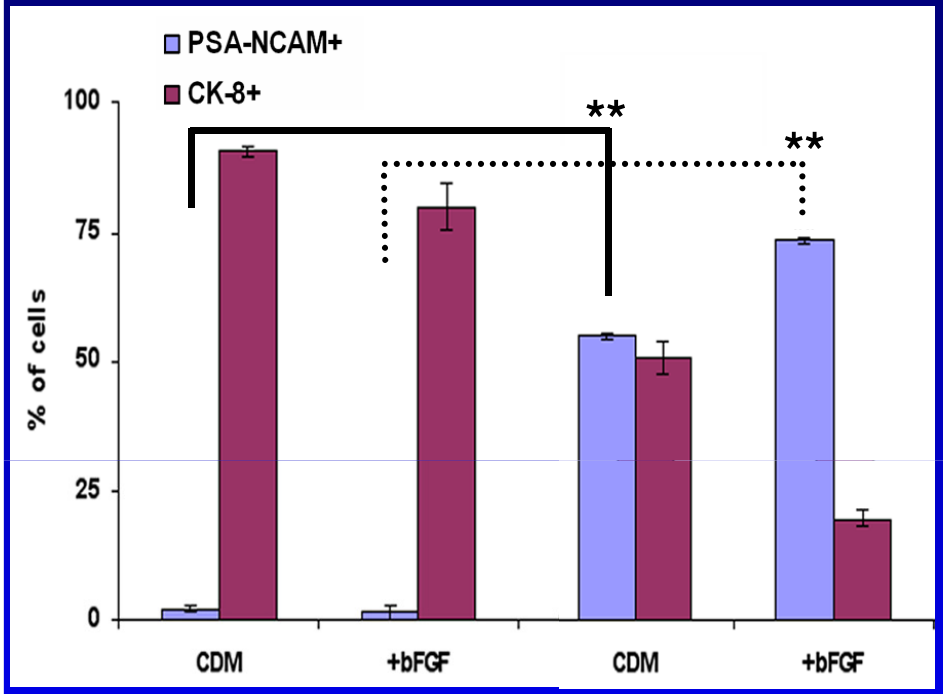
Non-Neural

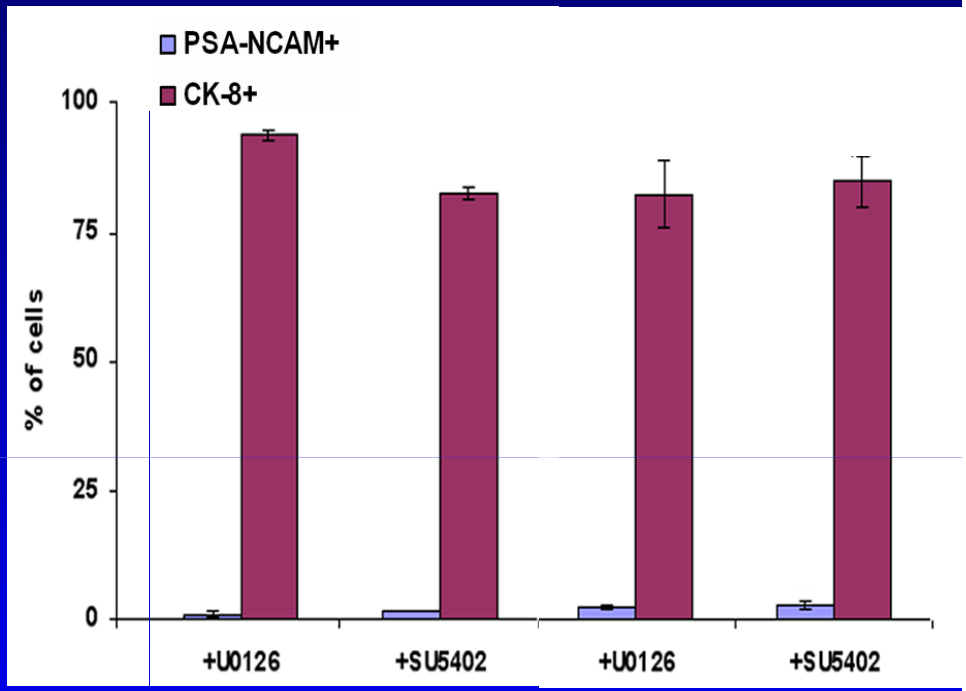




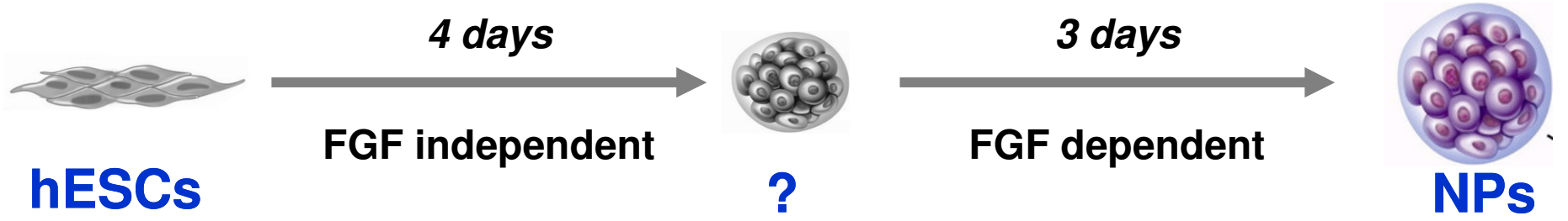
Cohen et al., *Dev. Biol. (2010)*

FGF-signaling induces neuralization during days 4 to 7 of differentiation

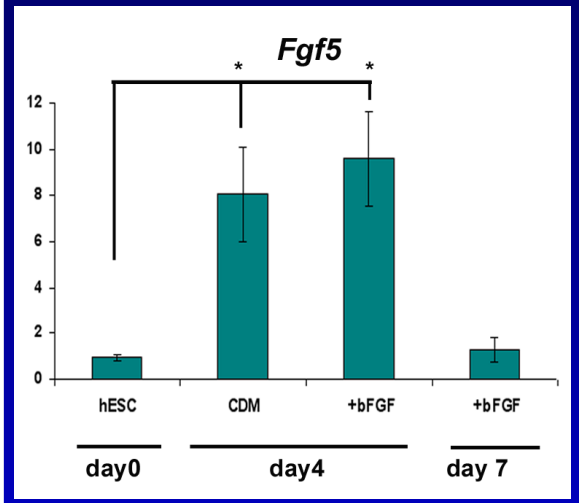


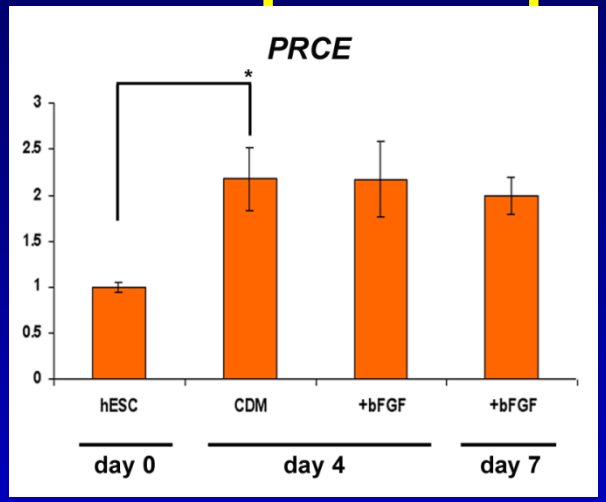


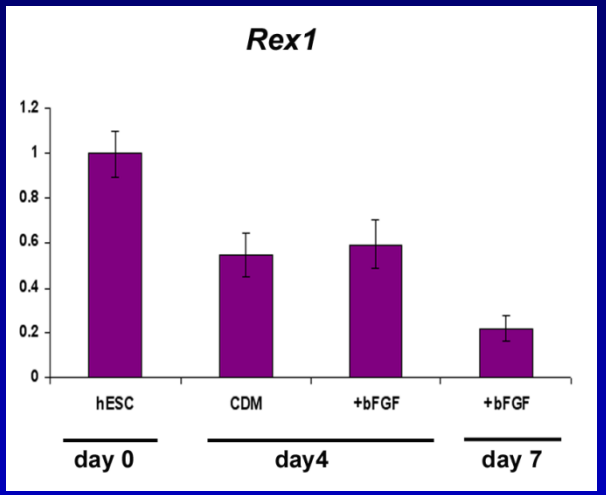
Cohen et al., *Dev. Biol. (2010)*

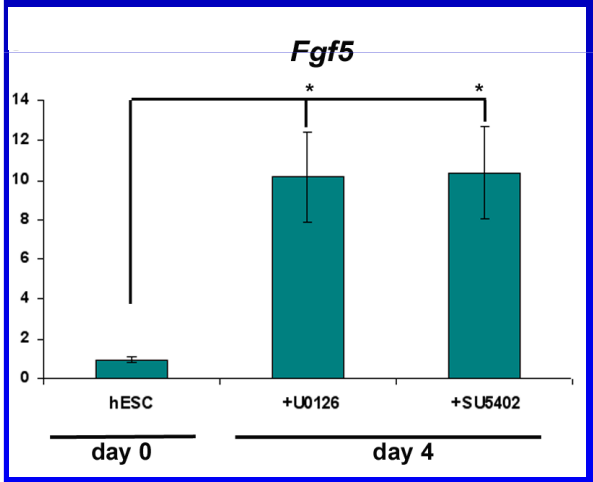


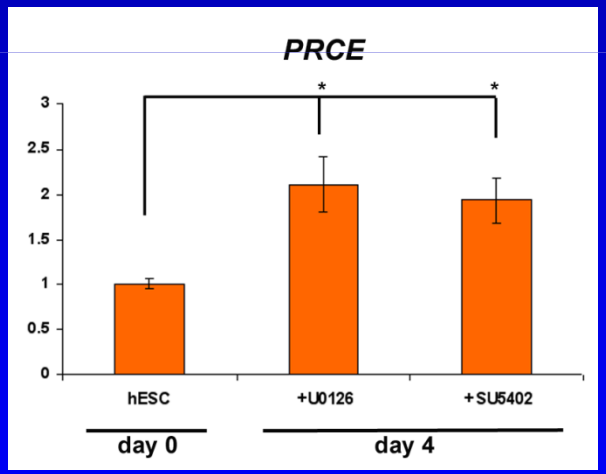
hESC clusters differentiate initially into primitive ectoderm in an FGF-independent process



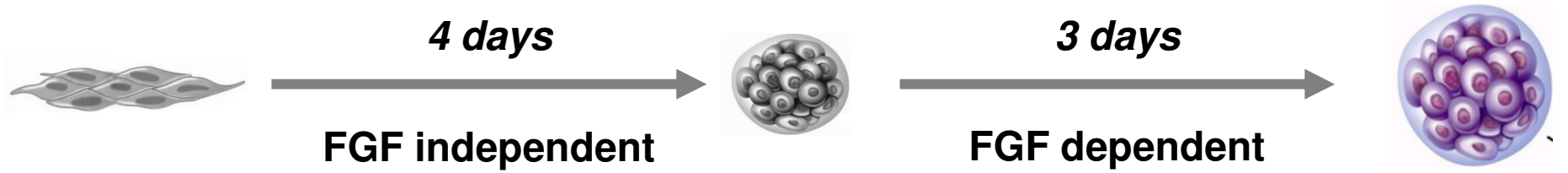






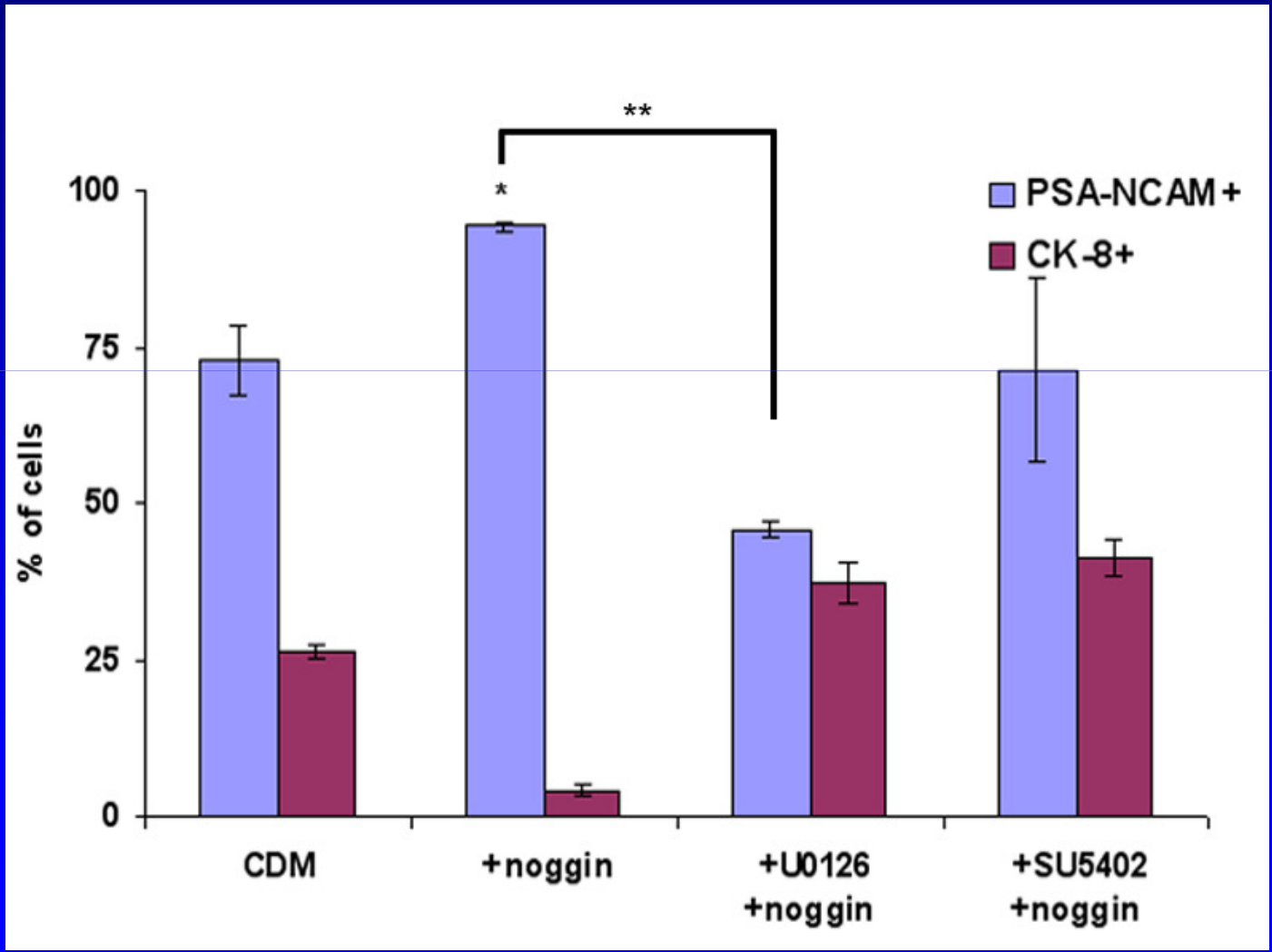


Cohen et al., *Dev. Biol. (2010)*



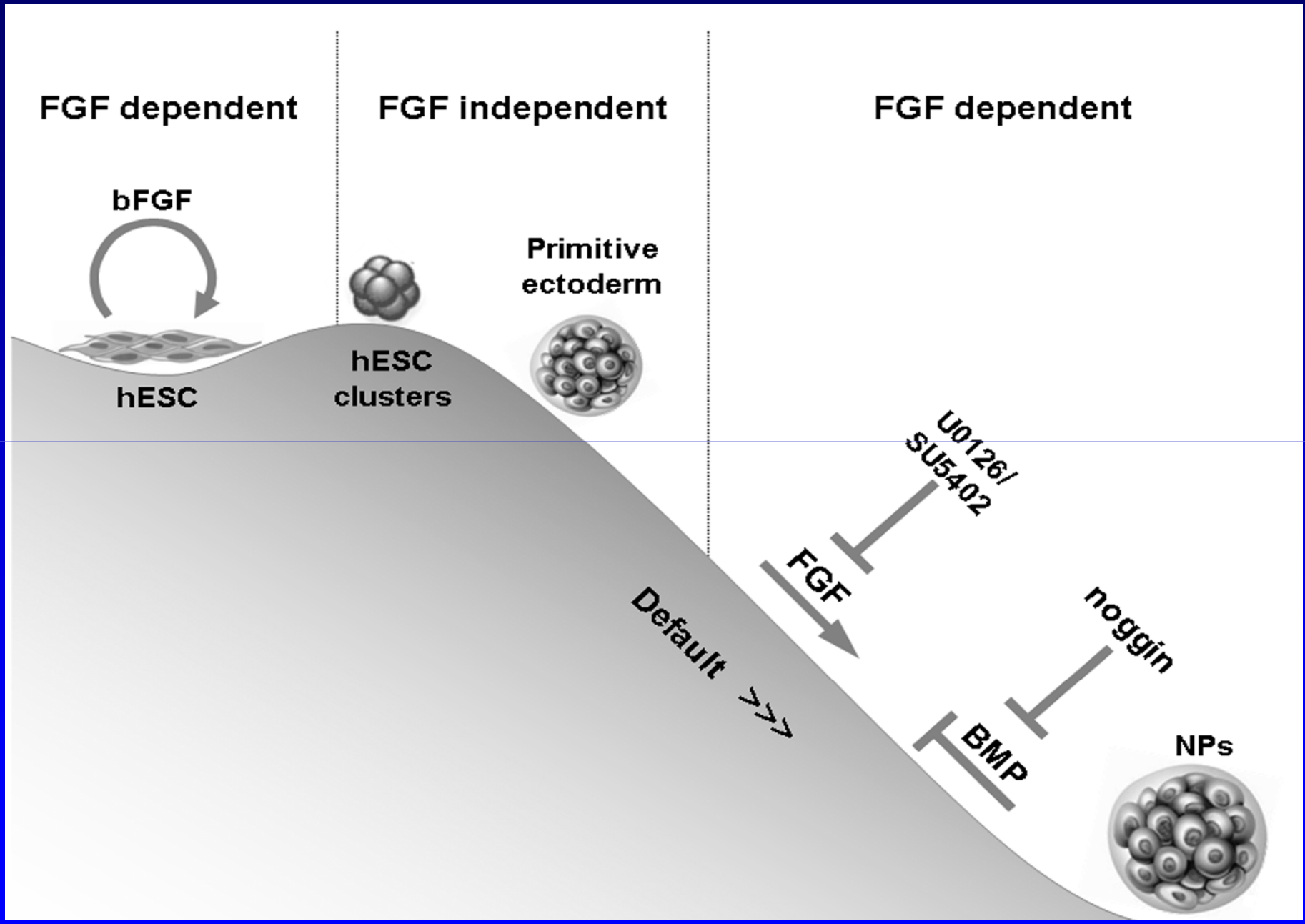
FGF signaling is not essential for neural specification of hESCs

day 14



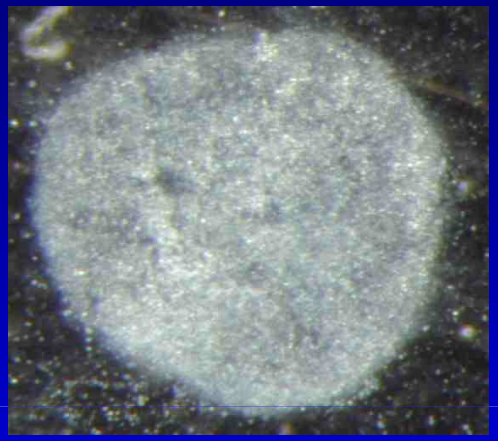
Cohen et al., *Dev. Biol. (2010)*

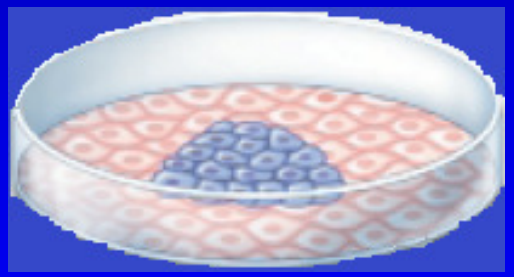
Conclusions



Cohen et al., *Dev. Biol. (2010)*

Controlled conversion of hES cells into neural precursors



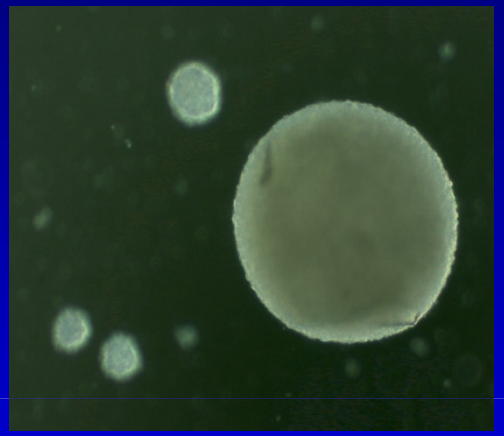


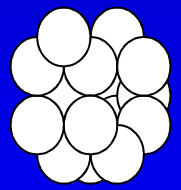
Undifferentiated hES cells

Defined

Culture Conditions

Noggin & FGF

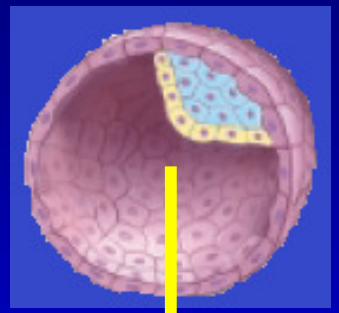


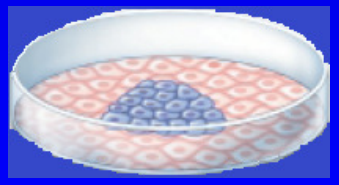


Neural spheres

Reubinoff et al., Nature Biot. 2001
Itzikson et al., MCN 2005
Cohen et al., Dev. Biol. 2010

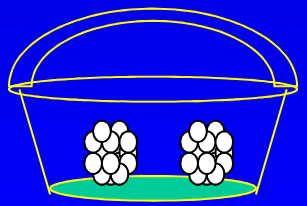
Road map for preclinical development of hESCs for transplantation in neurological and retinal disorders



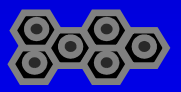


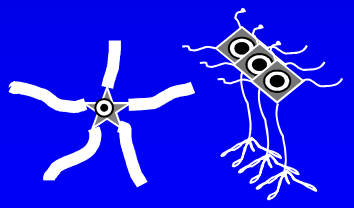
Clinical grade hES cells

Controlled defined process

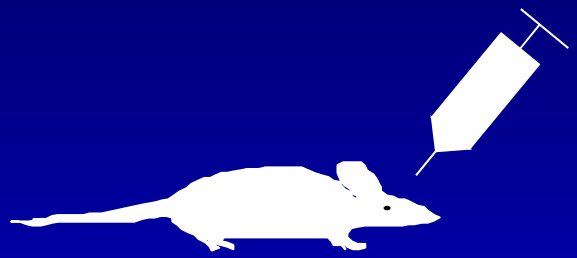


Neural Precursors





Neural Progenitors / Cells



Animal models

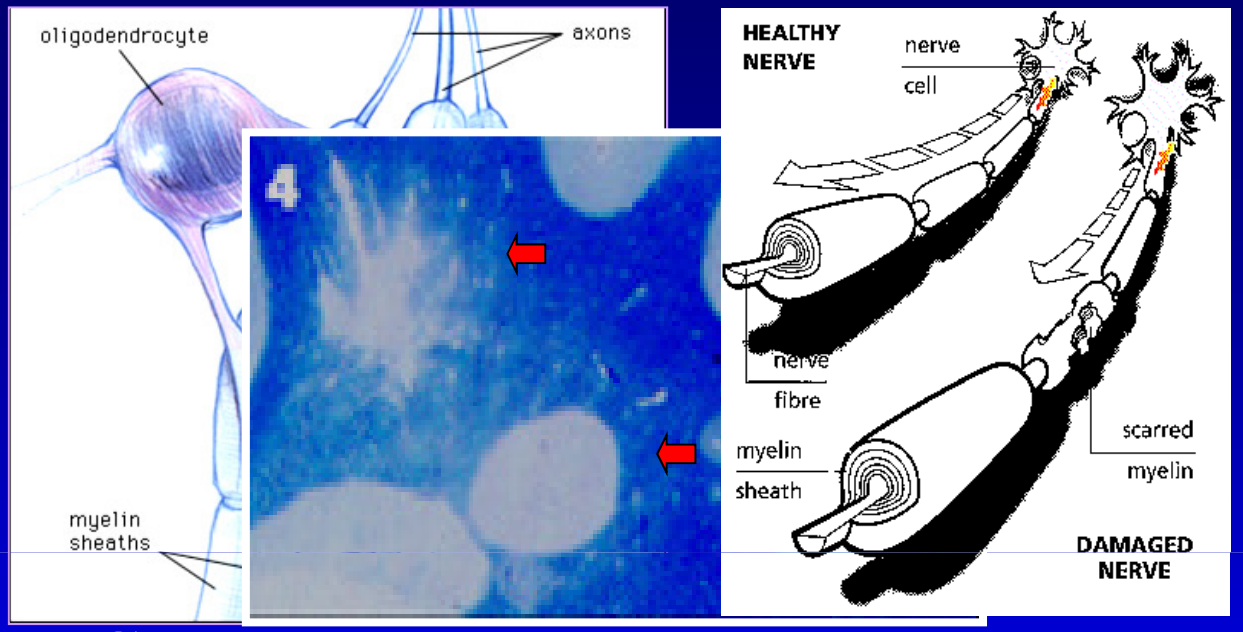
Multiple Sclerosis (MS)

- A multifocal, chronic, auto-immune disease of the CNS
- The leading cause of neurological disability in young adults
- Manifestations: paralysis, sensory disturbances, incoordination and visual impairment.

MS Is Characterized By Multifocal CNS Lesions

Inflammation

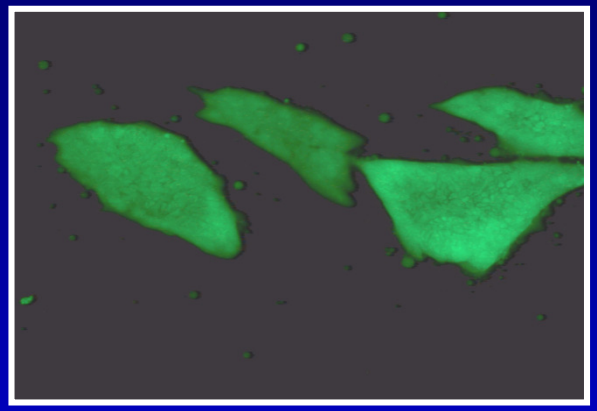
Demyelination



Axonal pathology- Correlated with the permanent disability of MS patients

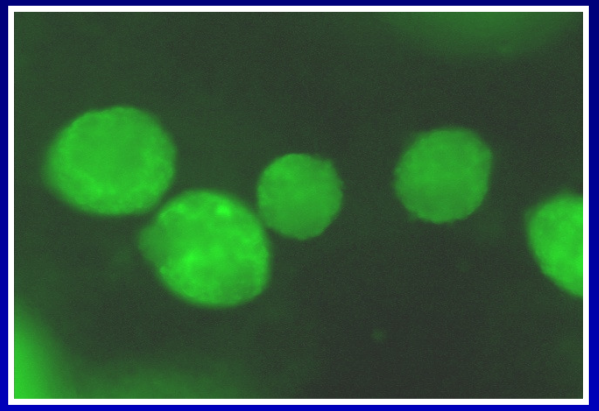
Gliosis

Transplantation of hESC in multiple sclerosis: Experimental design



GFP expressing hESC colonies

Noggin
3W



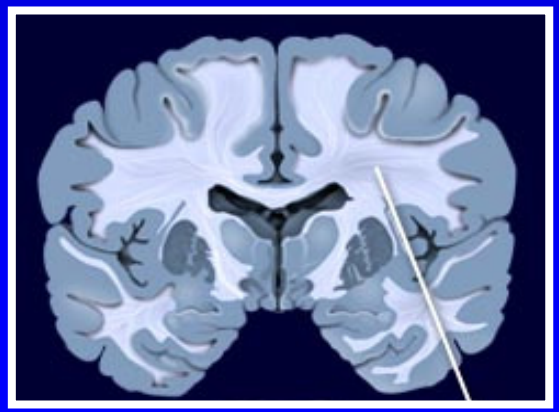
Neural Precursors

In-vitro differentiation potential

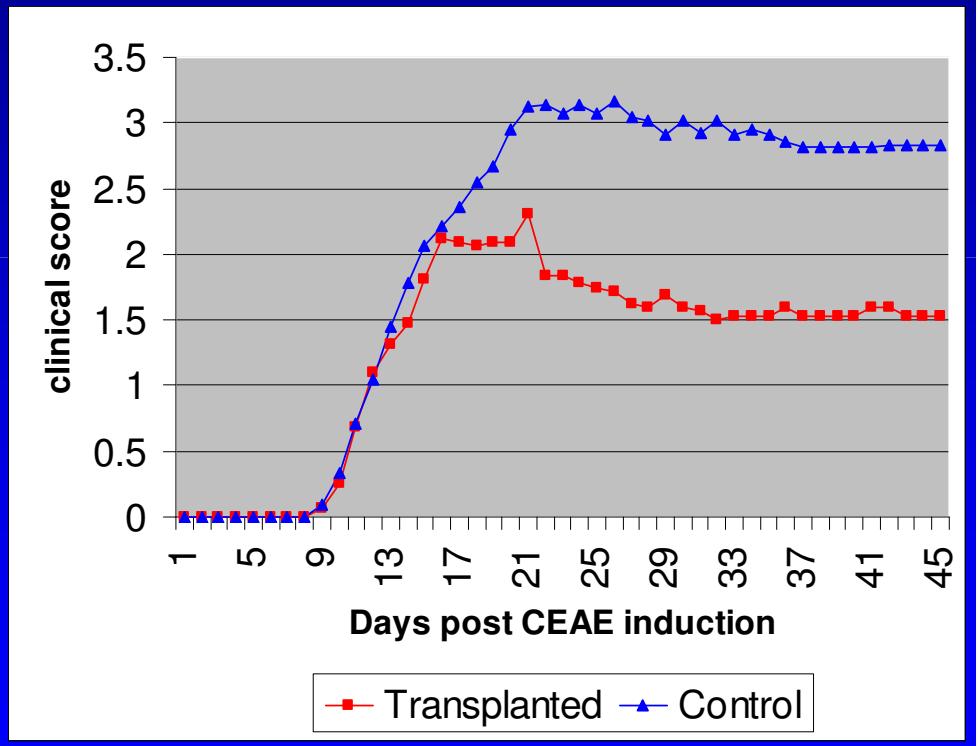
67 % neurons

12 % astrocytes

no oligodendrocytes

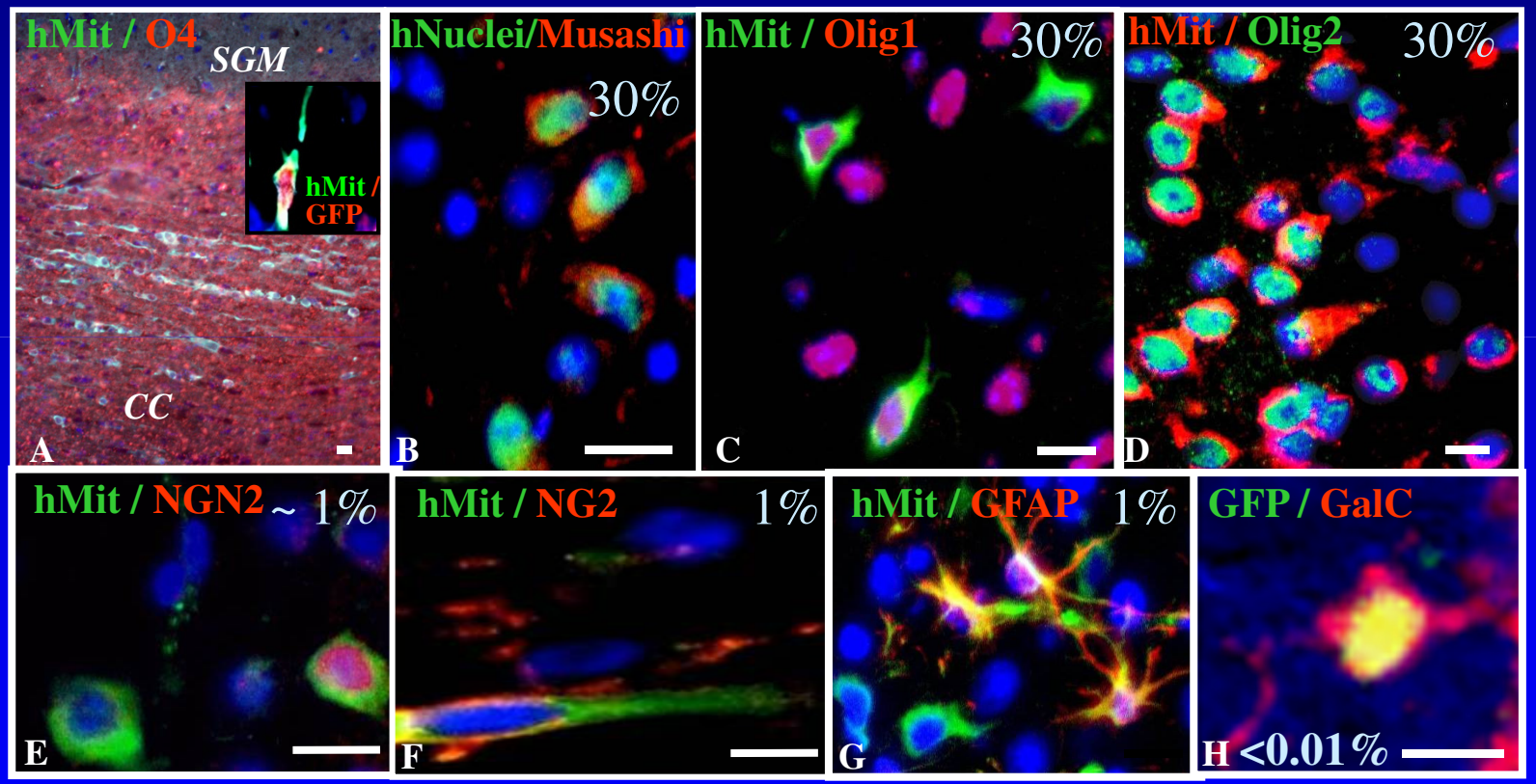


NPs Transplantation Attenuated Significantly The Clinical Signs Of CEAE



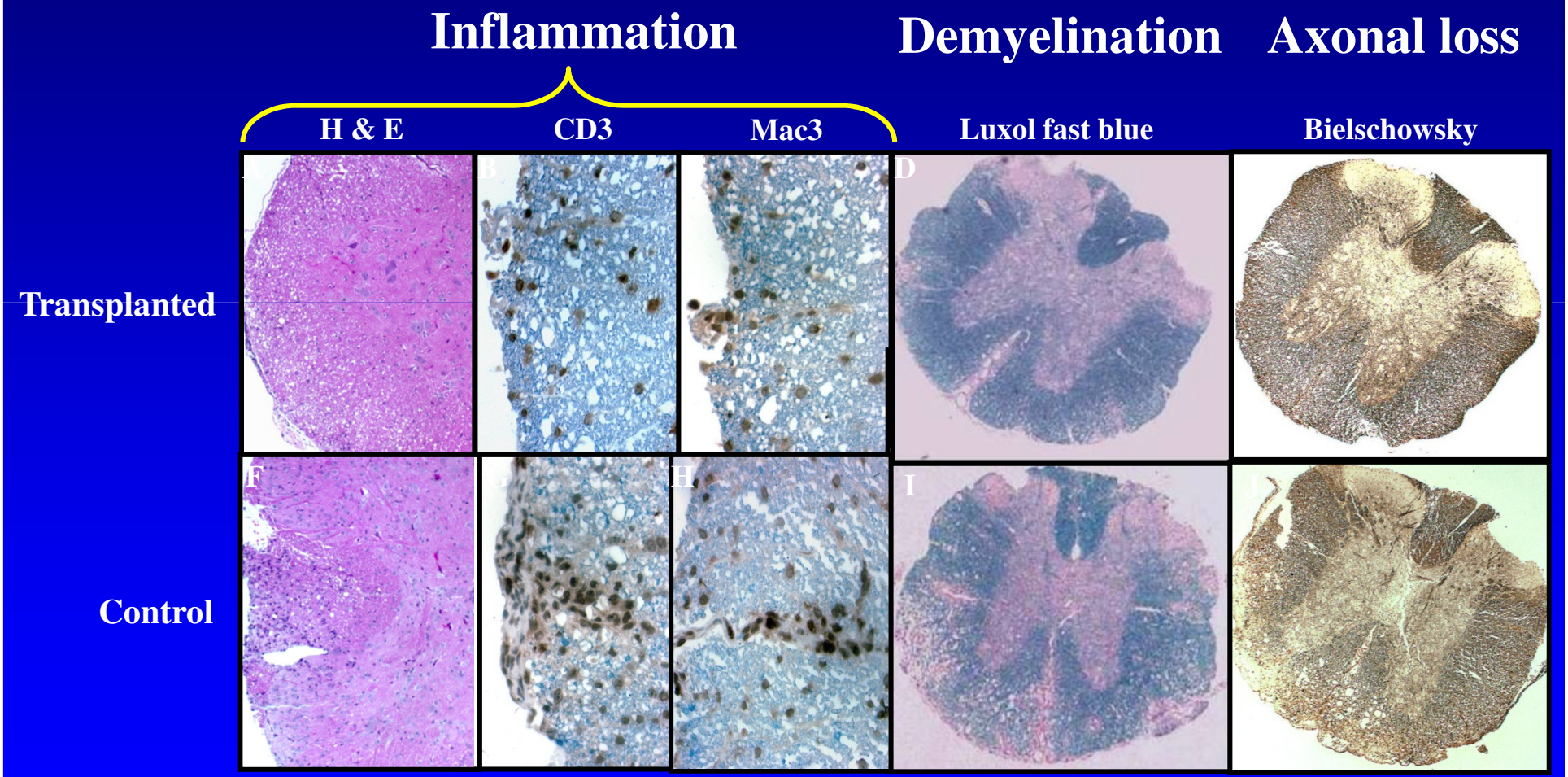
(Aharonowiz et al., PLoS ONE 2008)

In-vivo Differentiation Fate Of The Transplanted NPs



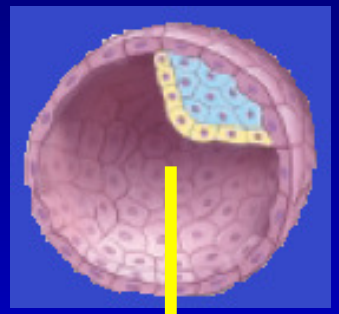
(Aharonowiz et al., PLoS ONE 2008)

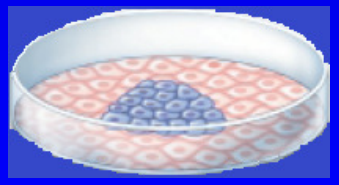
NPs Transplantation Attenuated Significantly The Pathological Features of CEAE

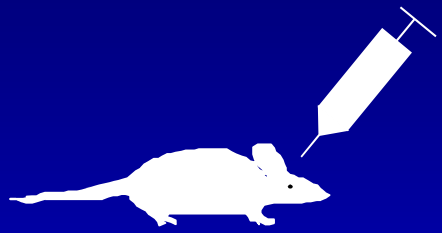


(Aharonowiz et al., PLoS ONE 2008)

Road map for preclinical development of hESCs for transplantation in neurological disorders



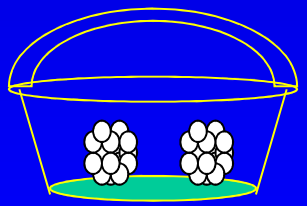


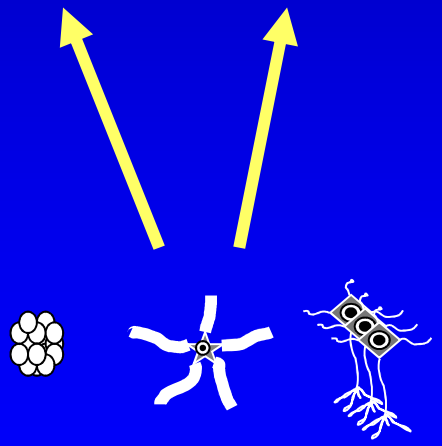


Immunomodulation
Host protection

Regeneration

Controlled defined process





Clinical grade hES cells

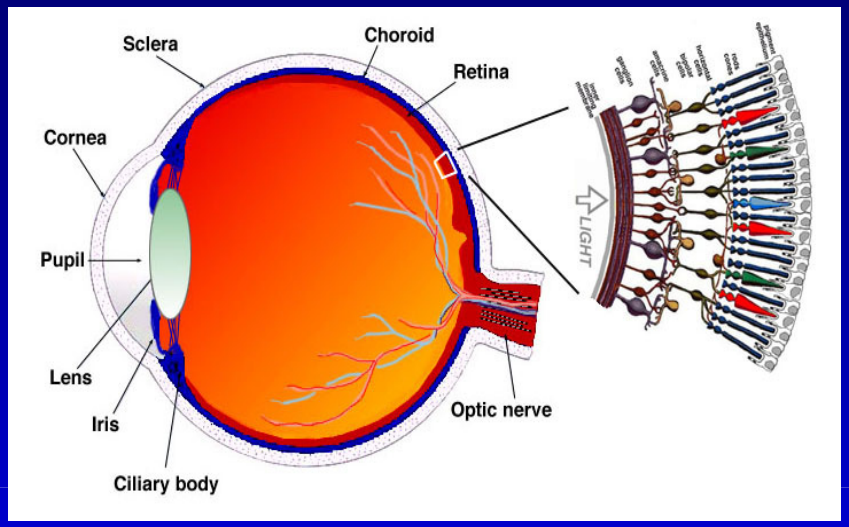
Neural Precursors

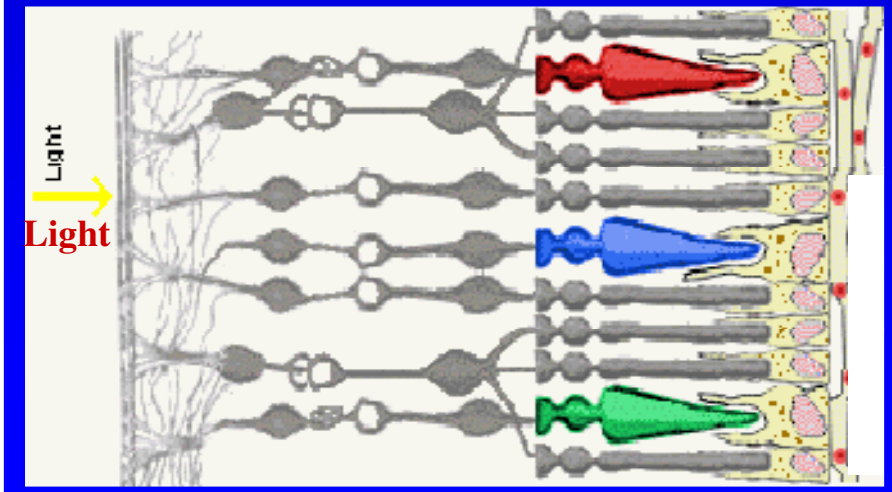
Neural Progenitors / Cells

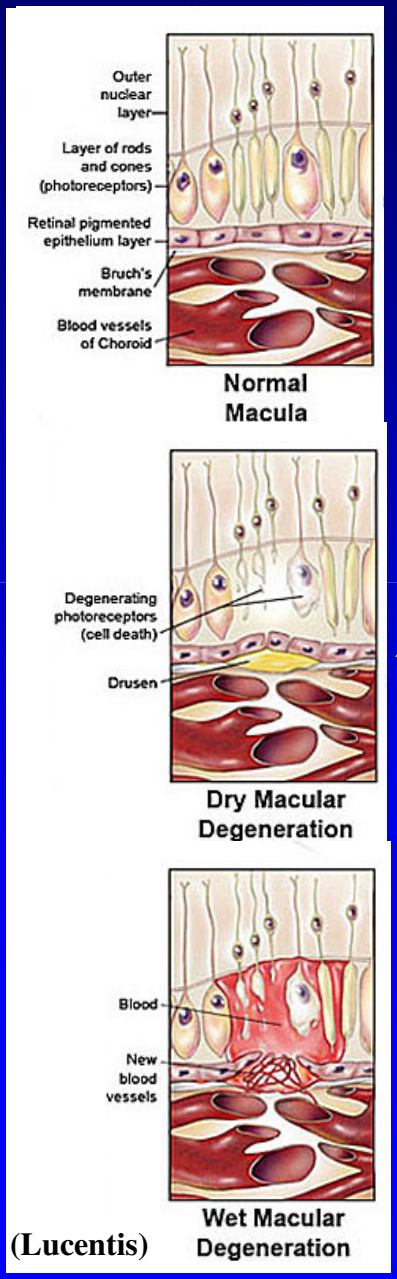
Age Related Macular Degeneration - AMD

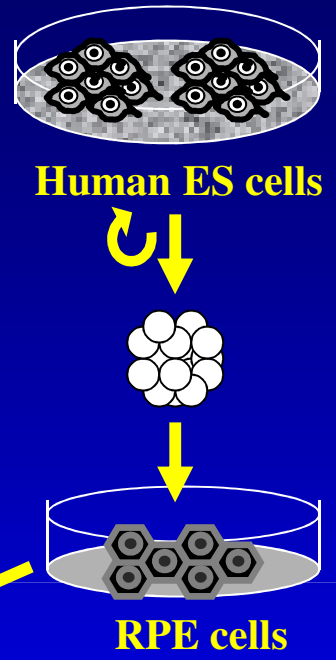
- The leading cause of blindness in the western world
- 30% of people > 75 yrs have clinical signs
- 6-8% of people >75 are legally blind
- No effective treatment

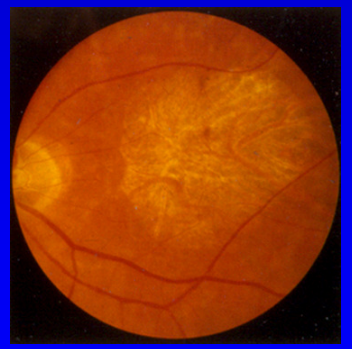
Human ES Cells For Age Related Macular Degeneration - AMD







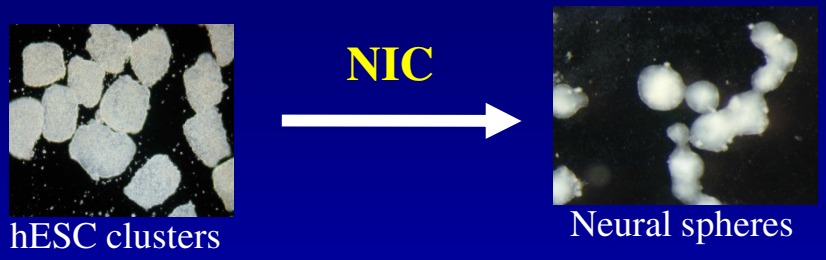


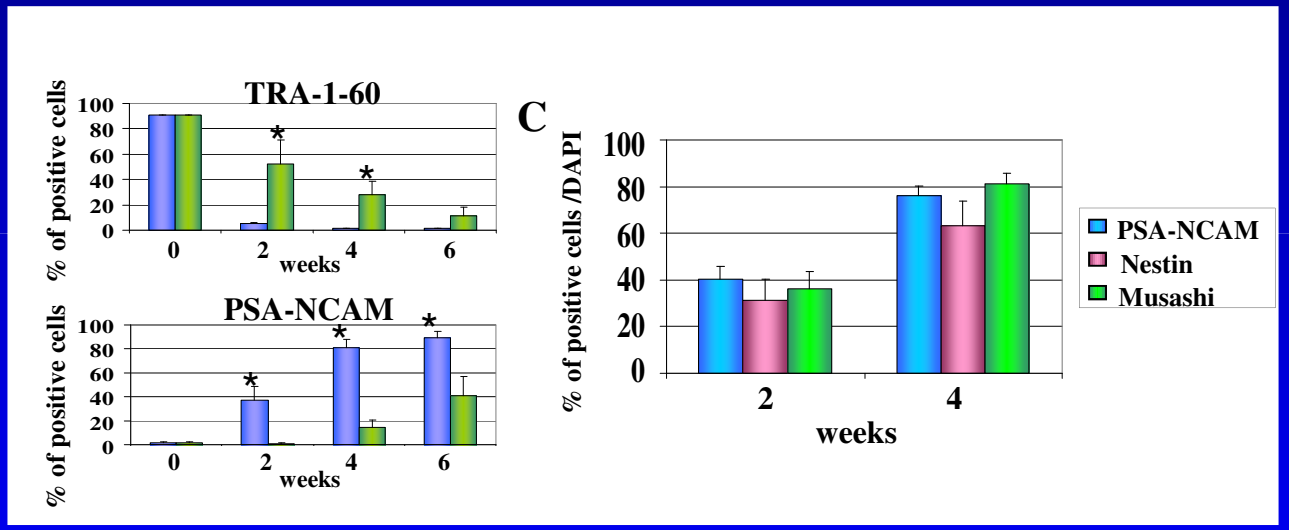


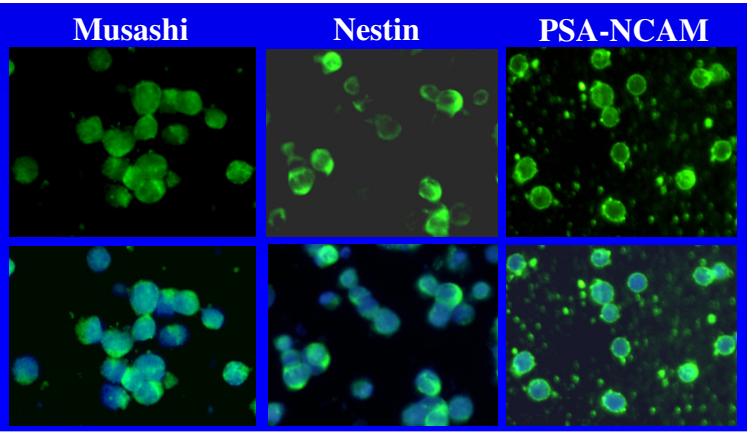
Nicotinamide (NIC;B3) promotes neural differentiation in a defined culture system

NIC increases cell proliferation, cell survival, and is neuroprotective *in vitro*

(Shen et al. 2004; Chong et al., 2005).



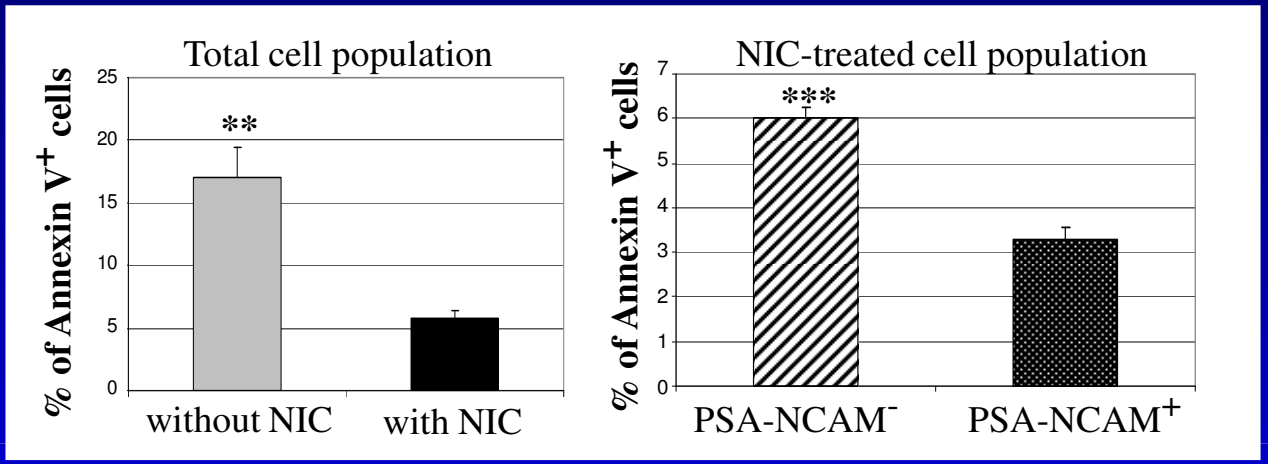




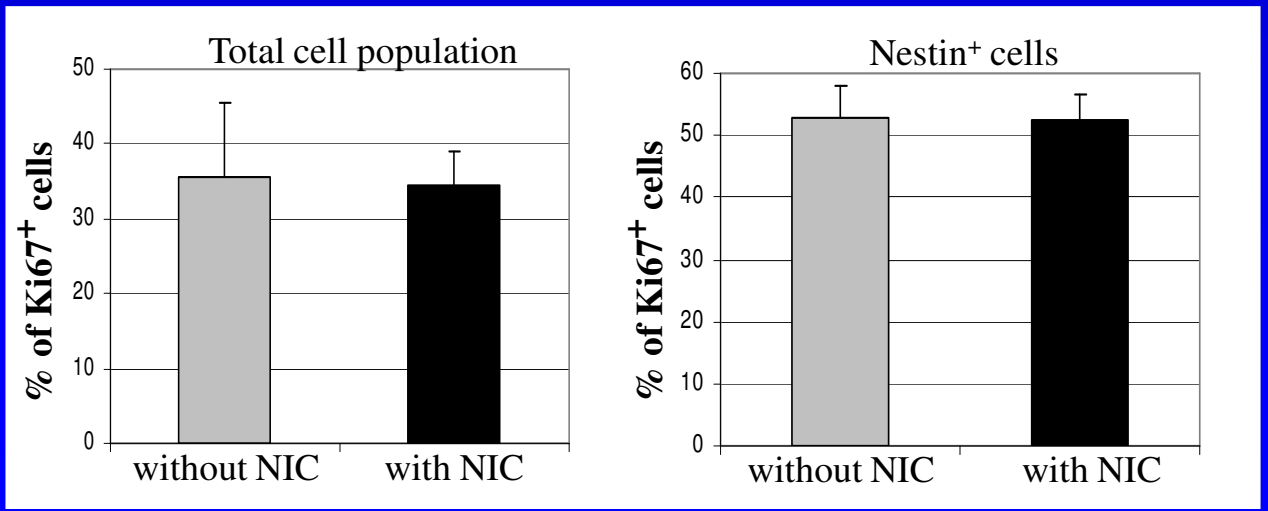
Idelson et al., Cell Stem Cell 2009

NIC promotes neuralization via prevention of apoptosis

Apoptosis

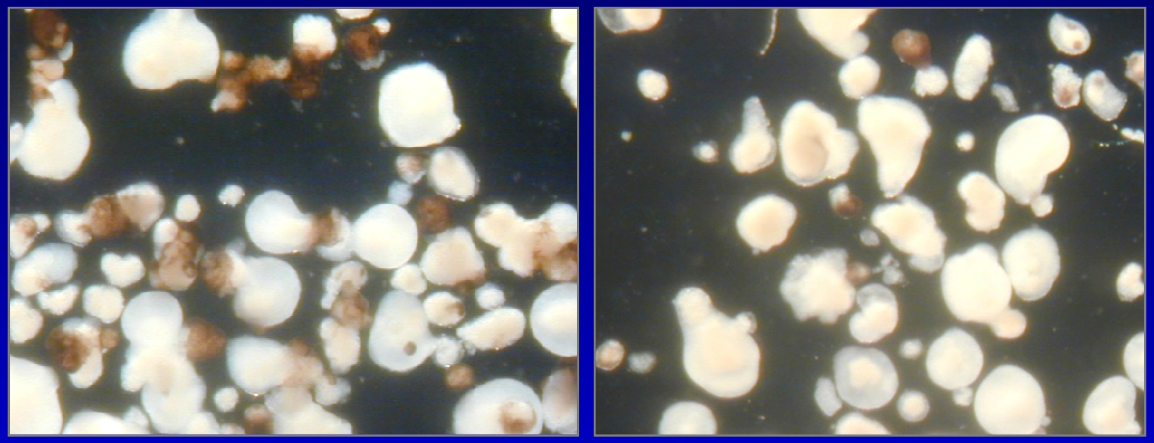


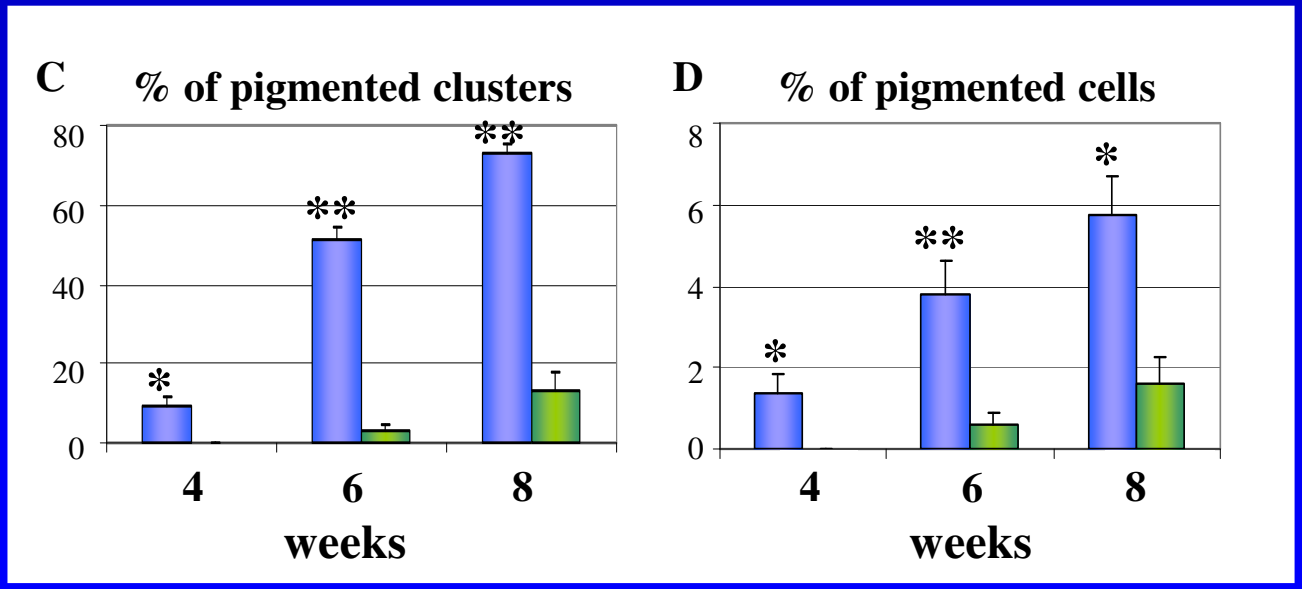
Proliferation



Idelson et al., Cell Stem Cell 2009

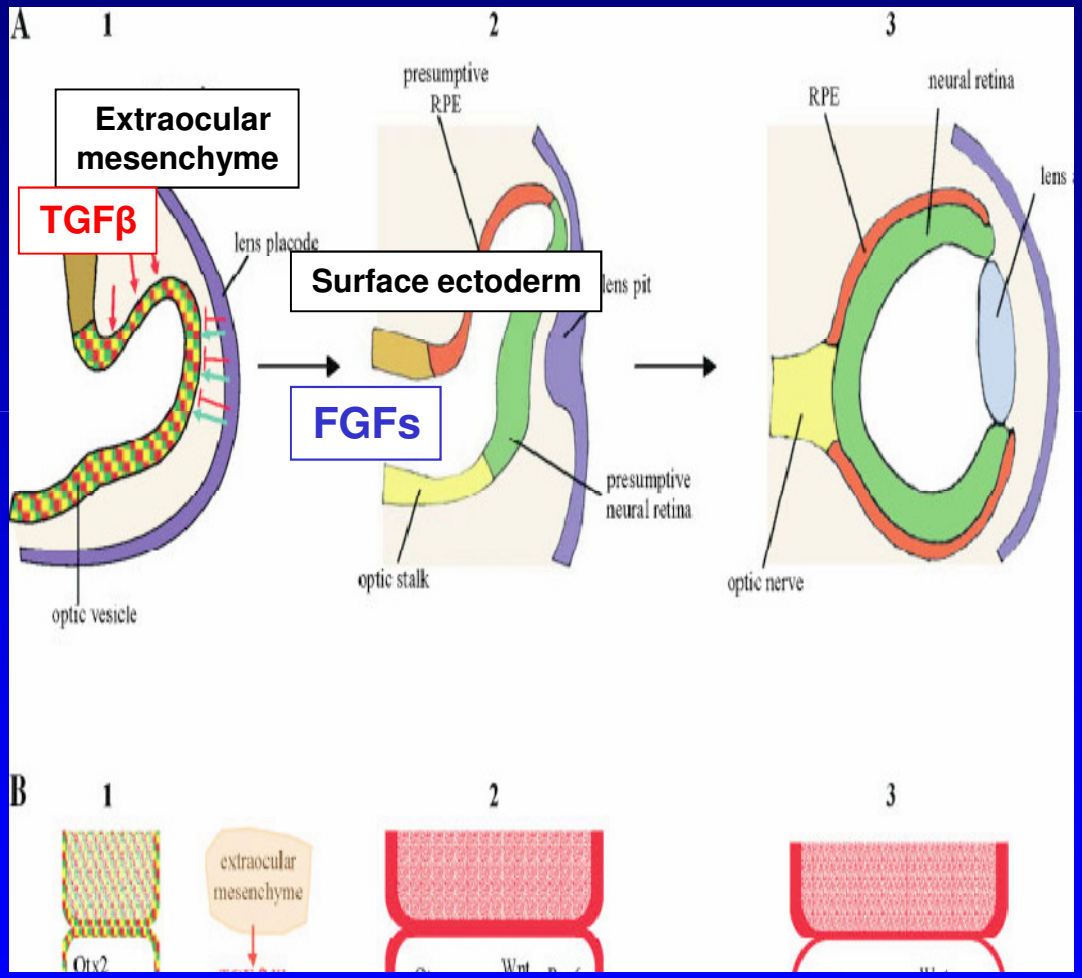
NIC promotes differentiation into pigmented cells





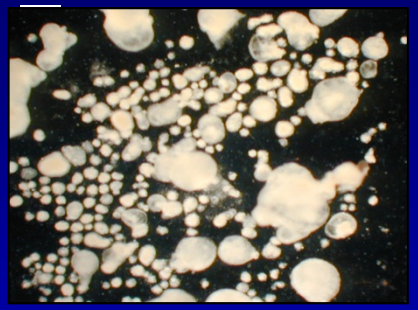
Idelson et al., Cell Stem Cell 2009

TGFβ and FGFs in retinal development

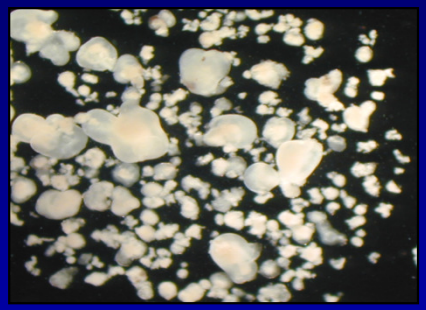


TGFβ factors promote while FGFs inhibit RPE differentiation

Activin A

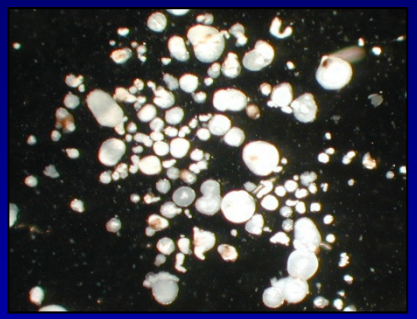


NIC alone

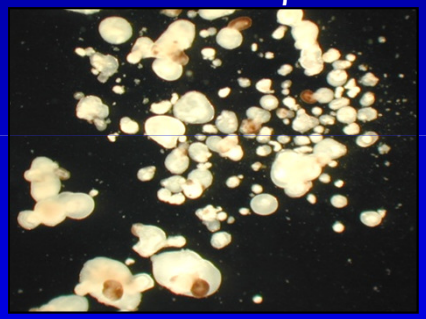


NIC+Activin A

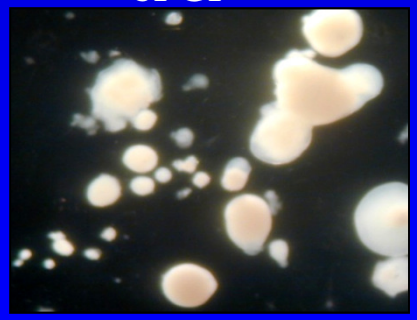
NIC+Act+SB431542



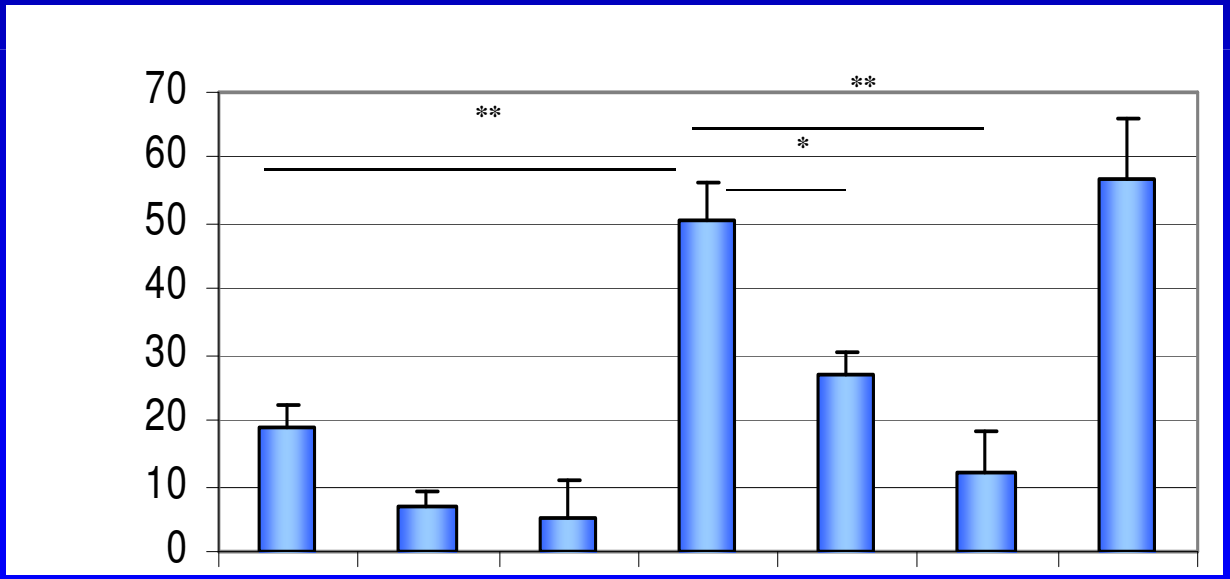
NIC+TGFβ1



bFGF

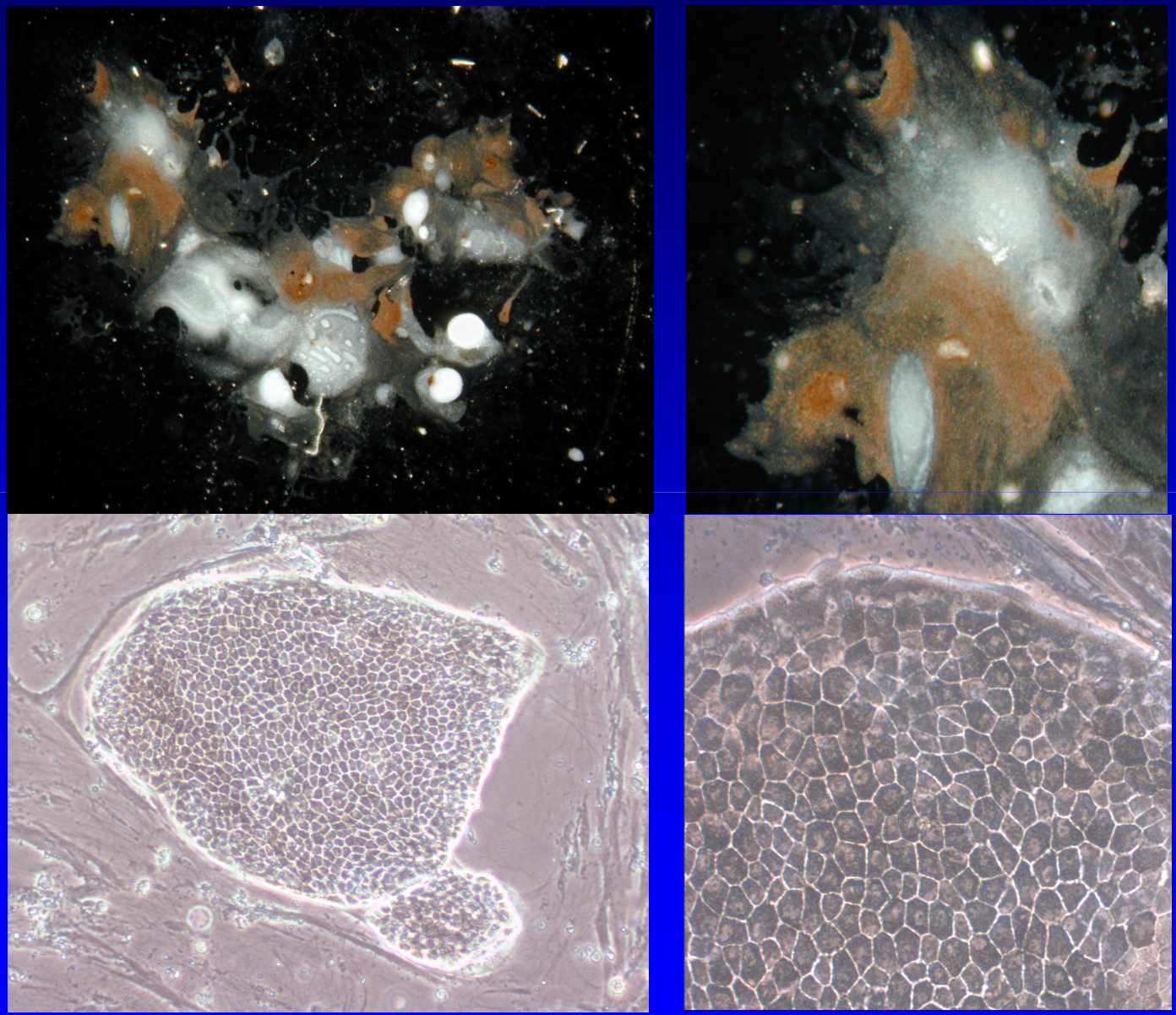


% of pigmented clusters



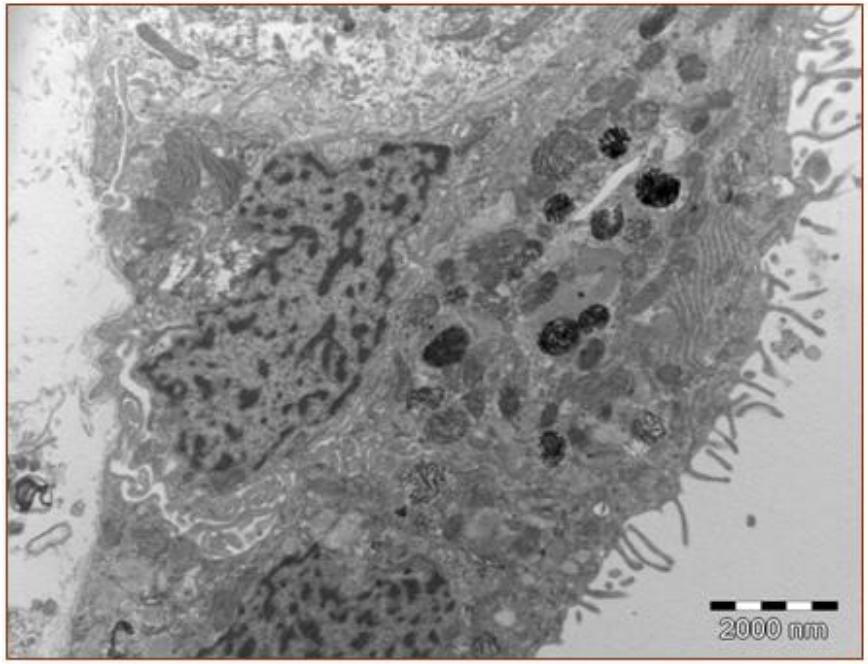
Idelson et al., Cell Stem Cell 2009)

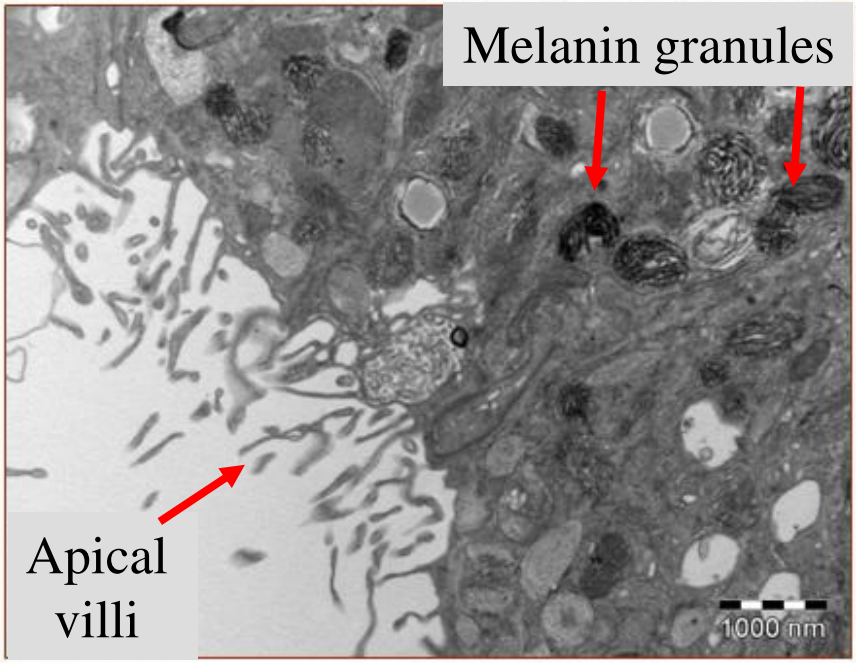
The Pigmented Cells are RPE-like

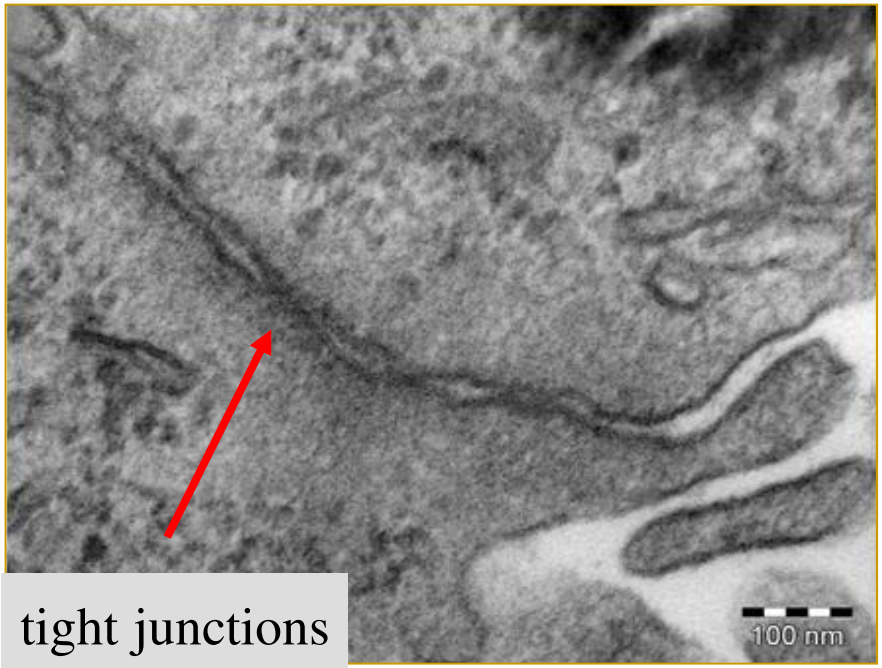


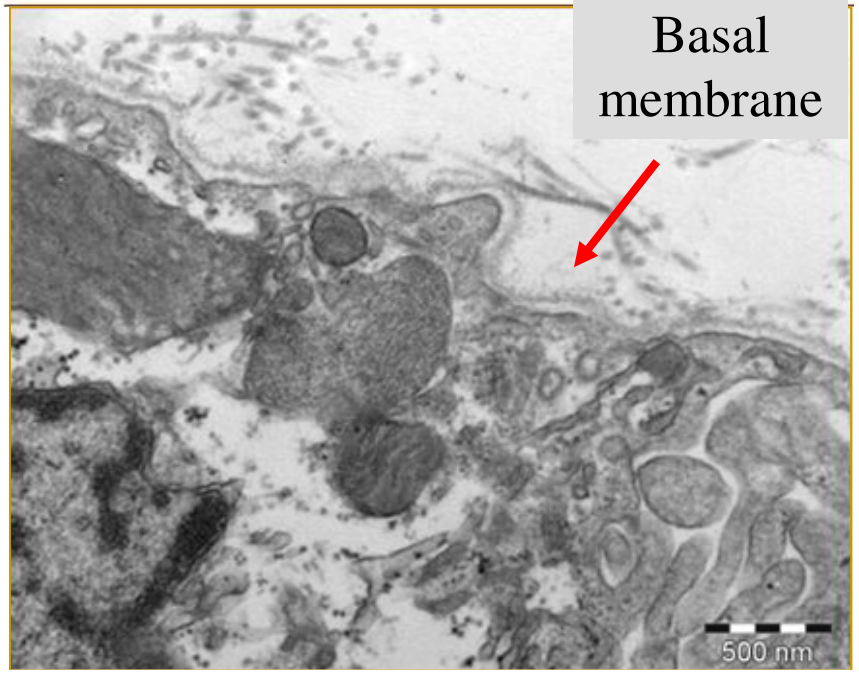
(Idelson et al., Cell Stem Cell 2009)

Electron microscopy

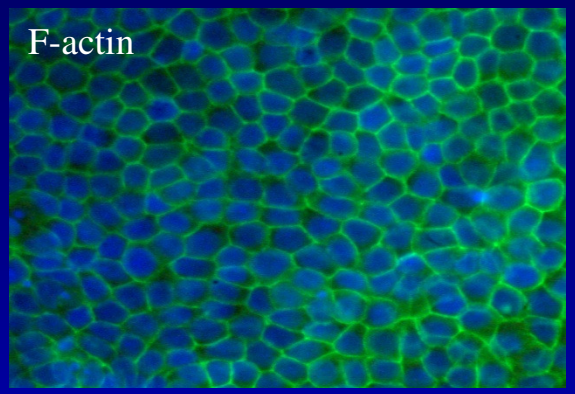


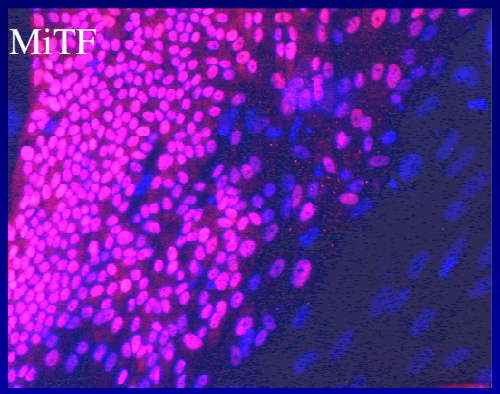


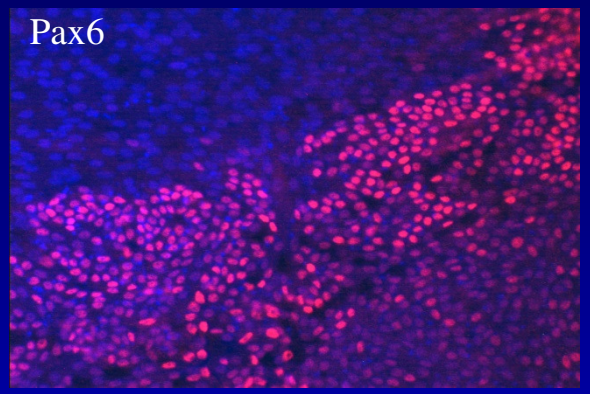


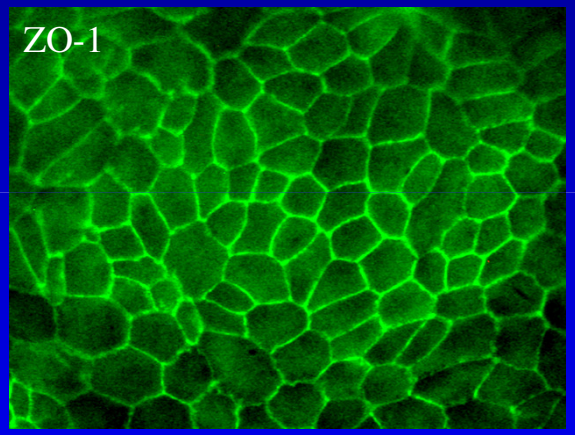


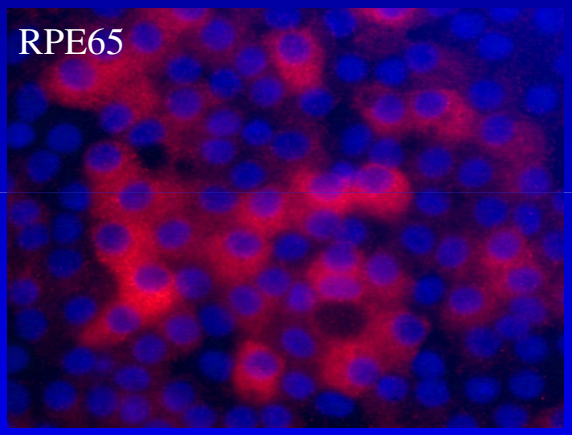
Immunostaining for RPE markers in-vitro

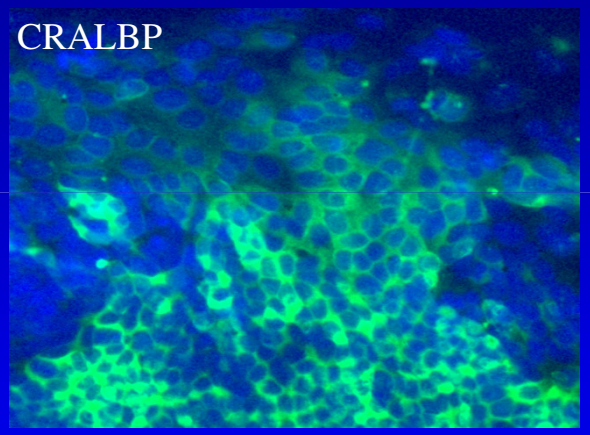


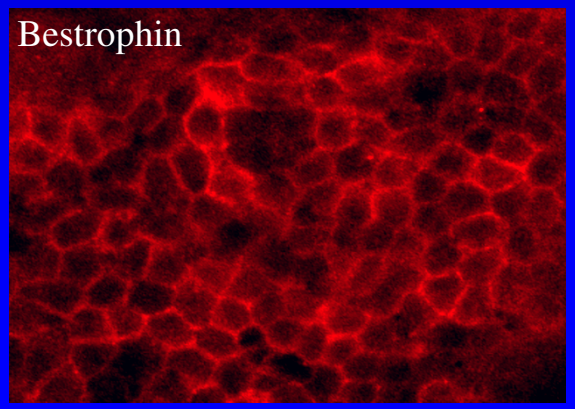


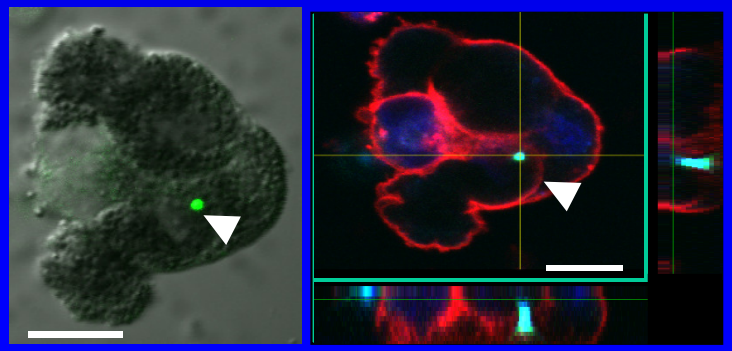








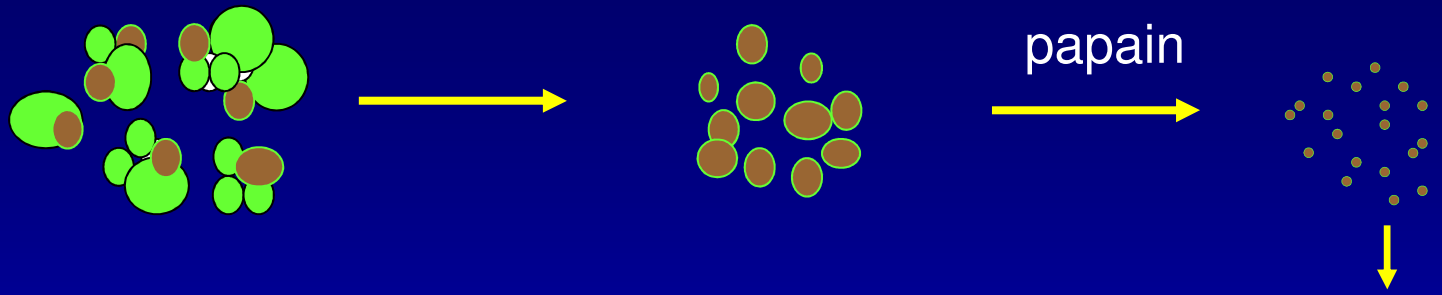


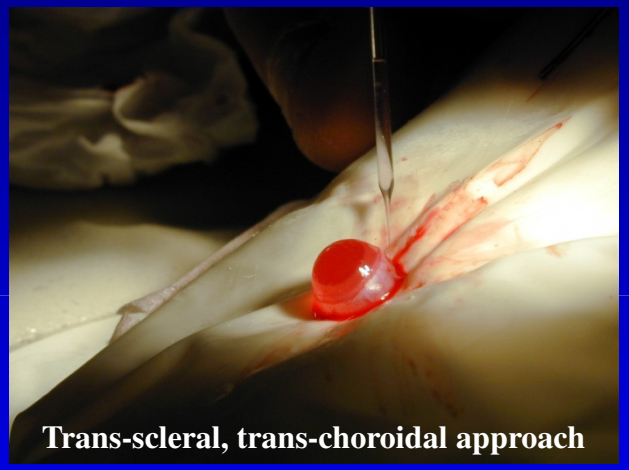


Fluorescent latex beads

Idelson et al., Cell Stem Cell 2009

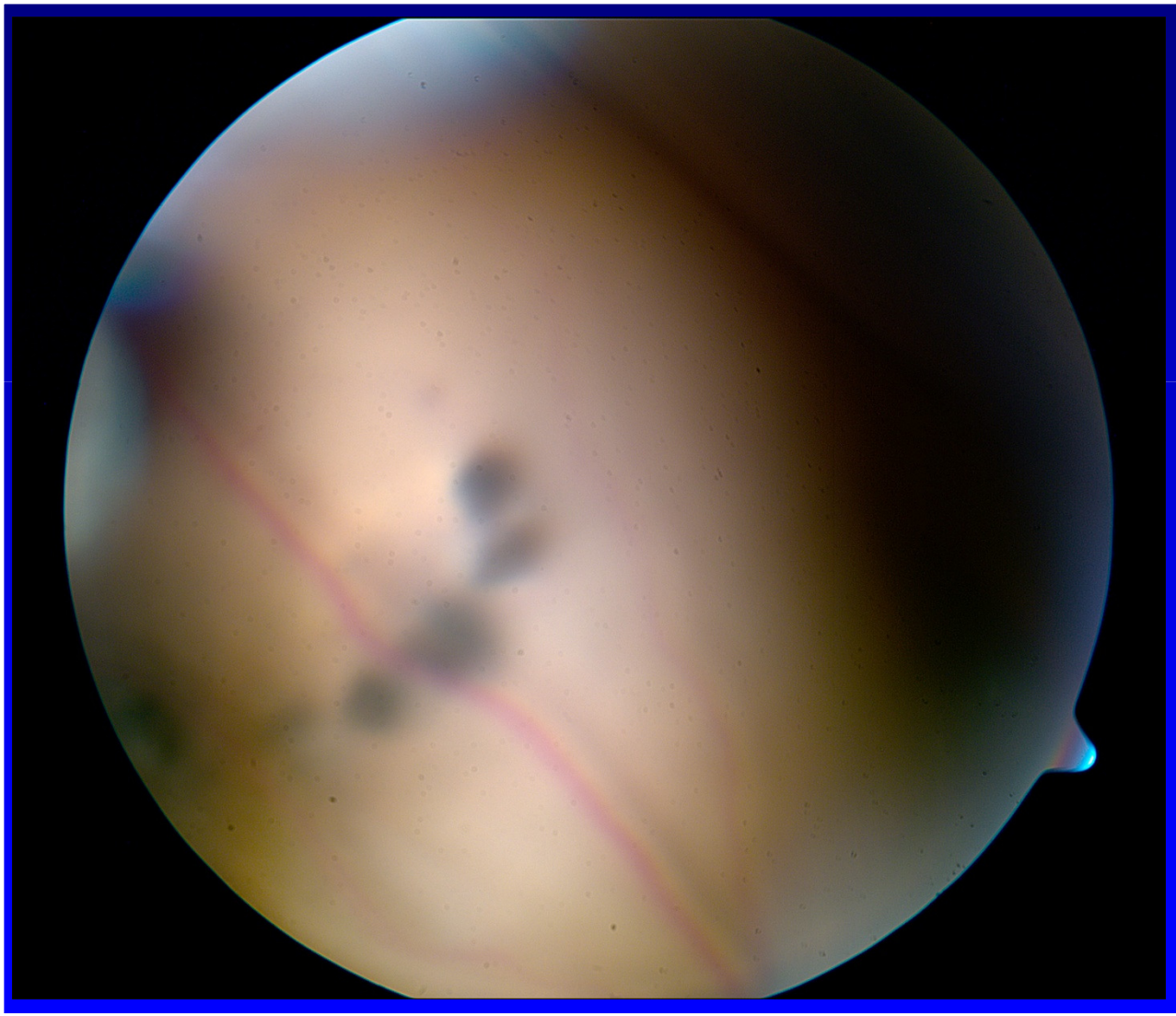
In-vivo transplantation of RPE-like cells derived from hESCs

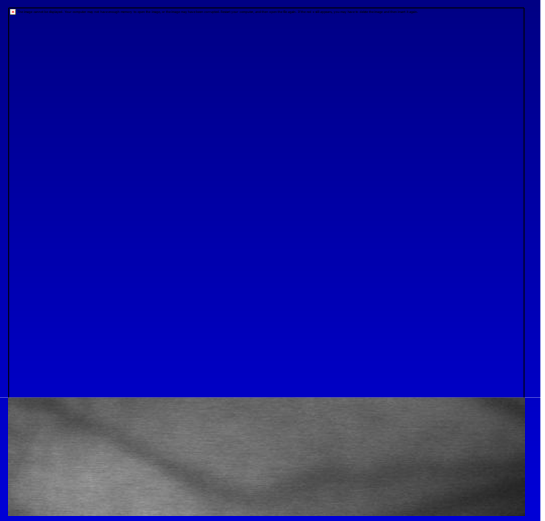


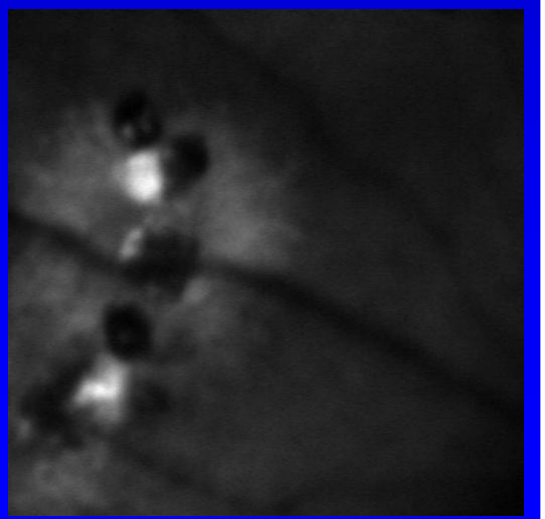


Royal College of Surgeons (RCS) rats with a mutation in the MERTK gene – a model of retinal degeneration caused by dysfunction of the RPE

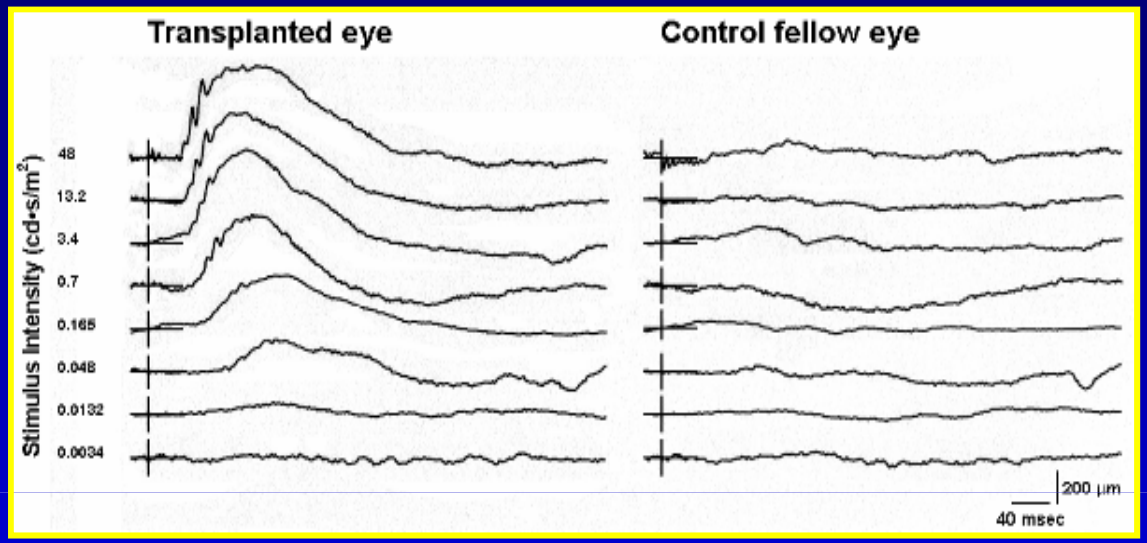
In-vivo imaging of transplanted pigmented cells in RCS rat eye

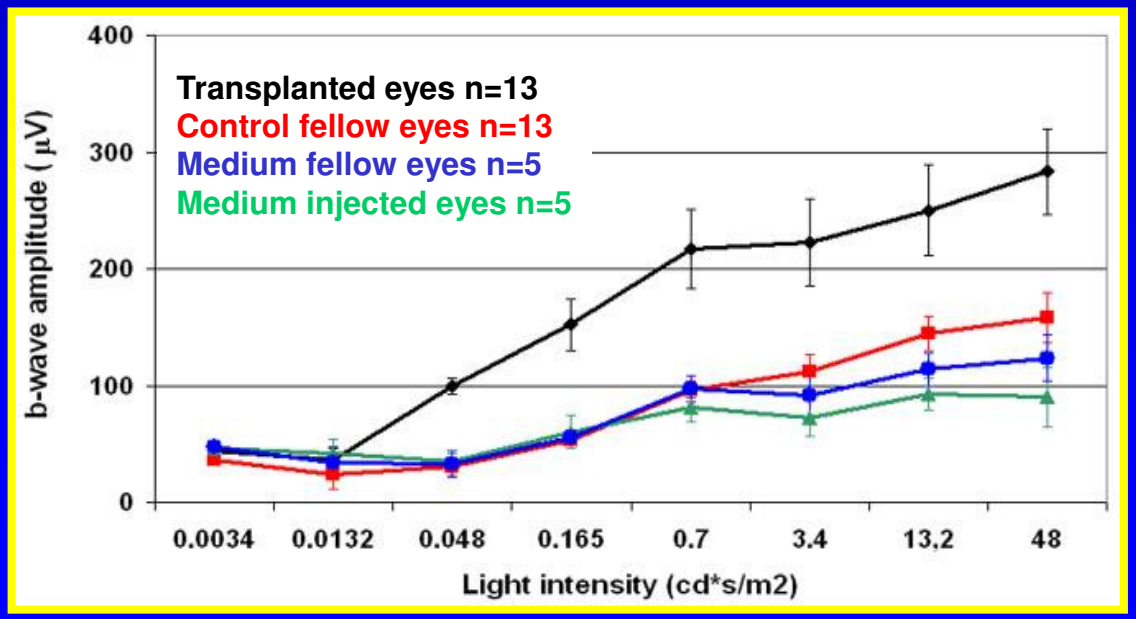






Transplanted Eyes Show Functional Rescue

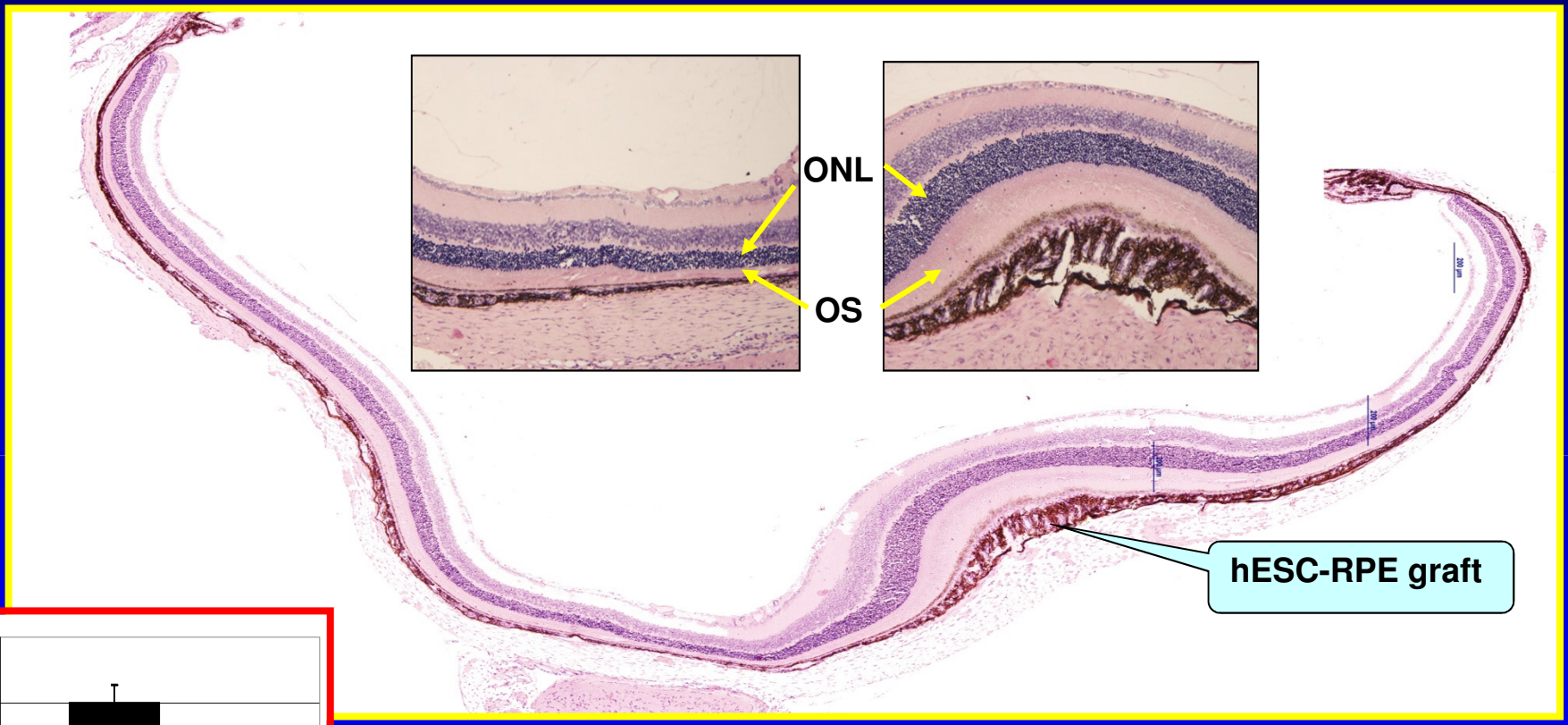


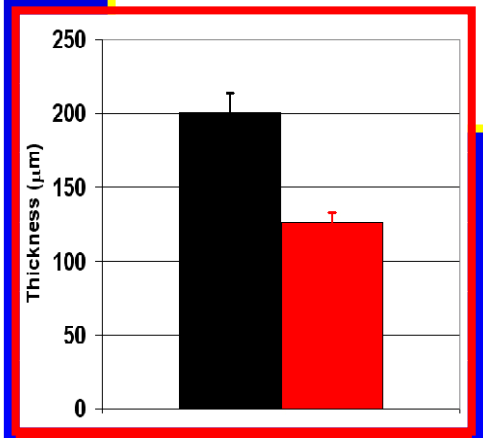


(Idelson et al., Cell Stem Cell 2009)

8 weeks

Structural Rescue of Host Retina

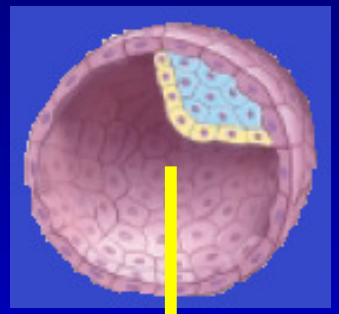


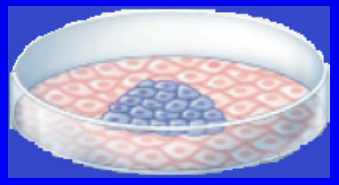


Total retinal thickness

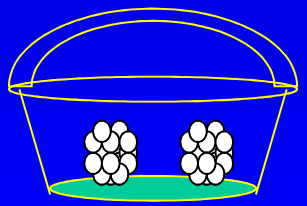
(Idelson et al., Cell Stem Cell 2009)

Road map for preclinical development of hESCs for transplantation in neurological disorders



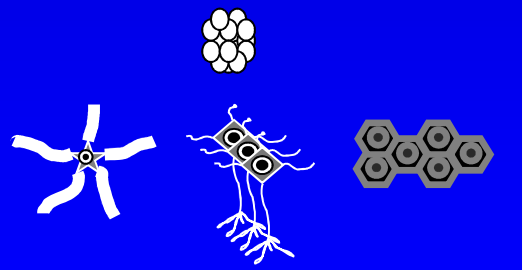


Controlled defined process



Trophic effect
Host protection

Regeneration

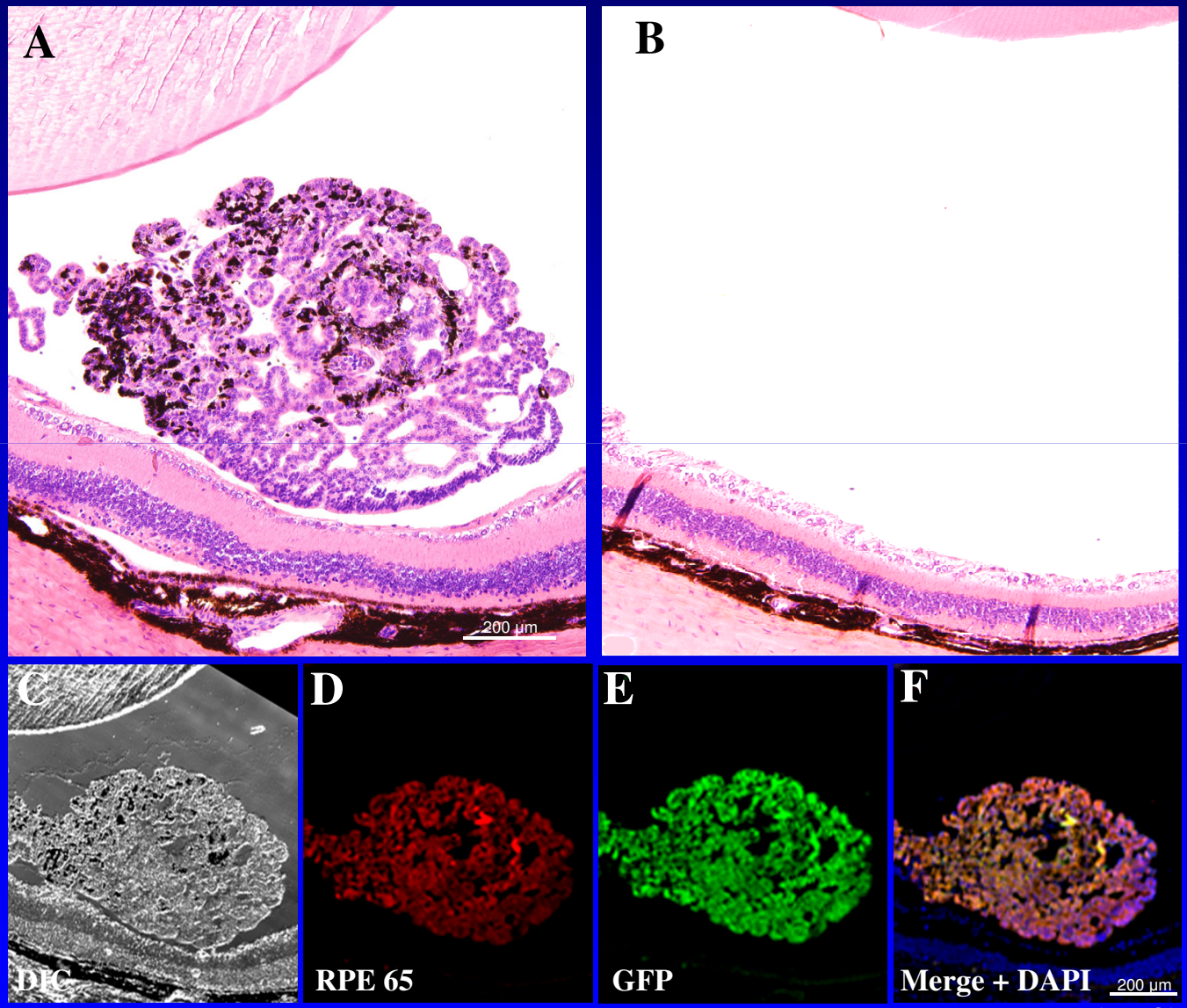


Clinical grade hES cells

Neural Precursors

Neural Progenitors / Cells

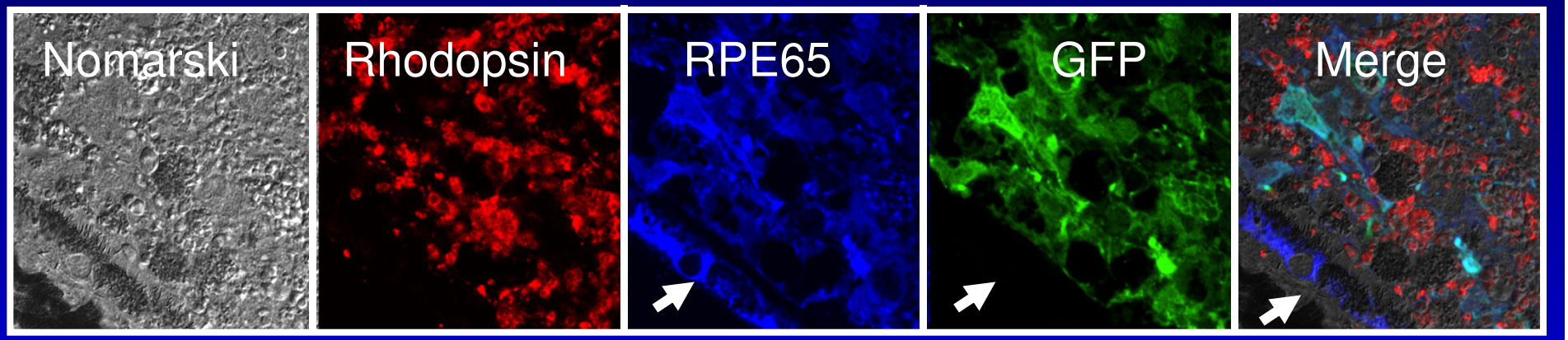
Lack of retinal rescue following intra vitreal hESC-derived RPE grafting

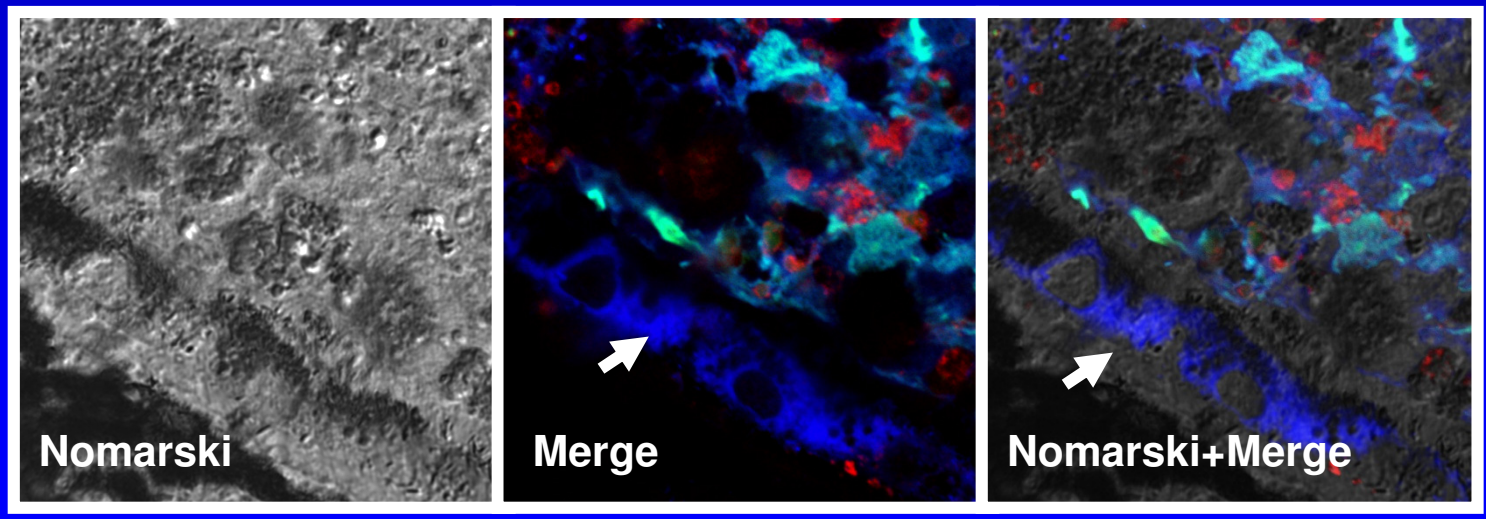


(n=9)

(Idelson et al., Cell Stem Cell 2009)

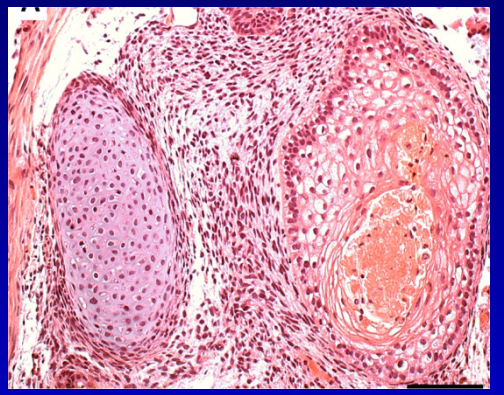
Rhodopsin within transplanted RPE-like cells supports phagocytic activity





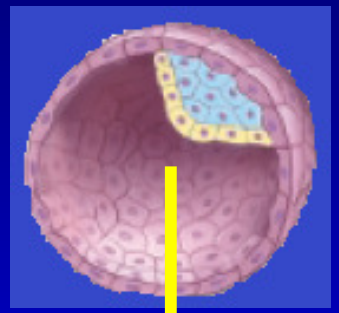
Arrows point to host RPE which expresses RPE65 but does not contain rhodopsin

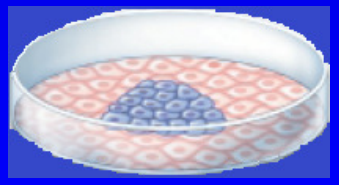
Safety



- No teratomas
- No evidence of non-neural tissues or neural rosette tumors
- Long term studies are required

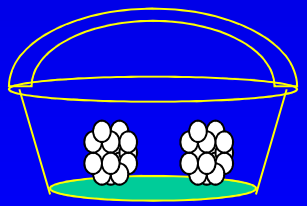
Road map for preclinical development of hESCs for transplantation in neurological and retinal disorders



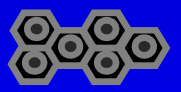


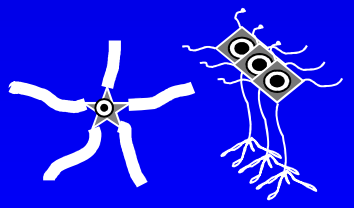
Clinical grade hES cells

Controlled defined process

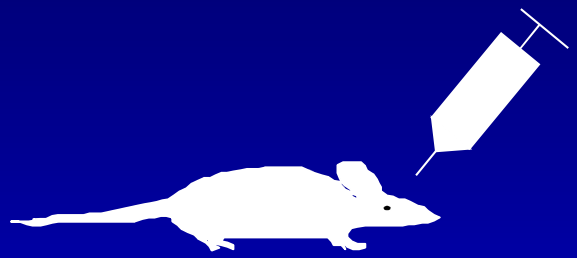


Neural Precursors





Neural Progenitors / Cells

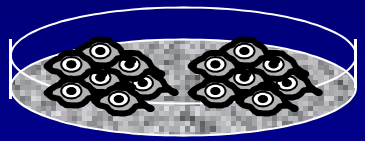


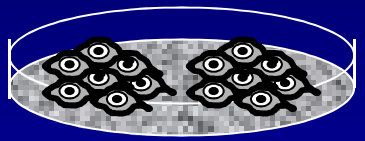
Animal models

HUMAN ES CELS – FROM THE LAB TO THE CLINIC

Basic research

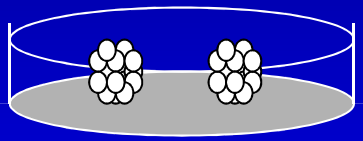
Translational research

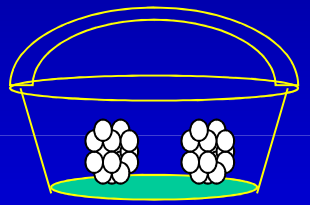




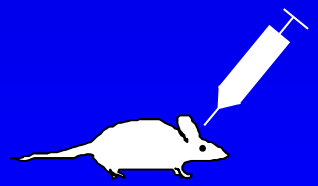
Clinical grade

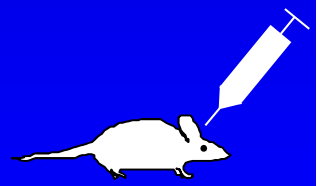
Controlled defined culture conditions

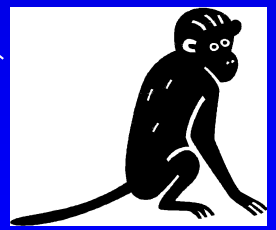


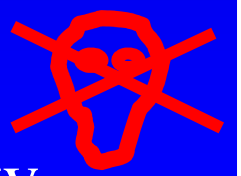


Bulk cultures
Storage









Validation of efficacy and safety

CellCure NeuroSciences Ltd.

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