Professor Richard Boyd

Monash University

Australia

Professor Martin Pera

Stem Cell Sciences

University of Melbourne

The Practical use of Stem Cell Therapy in a Clinical Setting - Concurrent Workshop

Saturday, 22 June 2013 Start 4:30pm Duration: 60mins Kandinsky

Stem Cells and Regenerative Medicine: Prospects and Challenges

Martin Pera
University of Melbourne
Walter and Eliza Hall Institute of Medical Research
Florey Neurosciences Institute

STEMCELLS AUSTRALIA

Stem Cells and Regenerative Medicine

- A new and rapidly growing field of biomedical research with widespread ramifications
- A potentially disruptive set of technologies whose future implications are difficult to predict
- A highly interdisciplinary field
- Scientific, clinical and economic basis for product development and health care delivery in this sector is evolving and remains largely undefined

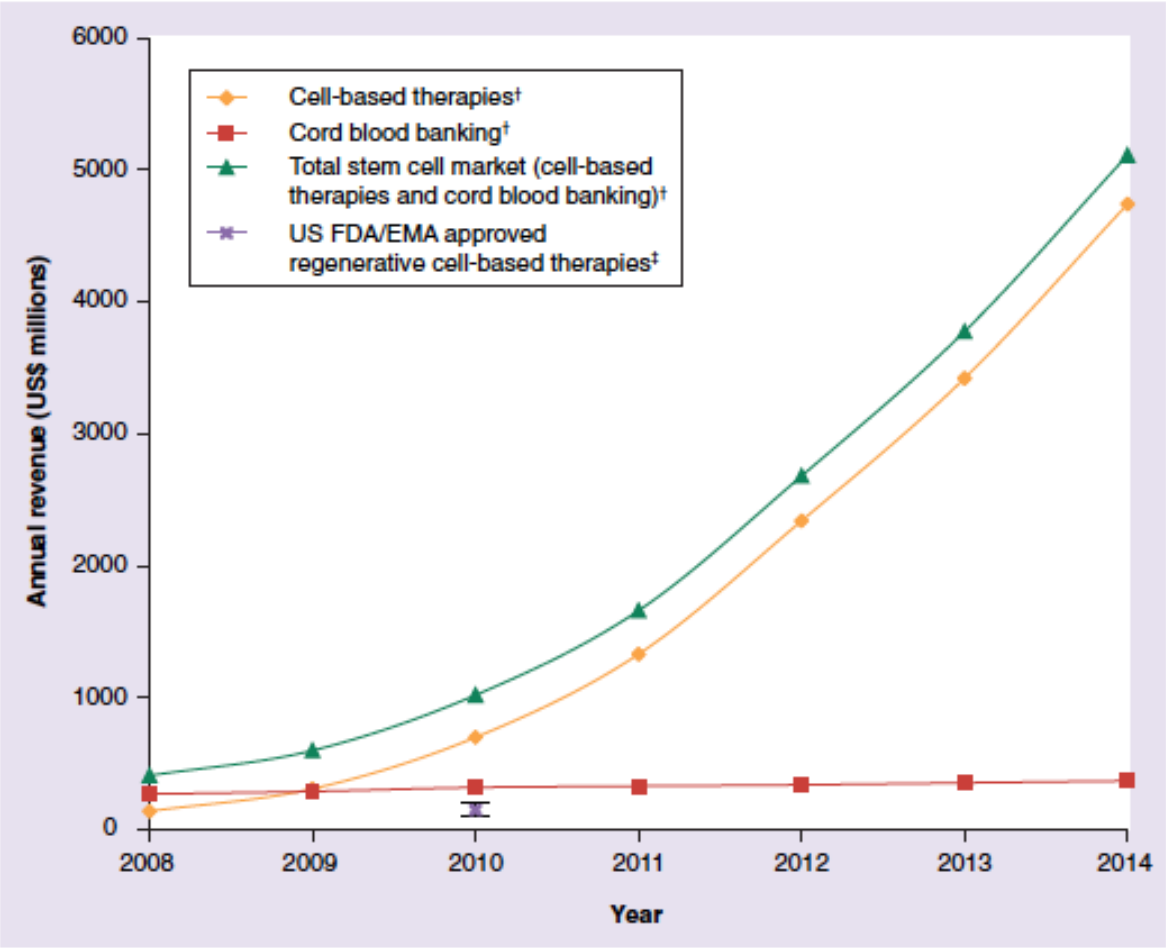


Figure 3. Cell therapy industry market (2008–2014). Graph showing the estimated CTI revenues from 2008–2014†, together with the value of the regenerative cell therapy market estimated for 2009–2010, based on actual sales of US FDA/EMA approved products‡.
CTI: Cell therapy industry.
†Data taken from [15].
‡Data taken from [16].

Mason et al. Regen Med 6: 265, 2011

Cell Therapy: A Billion Dollar Industry

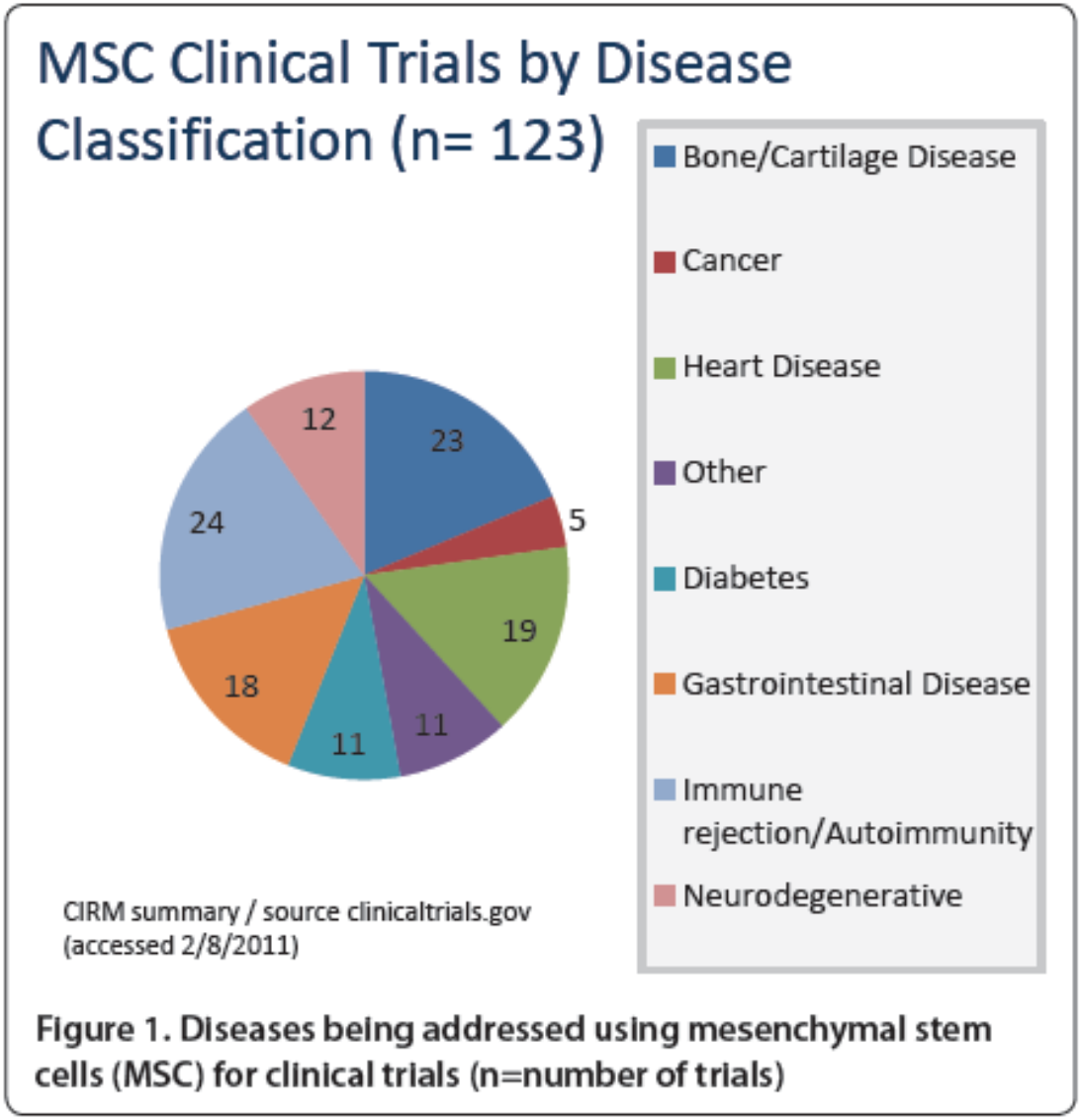
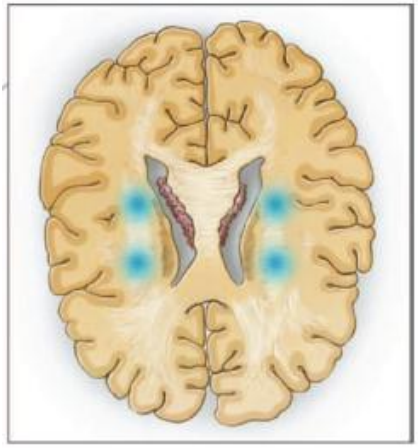


Figure 1. Diseases being addressed using mesenchymal stem cells (MSC) for clinical trials (n=number of trials)

2500 Clinical Trials
in the past ten years
50% Phase 2-3

Trounson et al. BMC Medicine 9: 52, 2011



Neural Stem Cell Transplant in Pelizaeus-Merzbacher Disease, A Demyelinating Disorder Caused by Deficiency in Proteolipid Protein 1

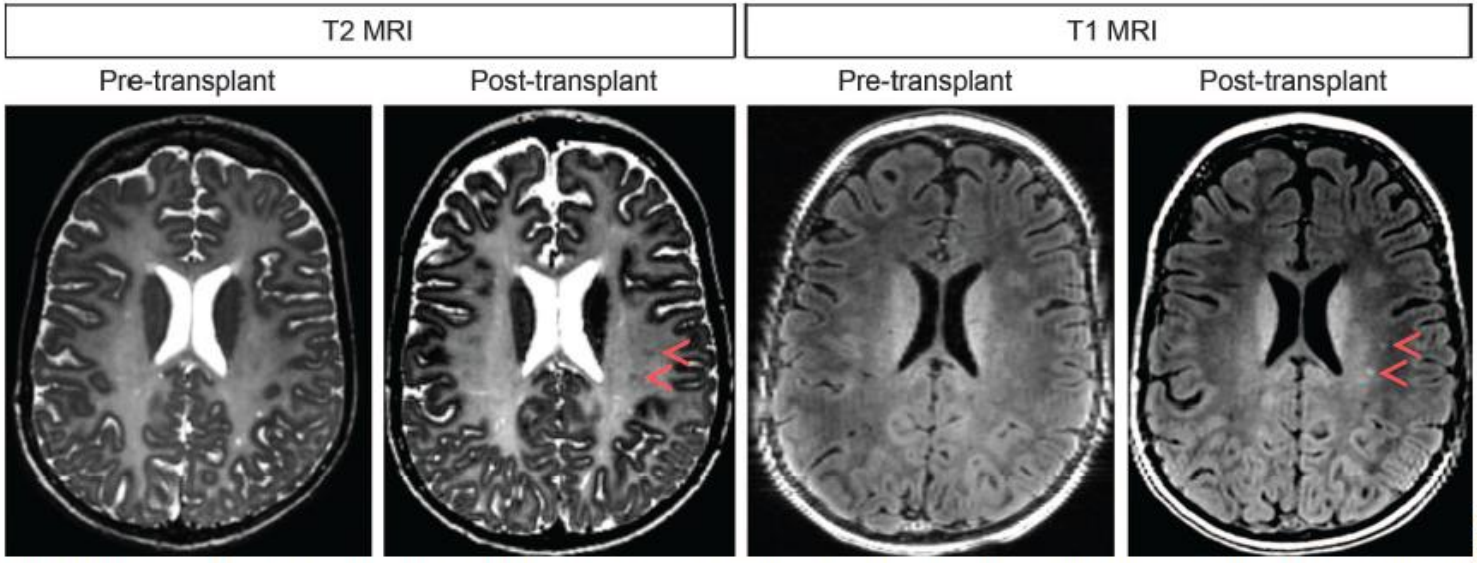


Fig. 4. MRI images at baseline (pretransplant) and 12 months after transplant for subject 2. Small ovoid areas of T2 hypointensity and T1 hyperintensity (red arrowheads) were not present before transplantation and then gradually became visible after transplant. These areas do not represent small cysts because decreasing T2 hyperintensity is observed.

Science Translational Medicine
AAAS

Neural Stem Cell Engraftment and Myelination in the Human Brain

Nalin Gupta *et al.*

Sci Transl Med **4**, 155ra137 (2012);
DOI: 10.1126/scitranslmed.3004373

Challenges to Clinical Translation

1-What is the right business model for development of cell based therapies?

Geron Corporation

- Largest in sector
- Very strong IP portfolio
- $100 millions invested in stem cell programs
- Strong science
- First Phase 1 trial for ES derived product in spinal cord injury

Geron Corporation Ends Stem Cell Program

- Former CEO Thomas B Okarma in 2009: US authorities' granting of approval to start the trial marked "the dawn of a new era in medical therapeutics" which placed Geron "at the forefront of the medical revolution”
- $181 million in cash and investments at Sept 30
- Stem cell program $25 million pa; $25 million loan from CIRM
- Two cancer drugs in Phase 2 trials

Some Australian Stem Cell Biotechs

- ES Cell International/Biotime
- Stem Cell Sciences/Stem Cells Inc
- Bresagen/Viacyte
- Mesoblast

- *Promising Technology vs. Having a Product*

New Models for Funding Research

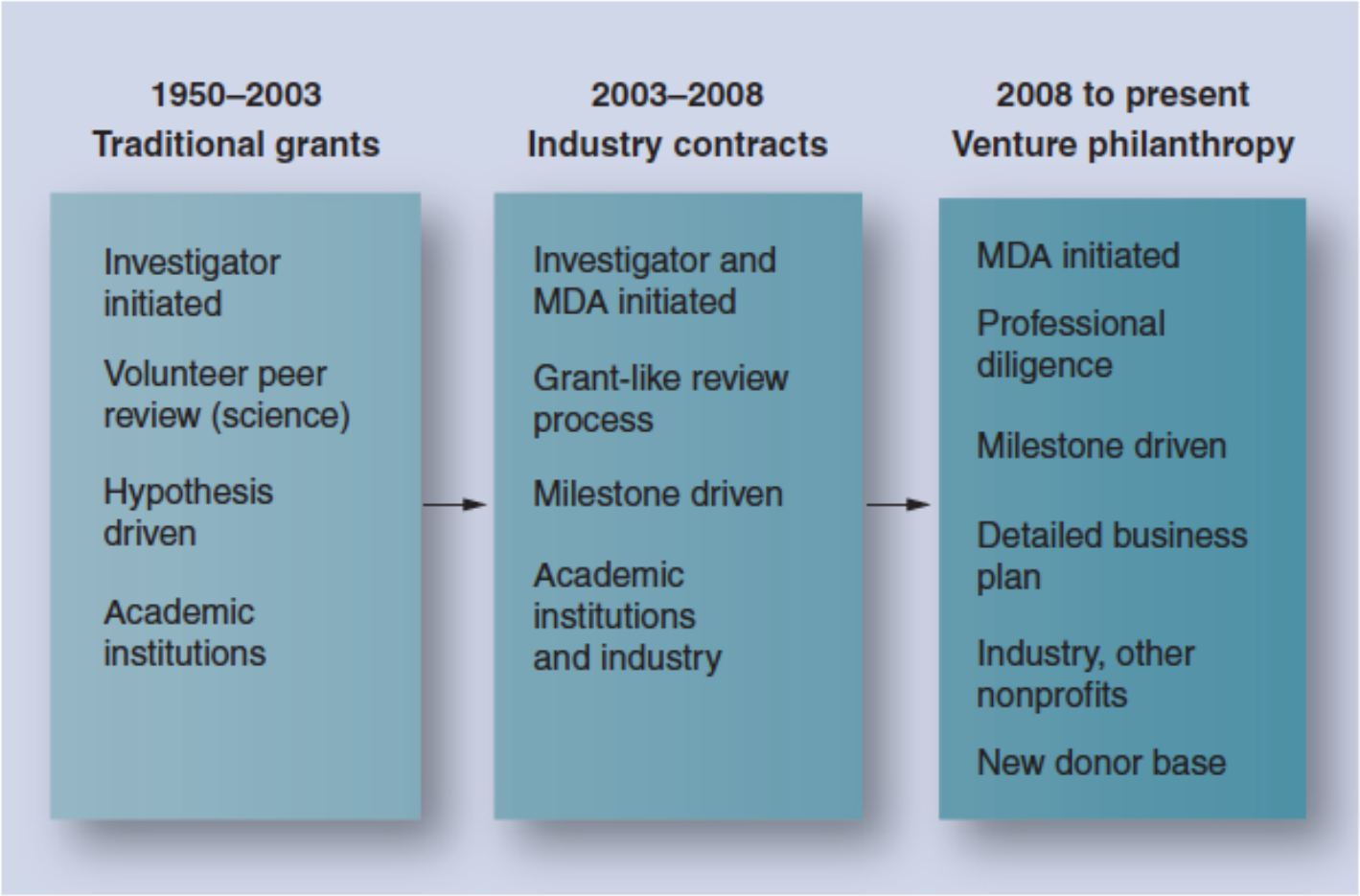
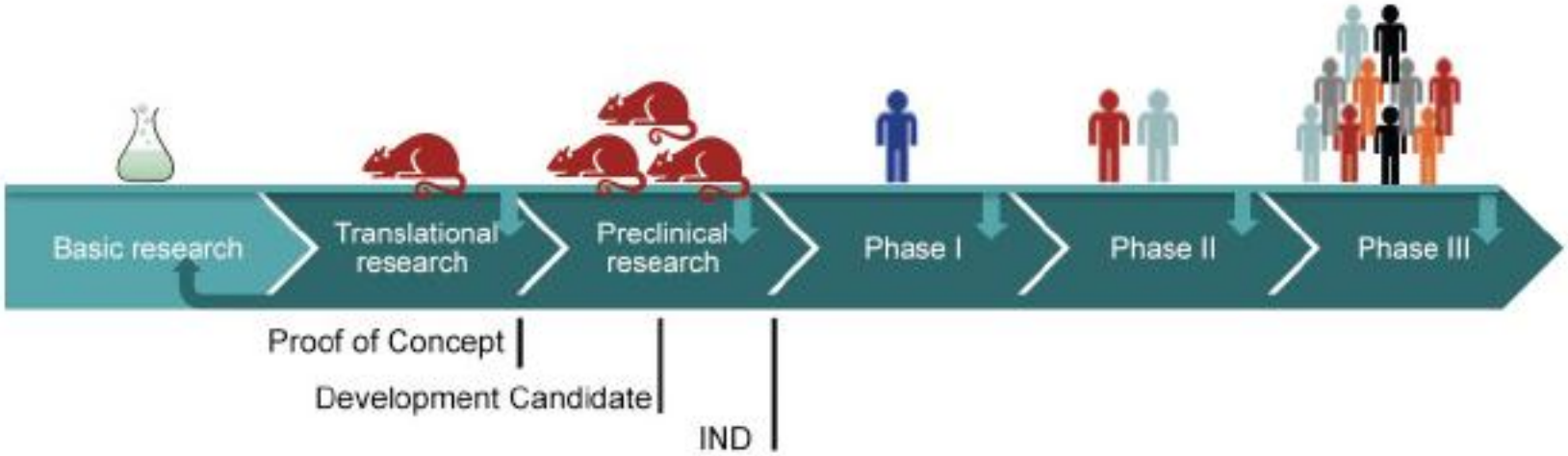


Figure 2. Evolution of a funding program.
MDA: Muscular Dystrophy Association.
Reproduced with permission from [105].

Reeve, BC Reg Med 7: 237,2012

California Institute of Regenerative Medicine: A Unique Initiative in Research Funding



A major goal of CIRM strategy is to drive translation of discoveries into treatments, and a key element of this approach is to bridge the valley of death.

CIRM Therapy Research in Progress

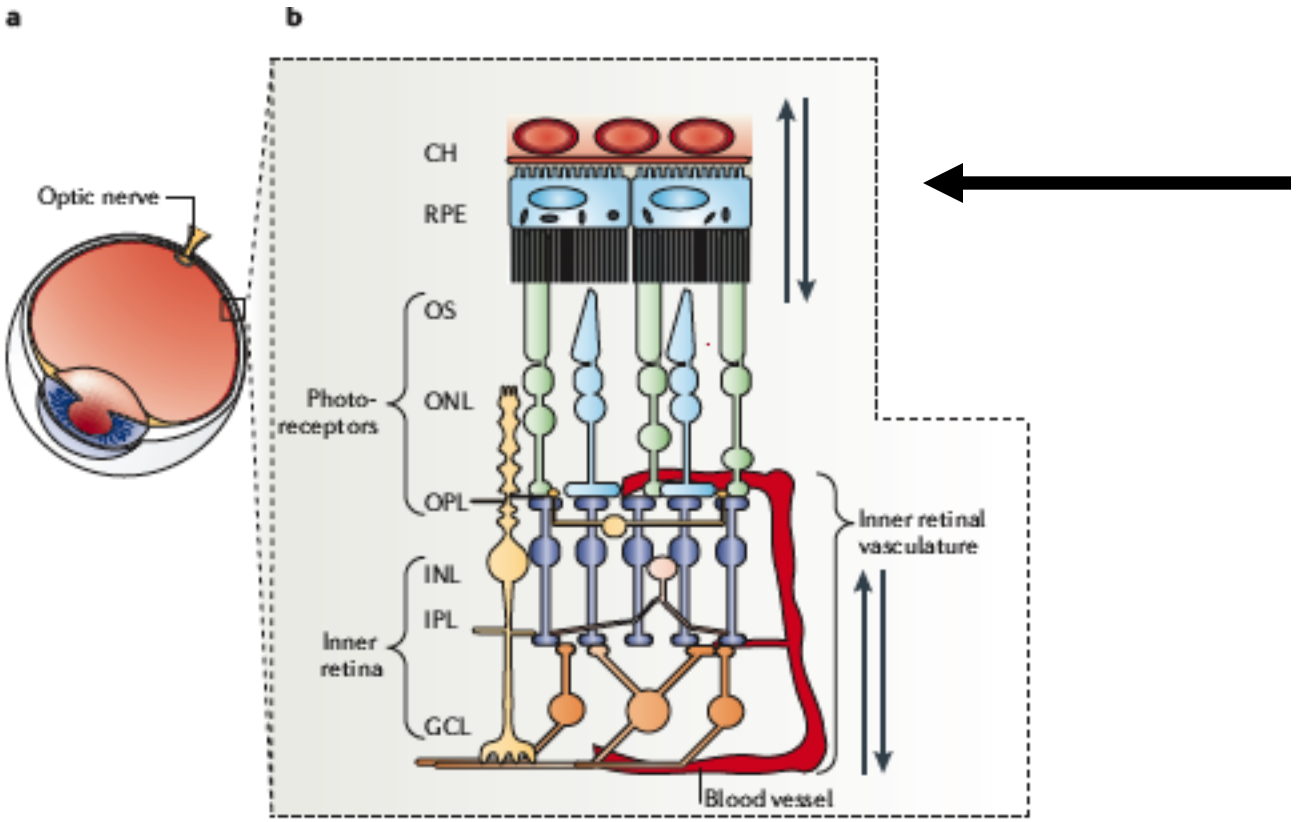
Disease	Millions	Proof of Concept / Development Candidate / IND / Phase I/II
Blood Disease		Project goal
Fanconi anemia	$6.6	
Sickle cell disease	$9.2	
Bone & Cartilage conditions		
Arthritis (osteoarthritis)	$3.1	
	$6.8	
Osteoporosis	$1.9	
Spinal fusion	$5.4	
Cancer		
Leukemia	$20	
	$20	
	$3.6	
	$3.3	
Brain Tumor	$18	
	$19.2	
	$3.4	
Solid Tumor (ovarian, colon)	$20	
Diabetes & Complications		
Diabetes	$20	
Diabetic ulcers	$4.5	
Eye Diseases		
Macular degeneration	$15.9	
	$5.9	
	$5.5	
Retinitis Pigmentosa	$3.9	
Cornea Damage	$1.7	
HIV/AIDS		
HIV/AIDS	$20	
HIV/AIDS	$14.6	
HIV/AIDS	$3.1	
Muscle & Skin conditions		
Muscular dystrophy	$2.3	
Skin disease (epidermolysis bullosa)	$11.7	

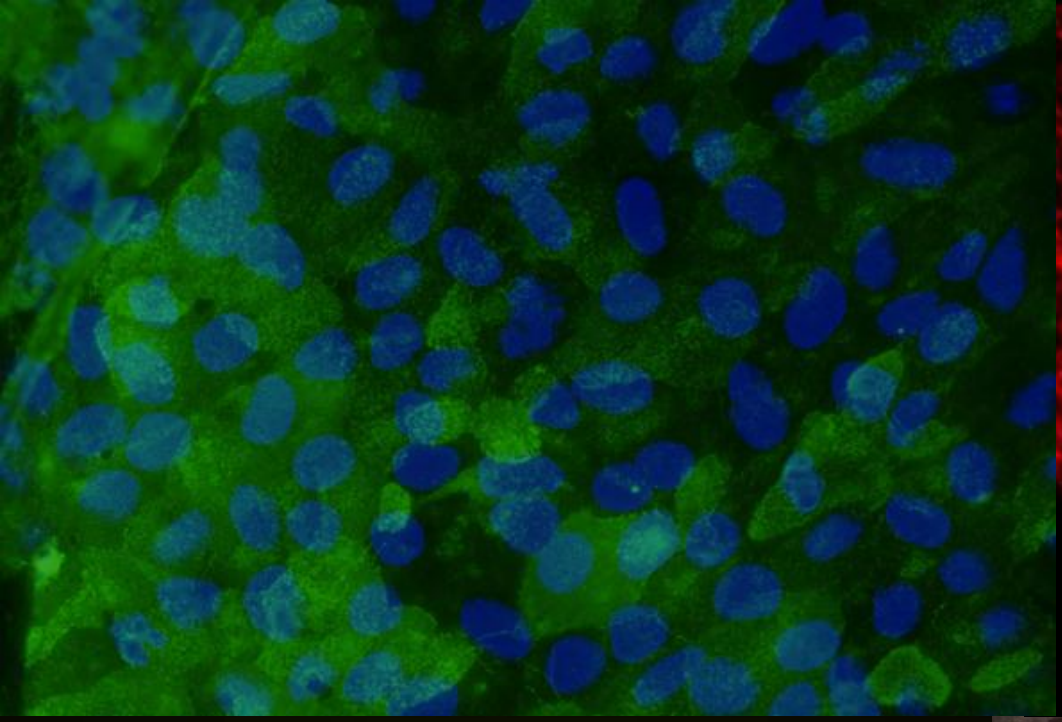
CIRM Investment in Translational and Clinical Research

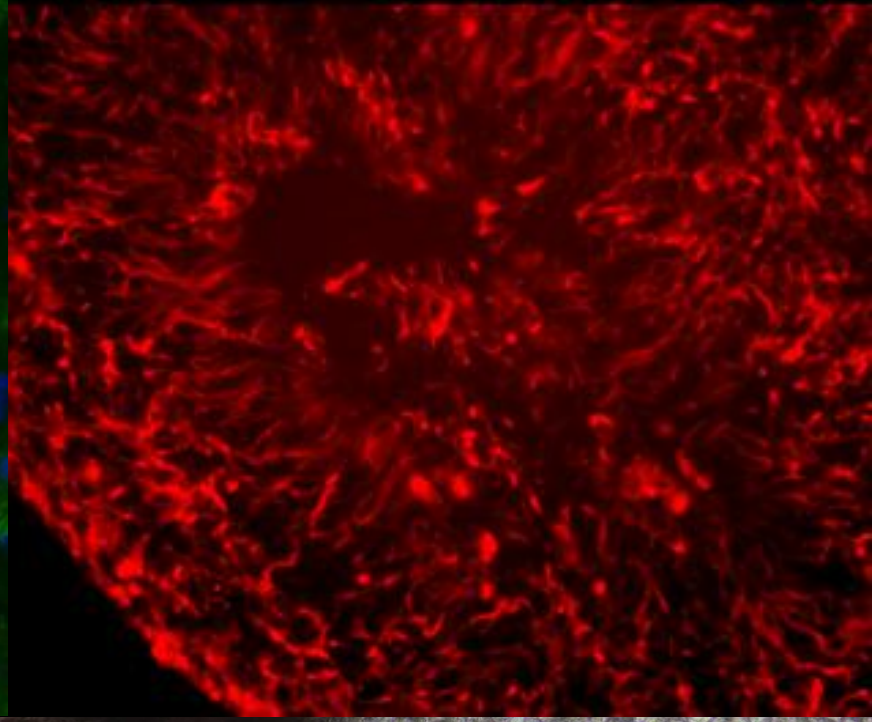
Blindness

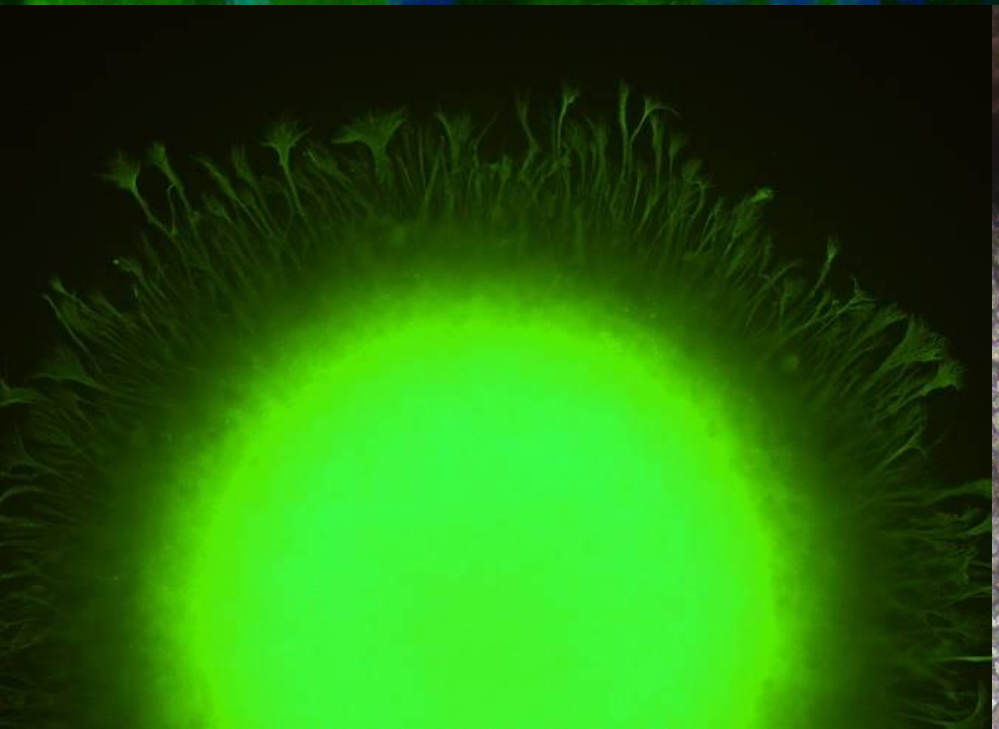
Macular degeneration is a major cause of blindness in the aging population

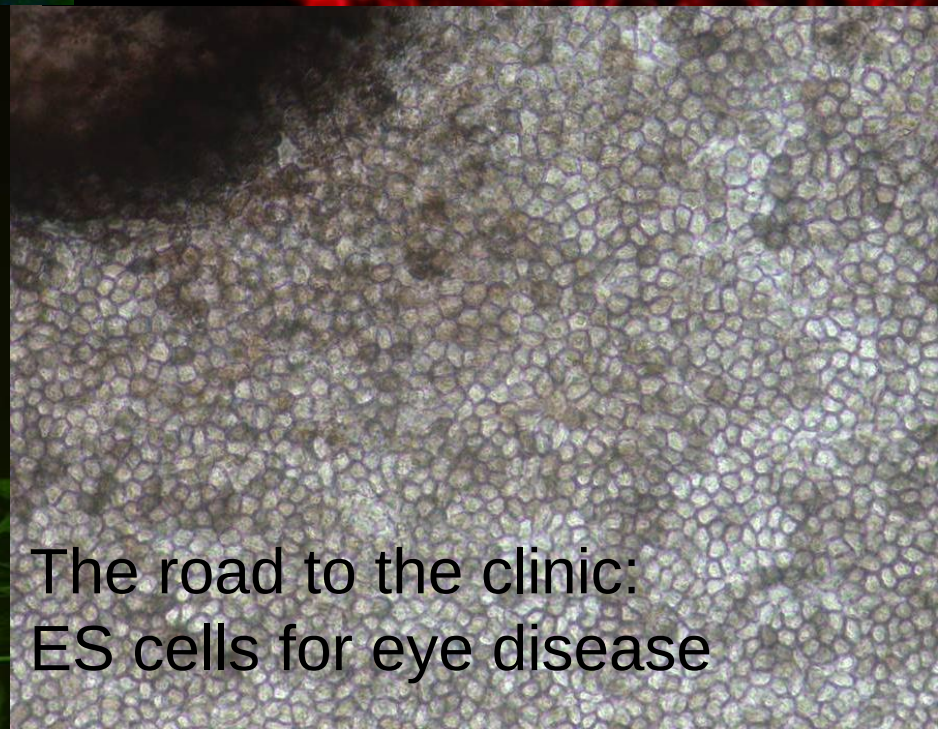
Retinal pigment epithelium and macular degeneration, a major cause of blindness











The road to the clinic:
ES cells for eye disease

Nov 98- human embryonic stem cells discovered

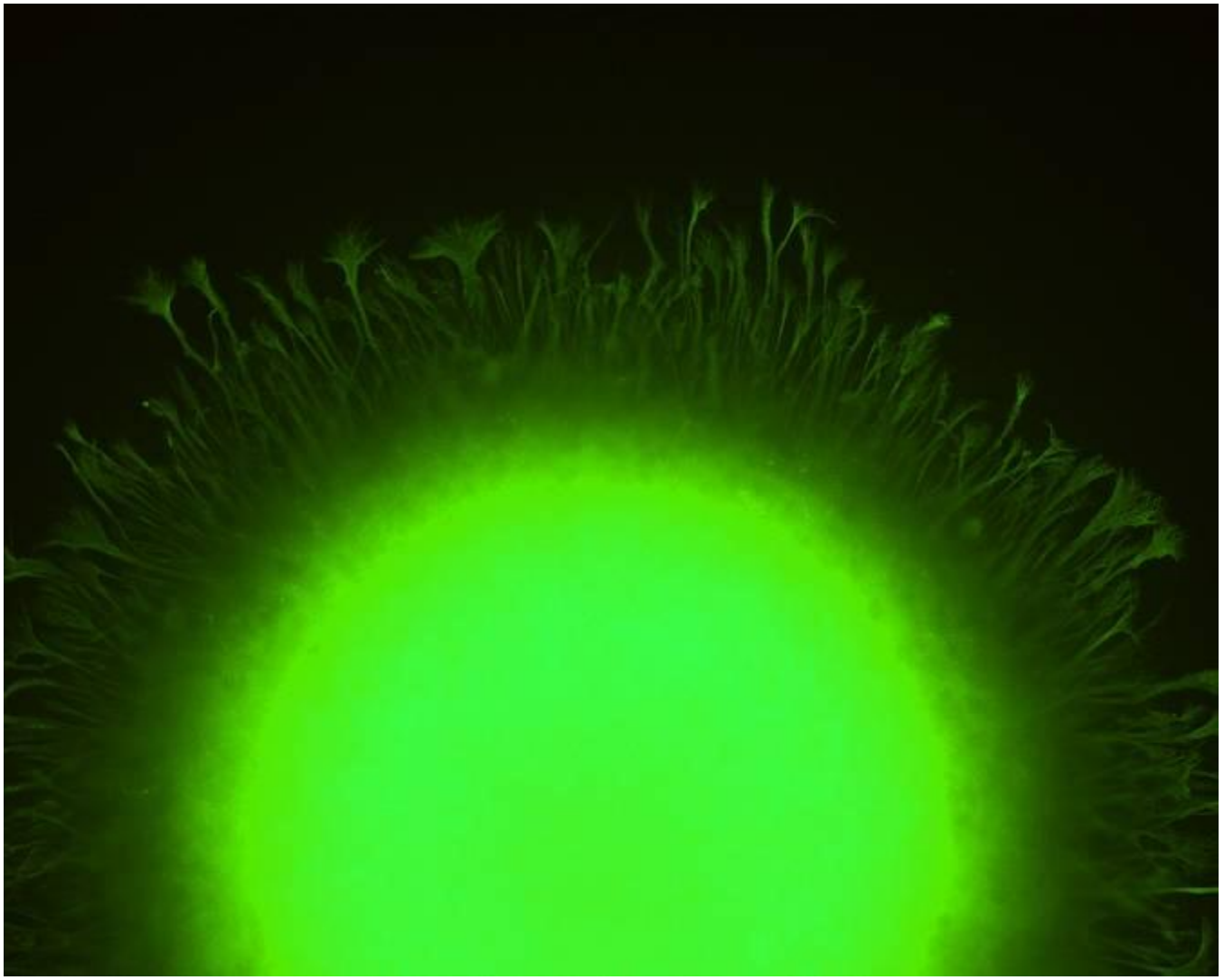
2102-First human trials of human embryonic stem cell therapeutics

Embryonic Stem Cell Lines Derived from Human Blastocysts

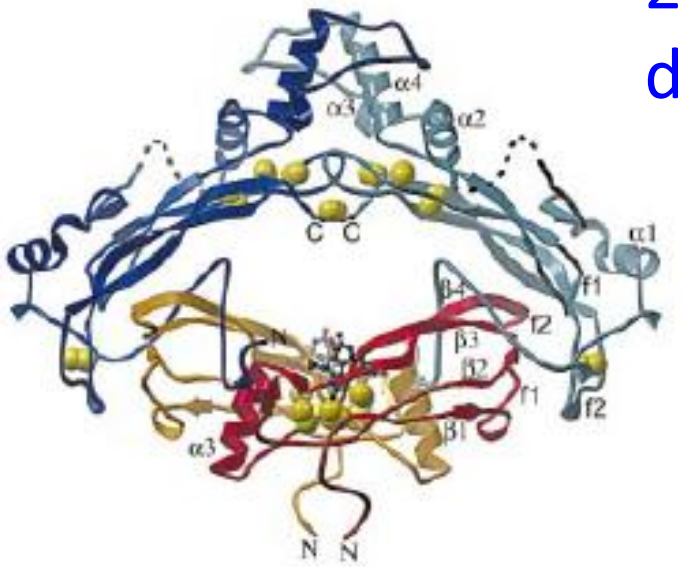
James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm). These cell lines should be useful in human developmental biology, drug discovery, and transplantation medicine.

2000- hESC can form neural tissue in vitro. The eye forms as an outgrowth of the embryonic brain

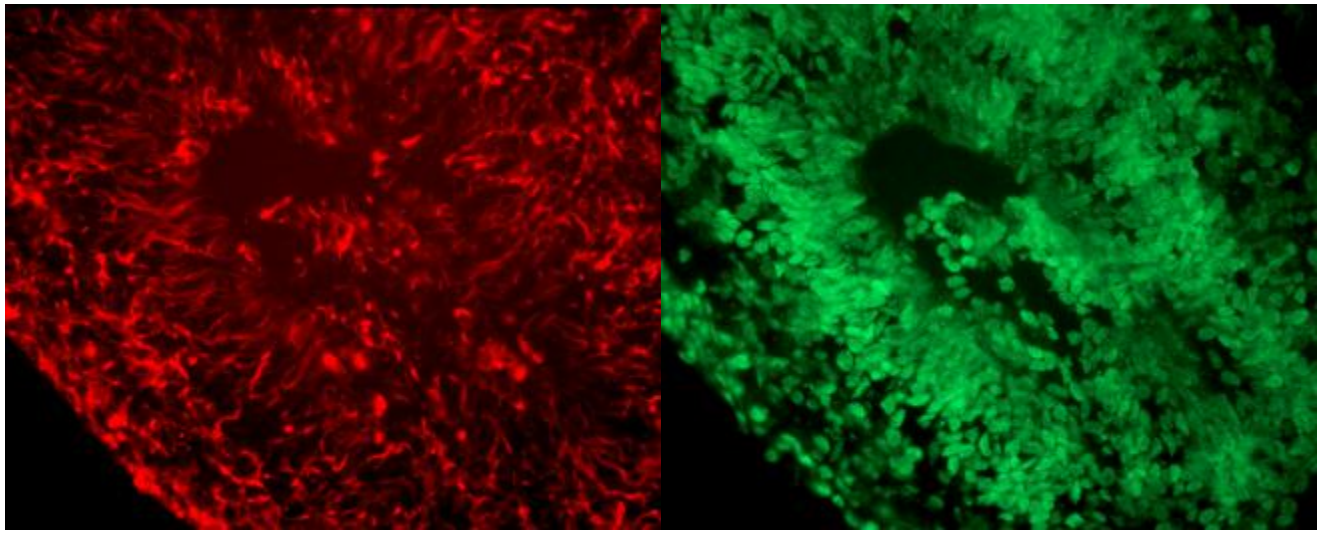


2004-directed neural differentiation

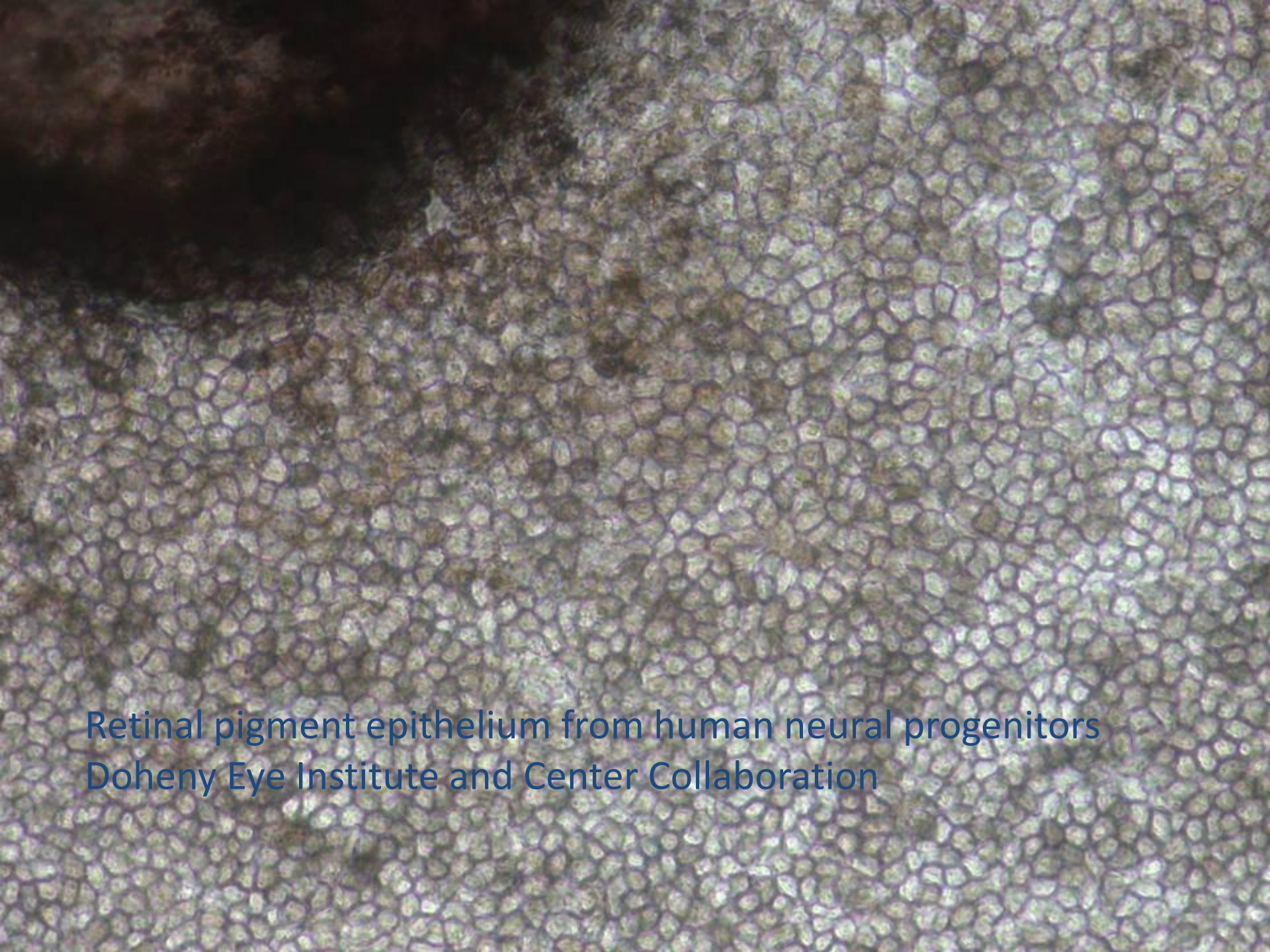


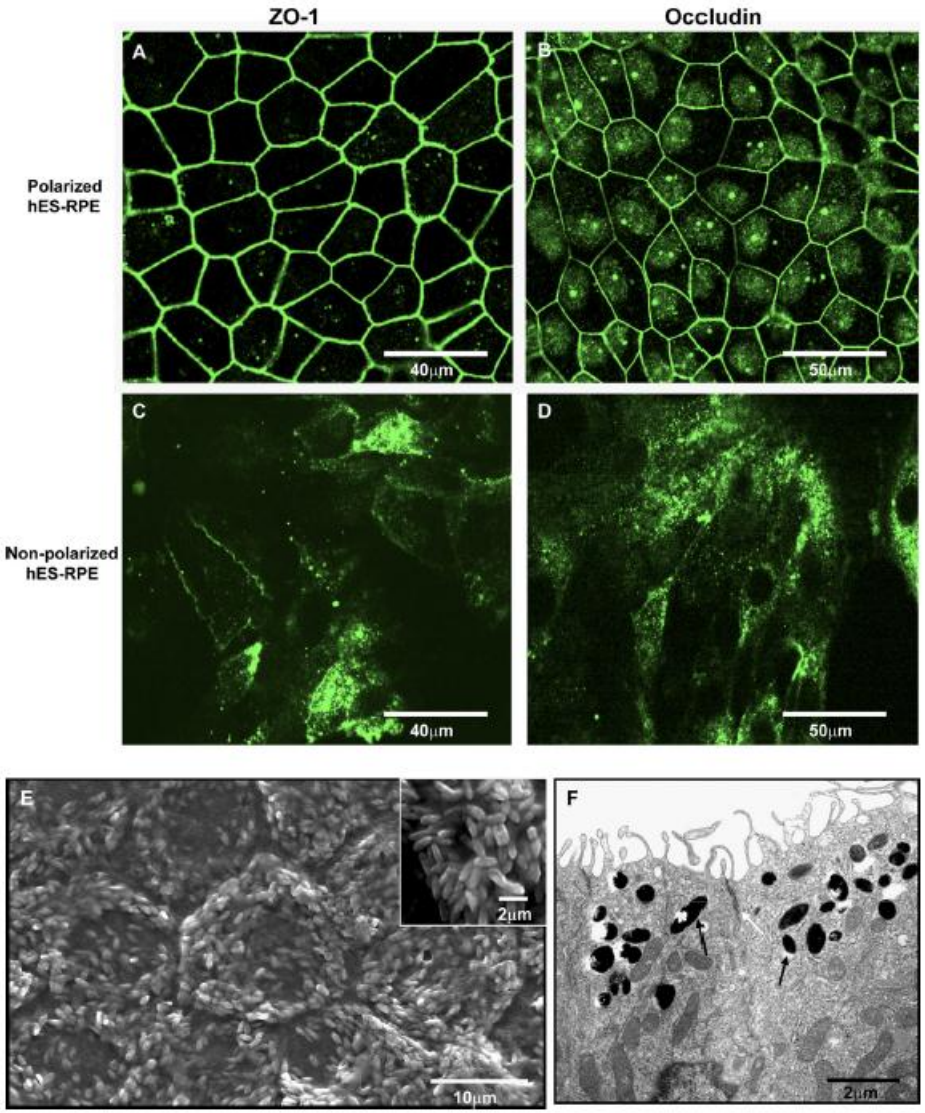
Treatment with the embryonic head inducer noggin
induces differentiation of human ES cells into primitive neural tissue
Nestin and Sox-2, markers of early neurogenesis

Groppe et al. Nature 420: 636, 2002



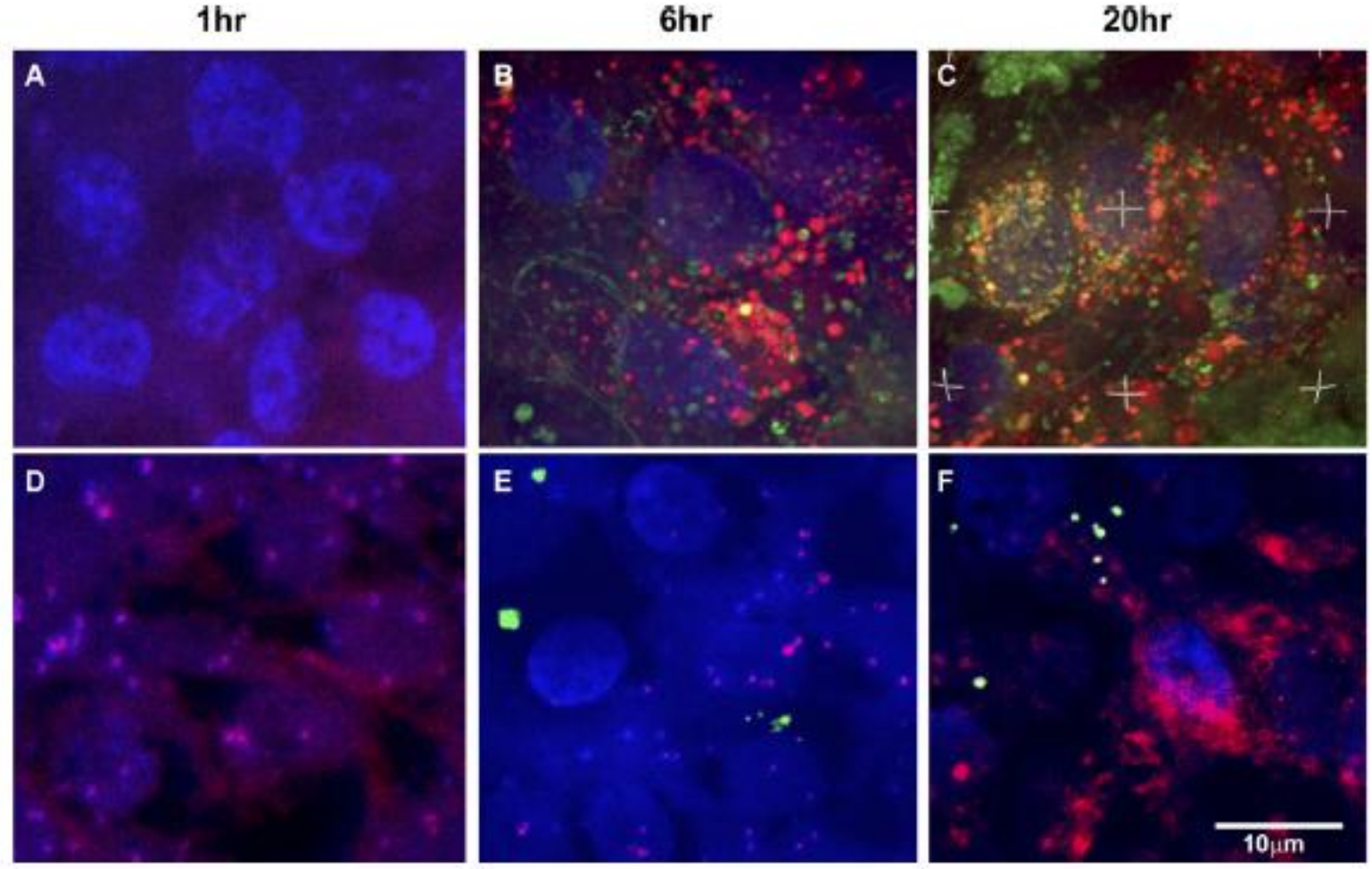
Conservation of developmental mechanisms


Retinal pigment epithelium from human neural progenitors
Doheny Eye Institute and Center Collaboration



ES-derived RPE forms a polarized epithelium

ES-derived RPE is functional: Phagocytosis of rod segments



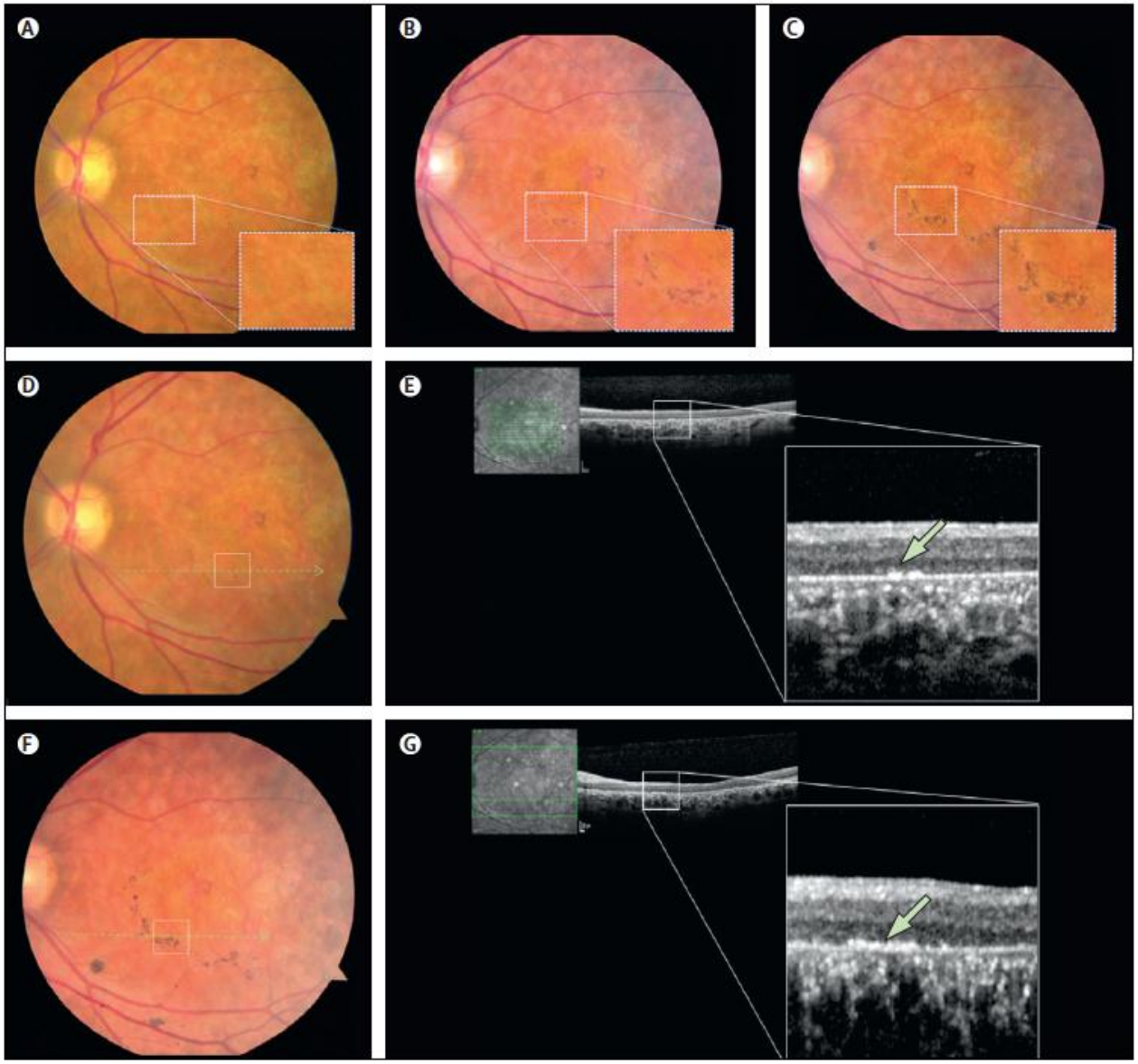
Macular Degeneration is a Promising Early Target

- Small amount of tissue to be replaced-not many cells required
- Pigment epithelium from ES cells is fully functional
- Eye is highly accessible for monitoring and intervention, imaging outstanding
- Localized immunosuppression is possible

CIRM Macular Degeneration Disease Team: The California Project to Cure Blindness- $16 million to bring the study to Phase 1 trial in four years

- USC Doheny Eye Institute (Mark Humayun, PI; David Hinton Co-PI; Vas Sadda, Biju Thomas, Martin Pera)
- UCSB Macular Degeneration and Stem Cell Centers (Dennis Clegg, Co-PI; Lincoln Johnson)
- UCL London Project to Cure Blindness (Pete Coffey, Partner PI funded by MRC)
- Caltech Biology and Chemistry (Scott Fraser, Bob Grubbs, Yu-Chong Tai)
- City of Hope Center for Biomedicine and Genetics GMP Facility (Larry Couture)

Phase 1 trials of hESC derived retinal pigment epithelium grafts in macular degeneration (ACT Trial)



Schwartz et al.
Lancet 379:, 713, 2012

Challenges to Translation

2-Tissue Matching

Induced pluripotent stem cells

provide a new approach to tissue matching for transplantation

but challenges remain

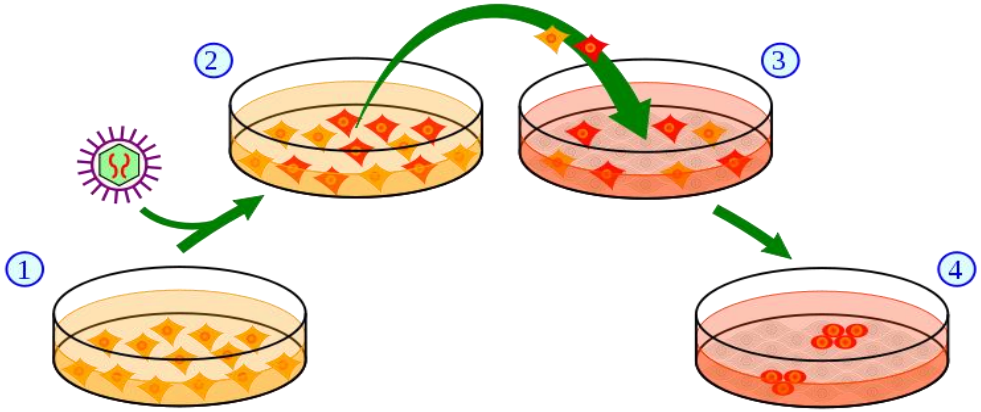
Reprogramming to Pluripotency

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

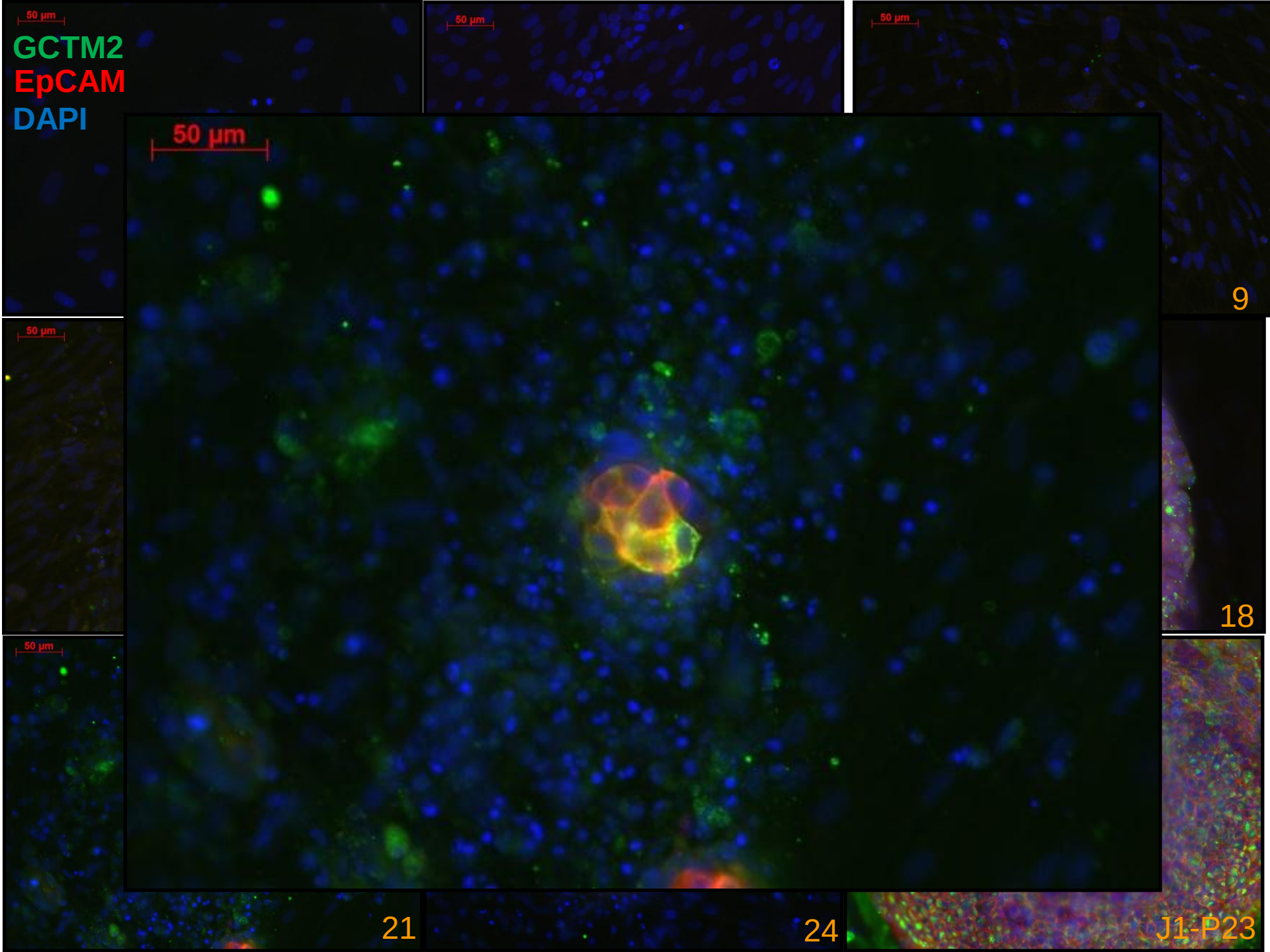
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

Induced Pluripotent Stem Cells (iPSC)

- Somatic cells “reprogrammed” by viral transfection
- ES-specific transgenes introduced into host cells Oct-4, Sox2, Klf-4, c-Myc
- Subset of cells: ES-like colonies = **iPS cells**
- Avoids use of embryos

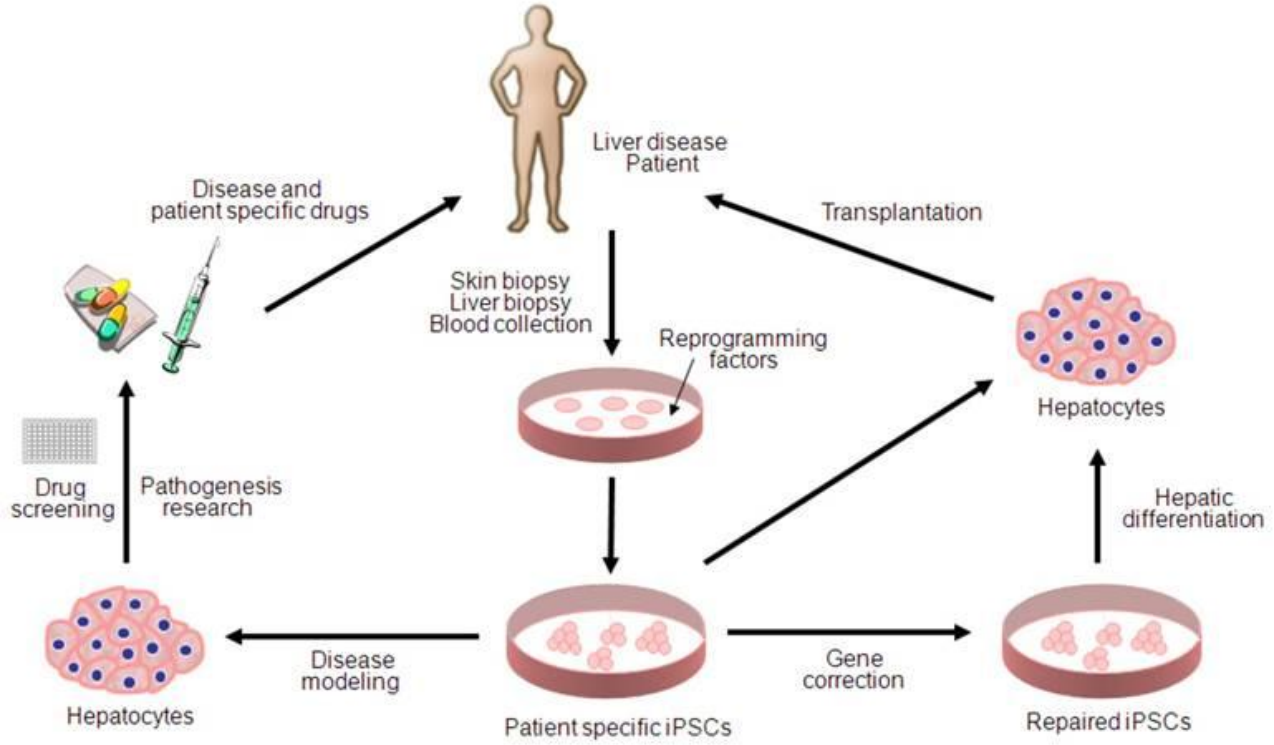


Yamanaka S et al *Cell* 2006 (mouse) and 2007 (human)

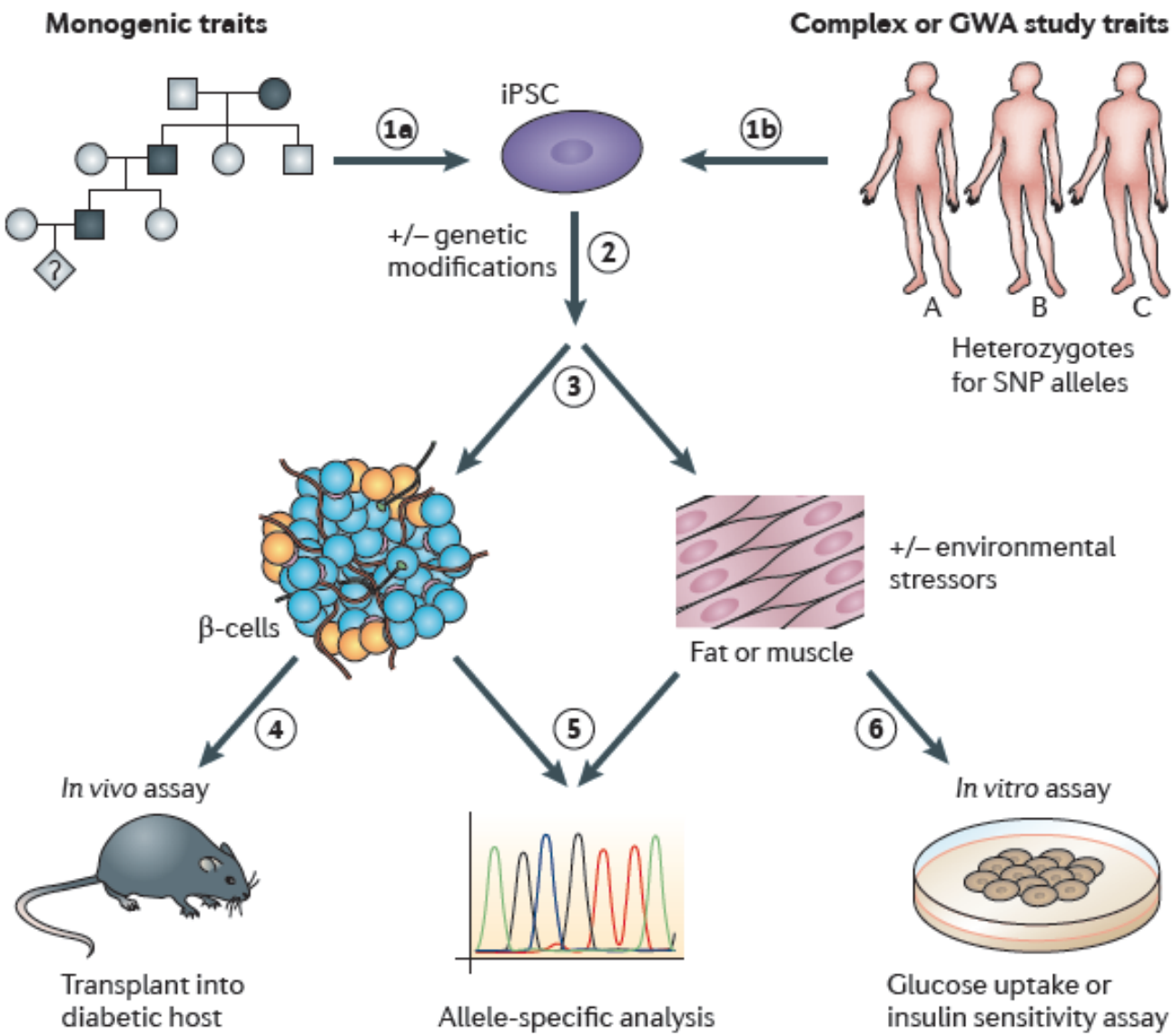

GCTM2
EpCAM
DAPI
50 µm
9
18
21
24
J1-P23

iPSC: applications

- Research: Disease Modeling
- Therapy: Tissue Matching



Yamanaka S et al *Cell* 2007
Chun YS et al *Int J Biol Sci* 2010

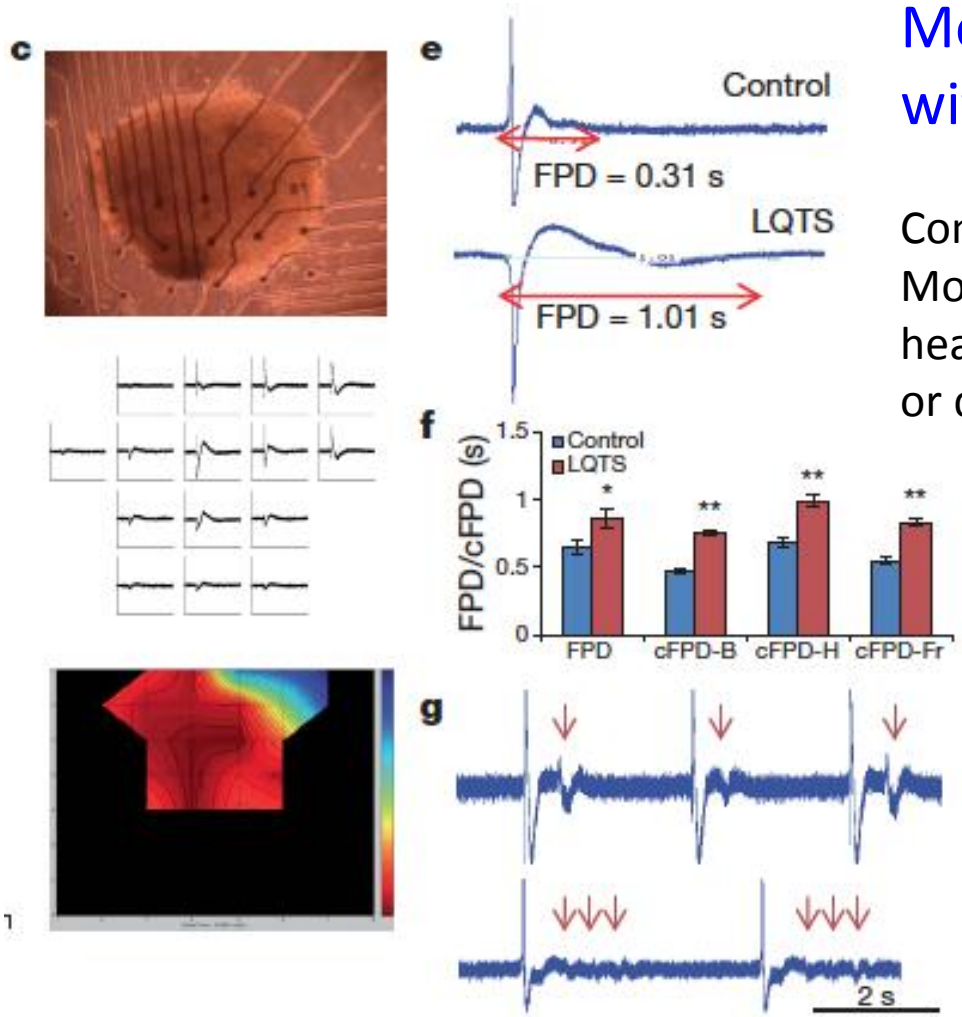


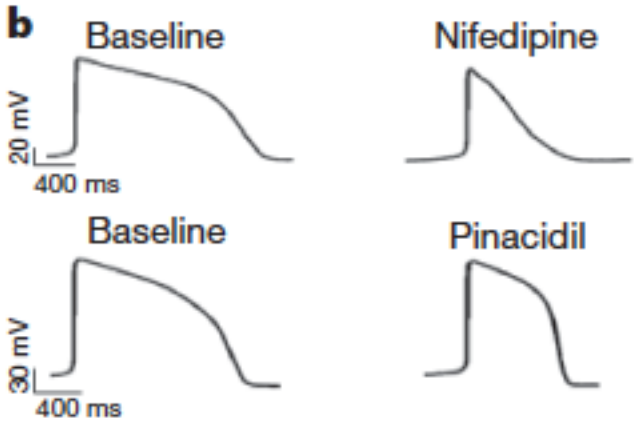
Zhu et al.
Nat. Rev. Gen 2011
Doi: 10.1038/nrg2951

Approaches to human functional genomics

Modeling the long Q-T syndrome with human iPSC

Congenital Type 2 LQTS:
Model for LQT caused by
heart failure, cardiac hypertrophy
or drugs





Itzhaki et al. Nature 471: 225, 2011

iPSC advantages

- No ethical issues around provenance
- Facile access to starting material
- Technology for reprogramming widely accesible

Stem Cell Ethics

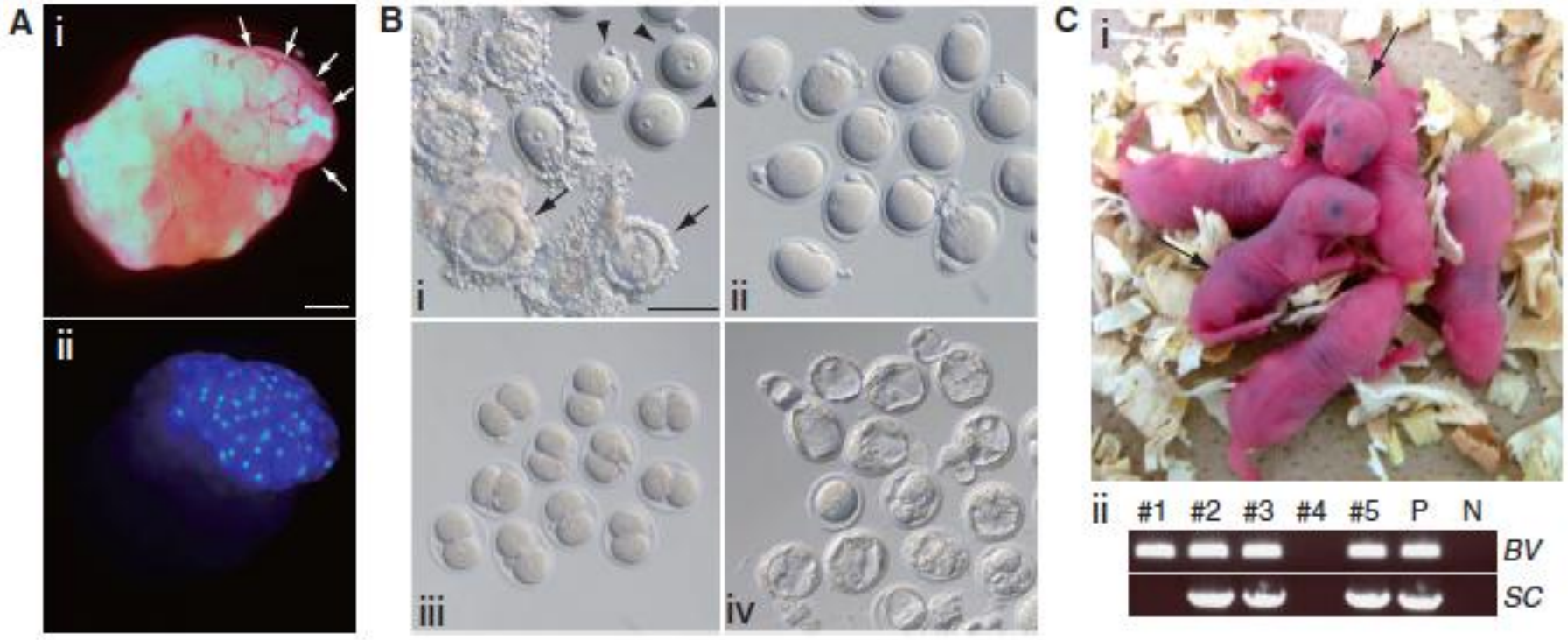
- The debate over the use of human embryos in research is not over, but it is of diminishing relevance to the field
- The availability of over 1000 ES cell lines and iPSC technology means that arguments for the use of embryos to achieve a new advance (that cannot be achieved by other means) must be very convincing
- Although iPSC provenance is ethically less challenging than embryo usage, there are many other issues around the use of human pluripotent cells in research and therapy

Ethics of Research with ES or iPS cells

- Experimentation in vitro-growth, differentiation, genetic manipulation, functional assessment, drug testing-raises issues of genetic privacy around iPS banks
- Inoculation of cells into adult or foetal animals with a view towards assessment of developmental capacity (eg teratoma formation) or ability to incorporate into and function within normal tissue or disease model-chimeras

Offspring from Oocytes Derived from in Vitro Primordial Germ Cell–like Cells in Mice

Katsuhiko Hayashi,[1,2,3]* Sugako Ogushi,[1,4] Kazuki Kurimoto,[1,5] So Shimamoto,[1] Hiroshi Ohta,[1,5] Mitinori Saitou[1,2,5,6]*



Differentiation of human iPS cells into gametes

- New possibilities for research on human germ line-infertility, early development
- But significant ethical questions over fertilisation and embryo production using IPS-cell derived gametes
- With IPS cells gametes could be created from individuals of any age, living or dead
- We could potentially make germline modifications in human

Induced Pluripotent Stem Cells Limitations to Technology

- Complete reprogramming to pluripotent state?
- Tissue of origin memory
- Differentiation capacity
- Genetic lesions induced during reprogramming
- Tumor formation

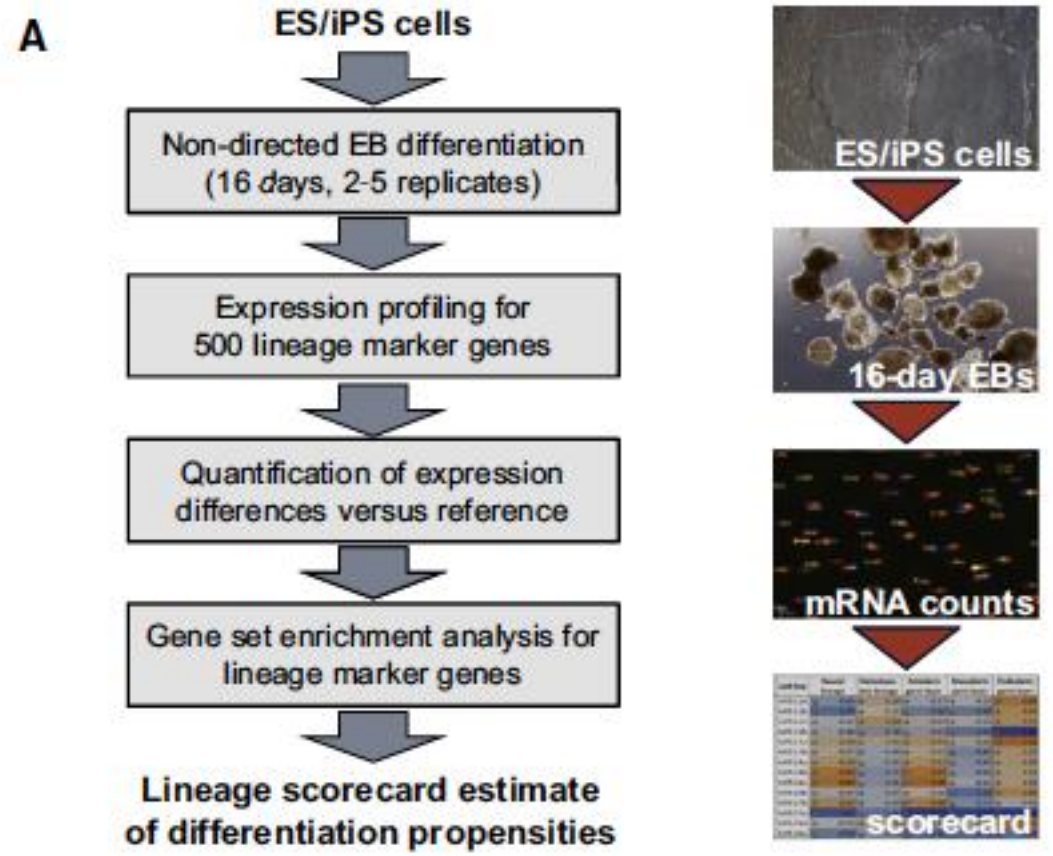
Challenges for Cell Therapy

3- Manufacturing a Product;
Safety Considerations
Versus
Patient Demands for Cures

Making the Right Stuff

Variations in Differentiation Capacity of Both ES and iPS cells in culture

Comparison of Differentiation Potential in Pluripotent Cells



Bock et al. Cell 2011 doi 10.1016/j.cell.2010.12.032

Variability in Differentiation Potential: iPSC

D

Cell line	Neural lineage	Hematopo-ietic lineage	Ectoderm germ layer	Mesoderm germ layer	Endoderm germ layer
hiPS 11a	-0.69	0.18	-0.37	-0.23	0.83
hiPS 11b	-1.17	-0.23	-0.96	-1.03	0.47
hiPS 11c	-0.22	0.40	-0.03	-0.16	0.37
hiPS 15b	-0.48	-0.78	-0.63	-1.11	-2.49
hiPS 17a	0.19	0.05	0.33	0.00	1.16
hiPS 17b	-0.07	-0.48	-0.02	-0.83	0.20
hiPS 18a	0.28	-0.52	0.31	-0.67	0.20
hiPS 18b	0.80	-0.72	0.84	-0.62	0.15
hiPS 18c	0.93	-0.65	1.05	-0.41	0.10
hiPS 20b	-0.37	-0.47	-0.30	-1.16	0.56
hiPS 27b	0.52	-0.50	0.68	-0.71	-0.42
hiPS 27e	-1.61	-1.04	-2.12	-1.82	-3.27
hiPS 29d	-0.25	-0.04	0.00	-0.11	0.83
hiPS 29e	-0.99	-0.60	-1.15	-1.14	-1.08

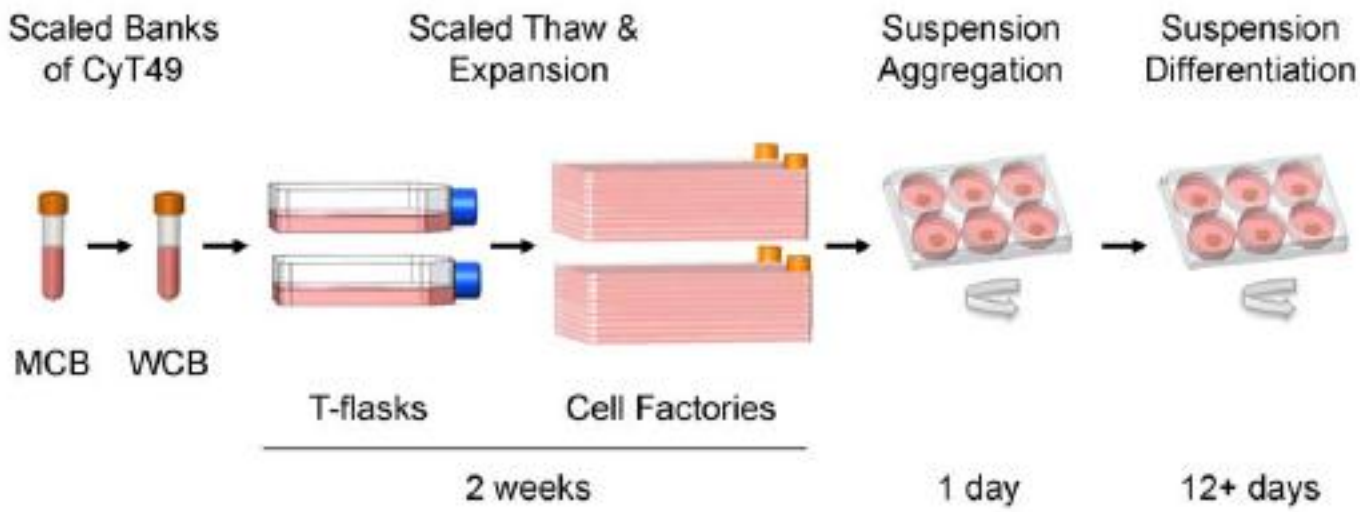
Differentiation propensity: high ↑ medium ↗ → ↘ low ↓

Bock et al. Cell 2011 doi 10.1016/j.cell.2010.12.032

Variability in Differentiation Potential of Cell Lines

- Variation in differentiation potential may require isolation and testing of multiple clones
- Variation probably relates not to gene expression in pluripotent state but rather to its stability-what is important is how cells exit pluripotent state

Scaleup of Production of Pancreatic Progenitors for Type 1 Diabetes: Viacyte



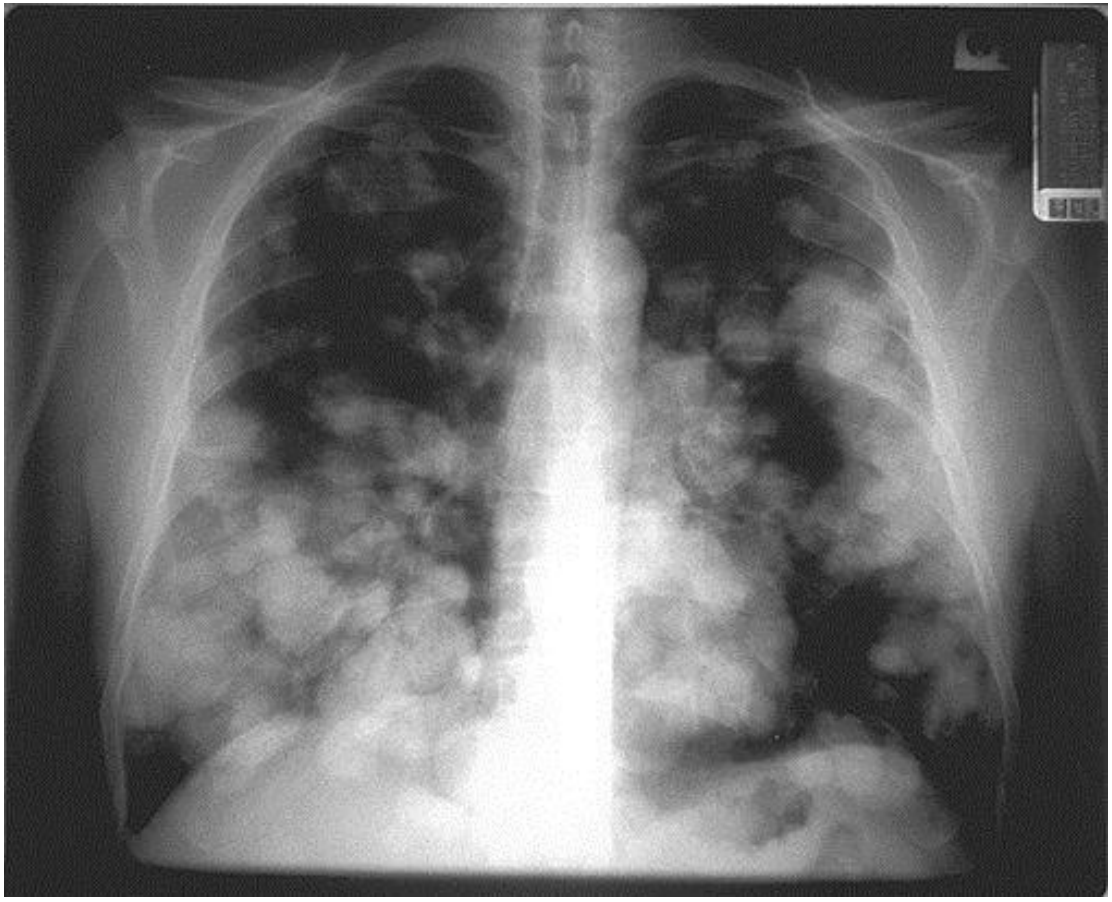
Strategy focuses on expansion in the stem cell state followed by mass differentiation

Schulz et al. PLoS One 7: e37004

Pancreatic Progenitors

- Protocol carefully optimized over years for one cell line that is particularly amenable to this differentiation lineage
- Nearly but not completely xeno-free
- End cells are not functionally mature
- $3x10^9$ progenitor cells produced, just sufficient for Phase 1 trial of 10 patients
- <2% are unknown cell types
- Costs not specified

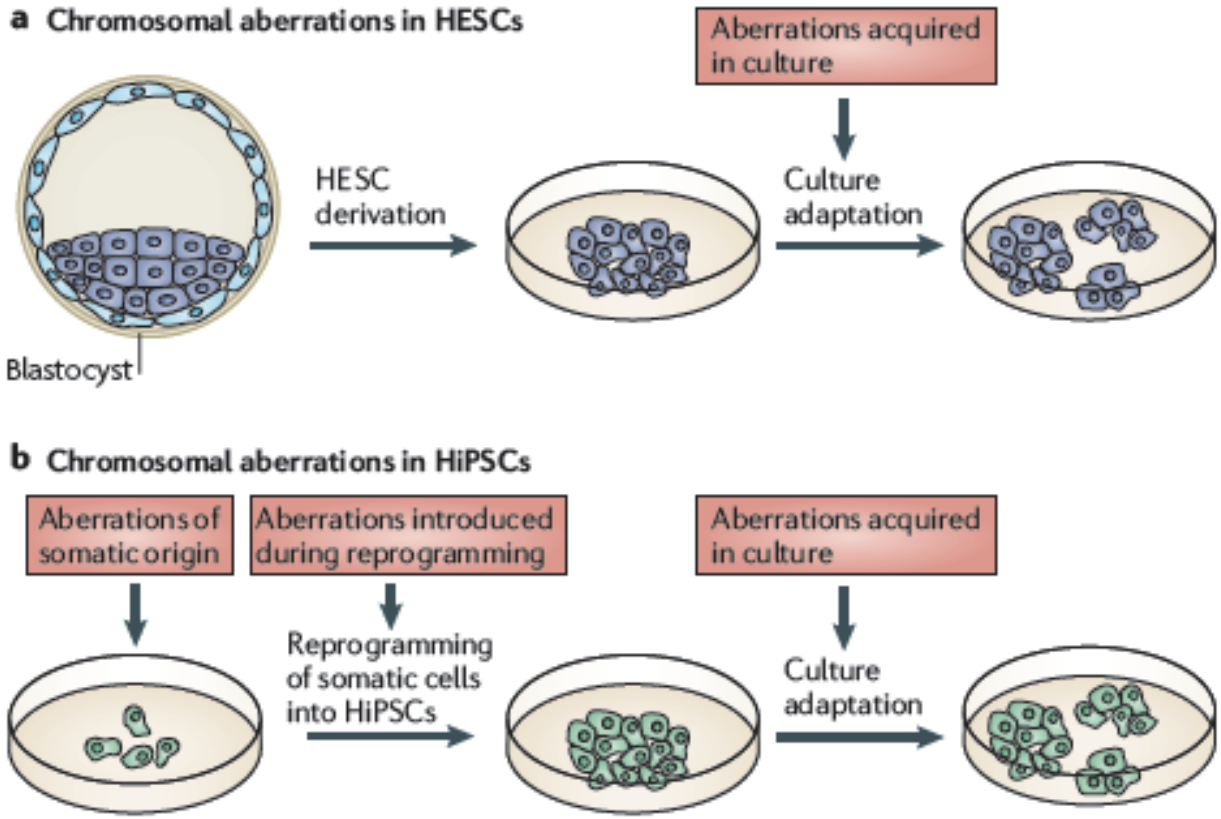
Genetic and epigenetic instability poses risk of cancer formation in grafts



Potential use in transplantation =stringent requirements for safety

Radiation Oncology, Peter Macallum Cancer Centre

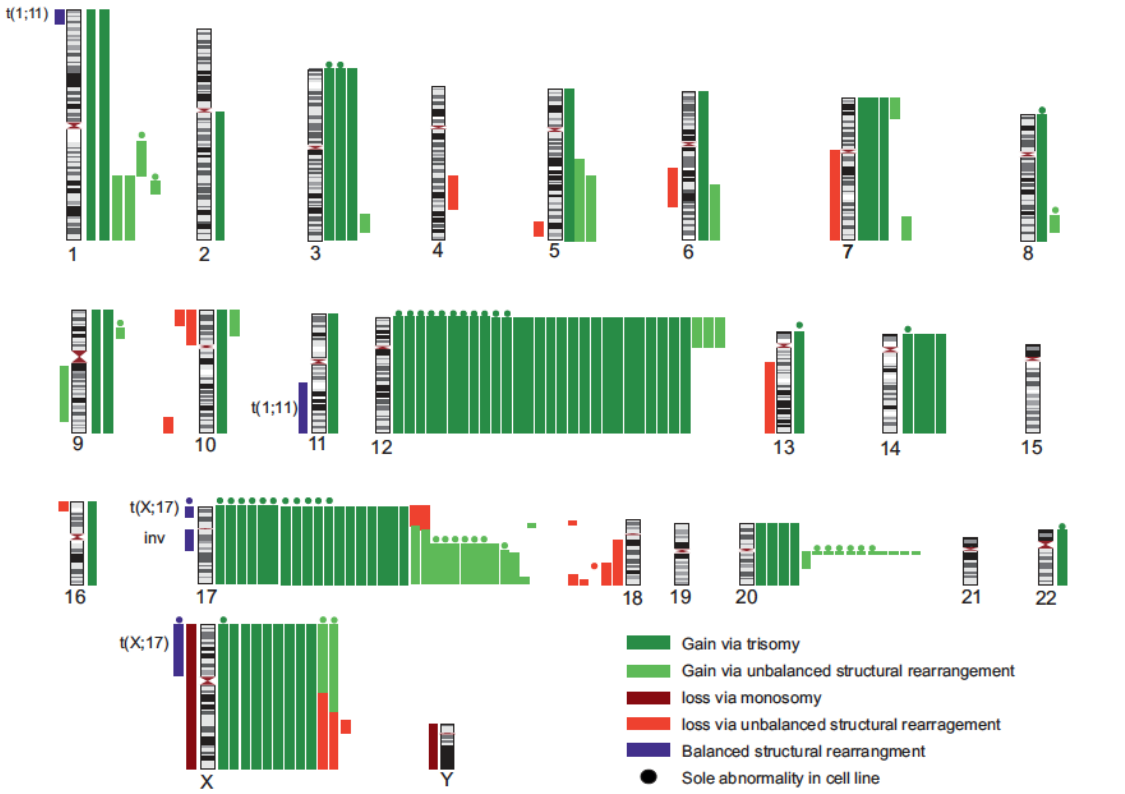
Genetic Lesions in hPSC



Functional significance of mutations not always clear.
Chromosomal rearrangements in later stages similar in ES and iPSC

Ben-David and Benvenisty Nat. Rev. Cancer 2011 doi: 10.1038/nrc3034

Chromosomal changes in ES +iPS cells resemble changes in cancer



Supplementary Figure 5(Andrews): Ideogram demonstrating all chromosome changes in hES cells reported in literature

ISCI, Nat Biotech 29: 1132, 2011

Most ES cell lines are remarkably stable

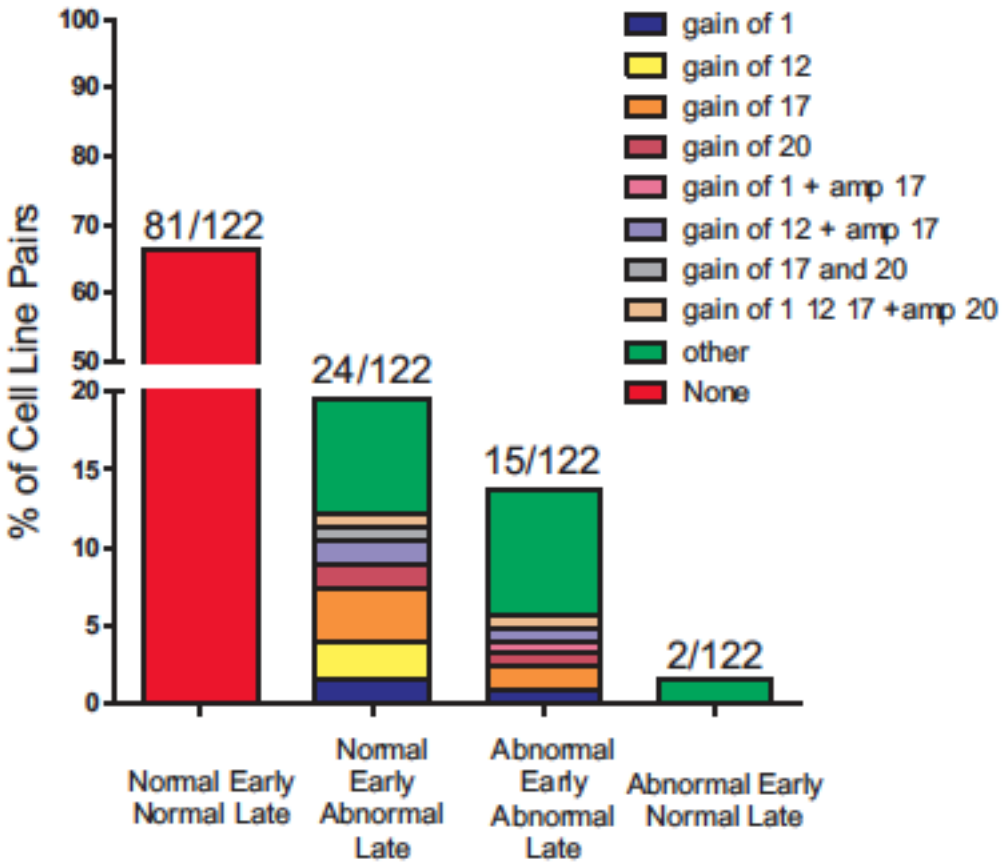


Figure 2a(Andrews): Cytogenetic changes occurring during prolonged passage of human ES cells

ISCI, Nat Biotech 29: 1132, 2011

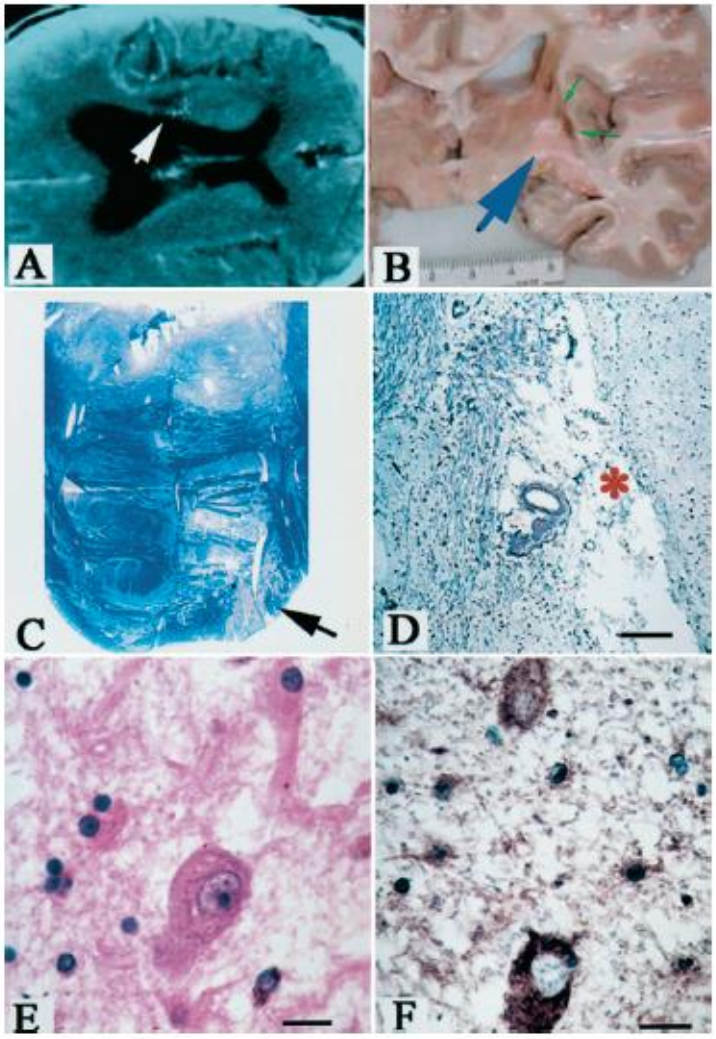
STEM CELLS

The dark side of induced pluripotency

Induced pluripotent stem cells have great therapeutic potential. But genomic and epigenomic analyses of these cells generated using current technology reveal abnormalities that may affect their safe use. SEE ARTICLES P.58, P.63 & P.68

Potential for harm due to inoculation of patients with pluripotent stem cell derived grafts containing cells with genetic lesions is a significant concern in the field of regenerative medicine

But the experiment has in fact already been done



Graft of EC-derived neurons in a patient 27 months after transplant

Nelson et al. Am J Path 160: 1201, 2002

R_X 2E6 EC derived neurons delivered sterotaxically to site of infarct

New and Sensitive Methods to Detect Contamination in Cell Therapeutics

Table 4 Methods for assaying residual pluripotent stem cells in clinical product or patient monitoring

Method of detection	Molecular compartment	Sensitivity	Limitations	Potential stage of application		
				Preclinical	CMC	Clinical
Methods with accepted clinical utility						
ASO-qRT-PCR	Tumor DNA	0.001%, that is, 1 CTC in 100,000 normal[83]	Requires large number of samples for repeated testing to assure statistical certainty	Yes	Yes	Yes
Flow cytometry	Tumor cell	0.01%, that is, 1 CTC in 10,000 normal[84]	Requires four- to six-color flow, necessitating multiple cell surface markers	Yes	Yes	Yes
ELISAs	Tumor protein	Ultrasensitive assays detect in sub-pg/ml range[85]	Requires unique protein expression & correlation of protein signal with cell number	Yes	Yes	Yes
MRI	Tumor cell	Masses >0.3 cm	Unknown effect of imaging labels on stem cell phenotype or genotoxic potential	Yes	No	Yes
FDG-PET	Tumor size	Masses >1 cm (ref. 86)	Poor spatial resolution	No	No	Yes
Methods with emerging evidence						
qRT-PCR	Tumor miRNA	Limit of detection down to 10 copies of miRNA[87]	Requires identification of miRNAs with known association with pluripotent cells	Yes	Yes	Yes
Immuno-PCR (TPA)	Tumor protein	Limit of detection in femtogram range[88]	Requires unique protein expression & correlation of protein signal with cell number	Yes	Yes	Yes
Fluorescent nanocrystals & cation exchange	Tumor miRNA	Limit of detection in femtomole range[89]	Requires identification of miRNAs with known association with pluripotent cells	Yes	Yes	Yes
Nanoparticle surface plasmon resonance	Tumor miRNA	Limit of detection in attomole range[90]	Requires identification of miRNAs with known association with pluripotent cells	Yes	Yes	Yes
Bioluminescence (BLI)	Tumor cell	Limit of detection to be determined[91,92]	Requires demonstration that vectors used to label cells have no effect on cell product profile	Yes	No	No

CMC, product chemistry, manufacturing and controls; CTC, circulating tumor cell; ELISA, enzyme-linked immunosorbent assay; miRNA, microRNA; MRI, magnetic resonance imaging; PET, positron emission tomography; TPA, TaqMan protein assay.

Lessons from human teratomas to guide development of safe stem cell therapies

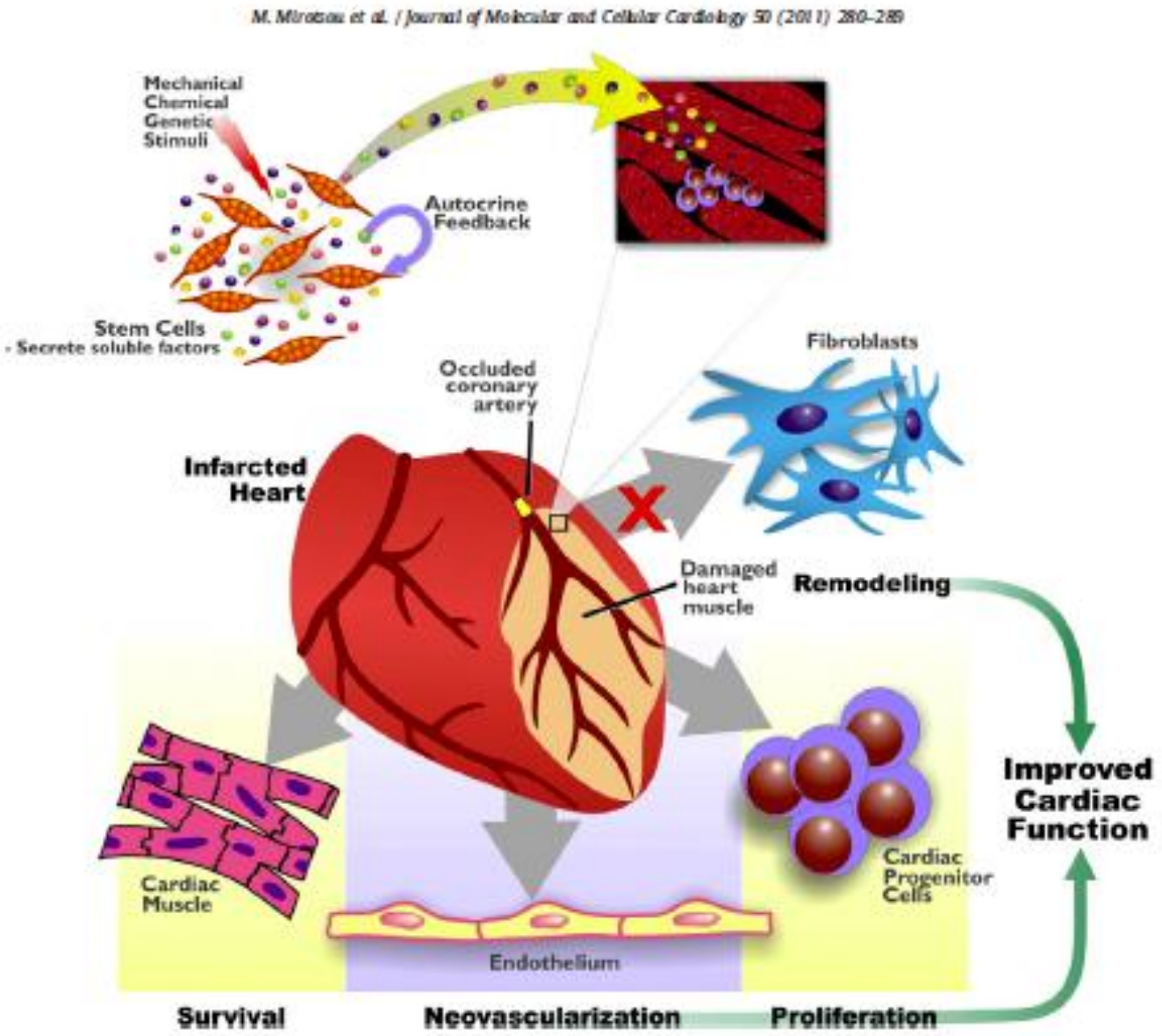
Justine J Cunningham[1], Thomas M Ulbright[2], Martin F Pera[3] & Leendert H J Looijenga[4]

Issues for Regulation of Cell Therapy

- Unlike drugs, cell therapies may have a half life in the body of decades
- Treatment may require administration of billions of cells to a patient; perhaps only 10^2 cells might cause tumor formation
- However, an overly cautious approach might delay introduction of potentially curative treatments
- Risk benefit assessment is crucial

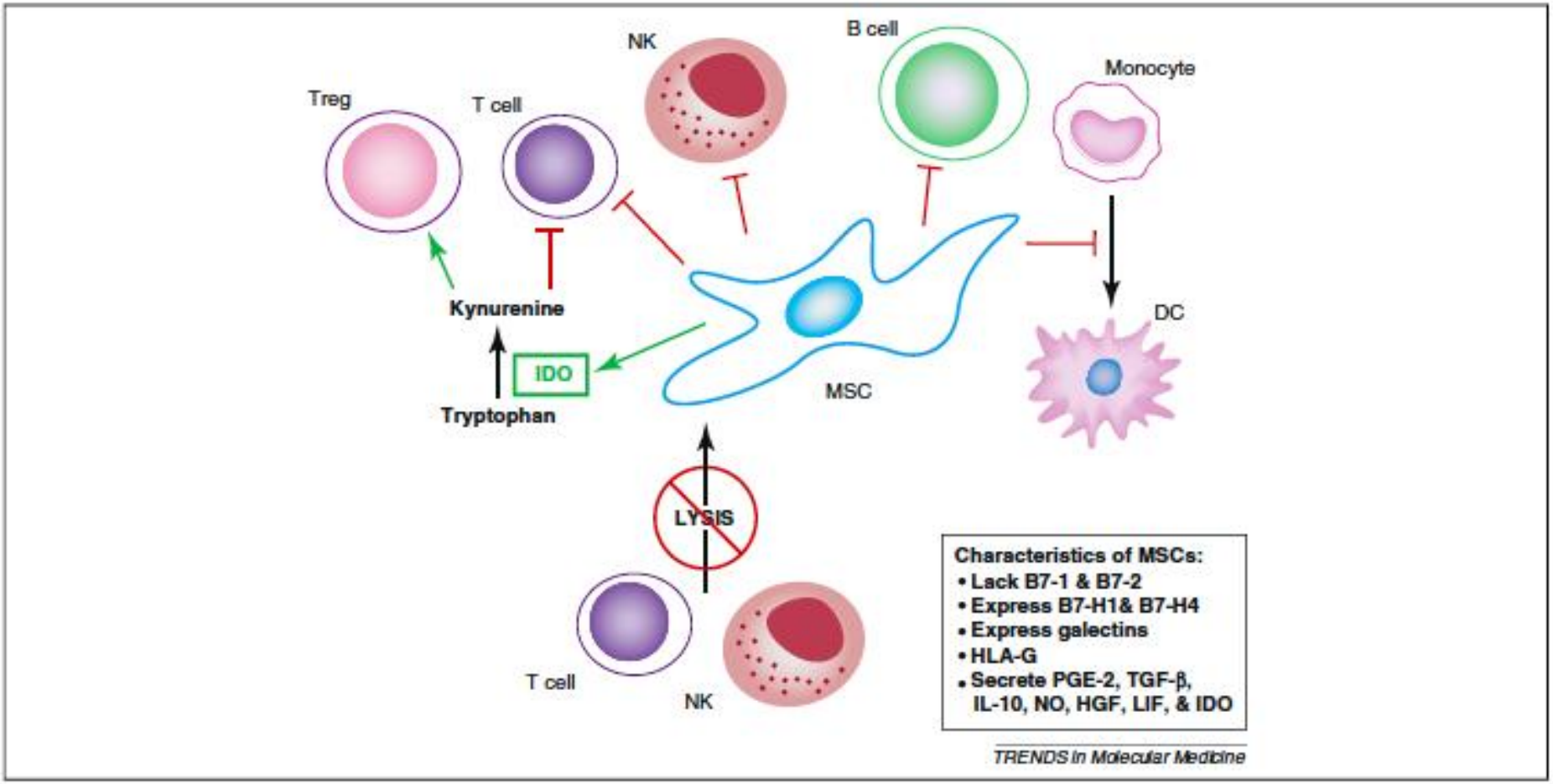
What Do Stem Cell Grafts Actually Do?

- The evidence for functional integration and actual replacement of lost tissue in preclinical or clinical studies is quite limited
- Production of protective or trophic or immunomodulatory factors is considered to be the mechanism of most cell grafts to date

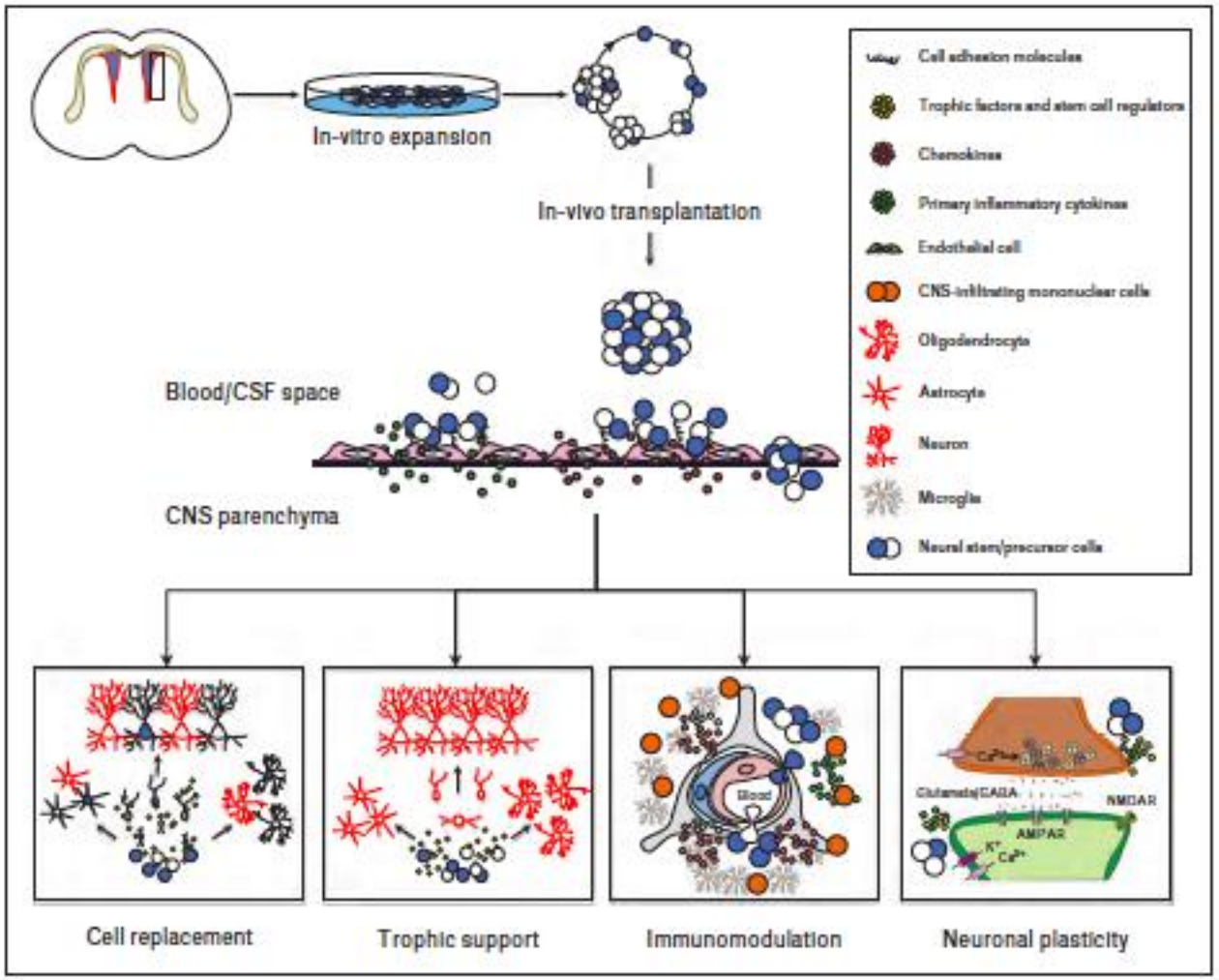


Stem cell mechanisms in myocardial infarction

Trends in Molecular Medicine February 2012, Vol. 18, No. 2



Mesenchymal stem cell immunomodulatory functions



Stem cell mechanisms in neural repair

Long-Distance Growth and Connectivity of Neural Stem Cells after Severe Spinal Cord Injury

Paul Lu,[1,3,*] Yaozhi Wang,[1] Lori Graham,[1] Karla McHale,[1] Mingyong Gao,[1] Di Wu,[1] John Brock,[1] Armin Blesch,[1,5] Ephron S. Rosenzweig,[1] Leif A. Havton,[4] Binhai Zheng,[1] James M. Conner,[1] Martin Marsala,[2] and Mark H. Tuszynski[1,3,*]
[1]Department of Neurosciences
[2]Department of Anesthesiology
University of California, San Diego, La Jolla, CA 92093, USA
[3]Veterans Administration Medical Center, San Diego, CA 92161, USA
[4]Departments of Anesthesiology and Perioperative Care and Anatomy and Neurobiology, Reeve-Irvine Research Center, University of California, Irvine, Irvine, CA 92697, USA
[5]Spinal Cord Injury Center, Heidelberg University Hospital, 69120 Heidelberg, Germany
*Correspondence: plu@ucsd.edu (P.L.), mtuszynski@ucsd.edu (M.H.T.)
http://dx.doi.org/10.1016/j.cell.2012.08.020

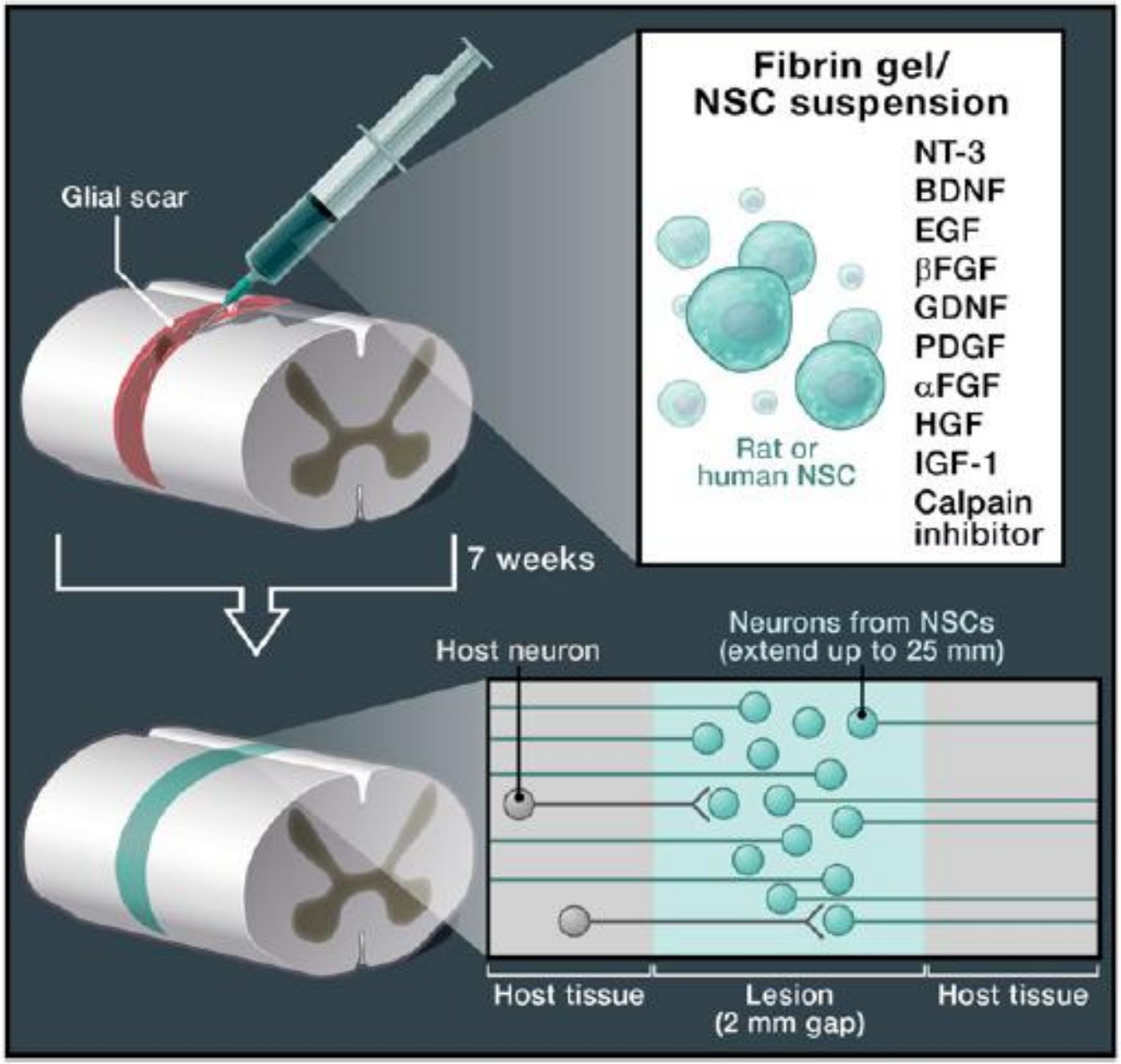
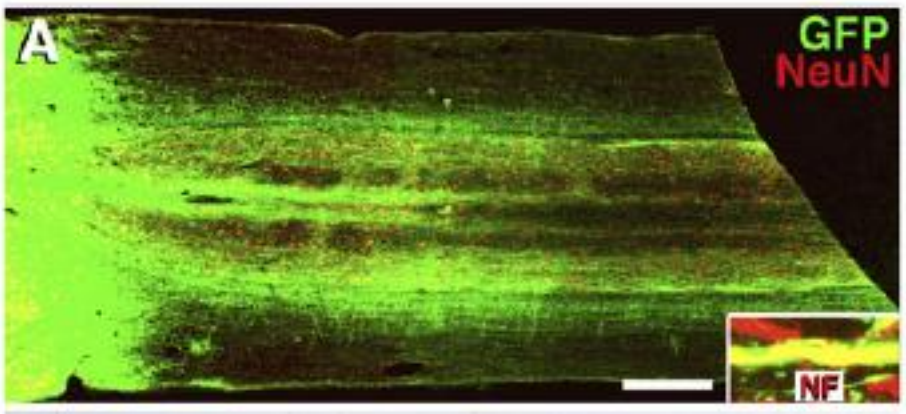
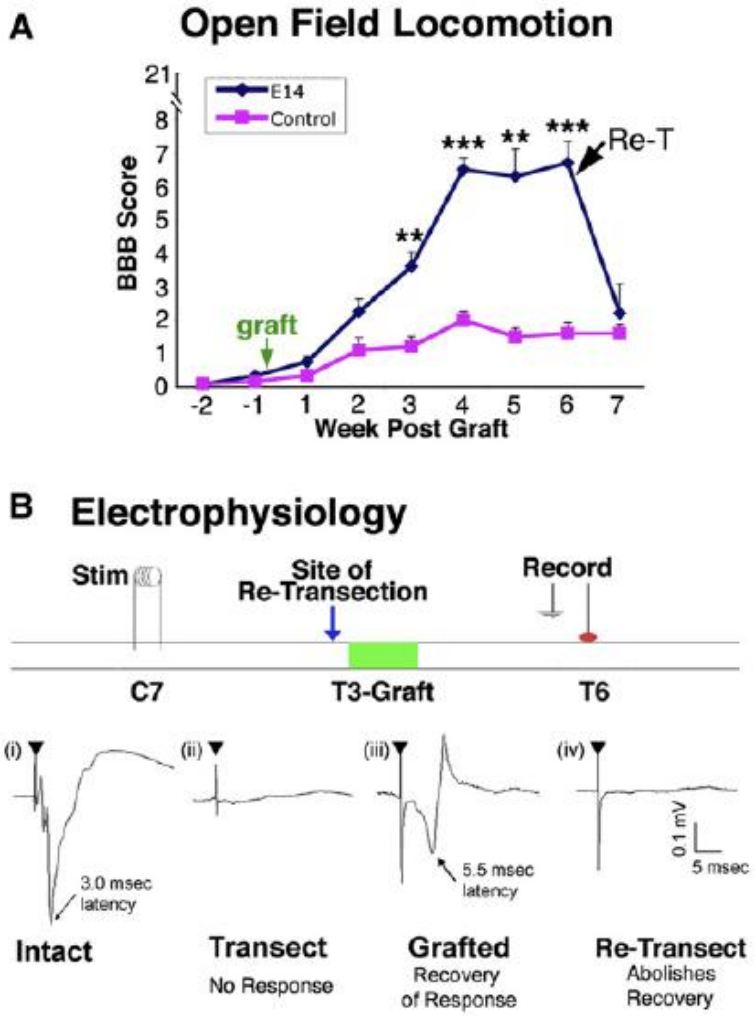
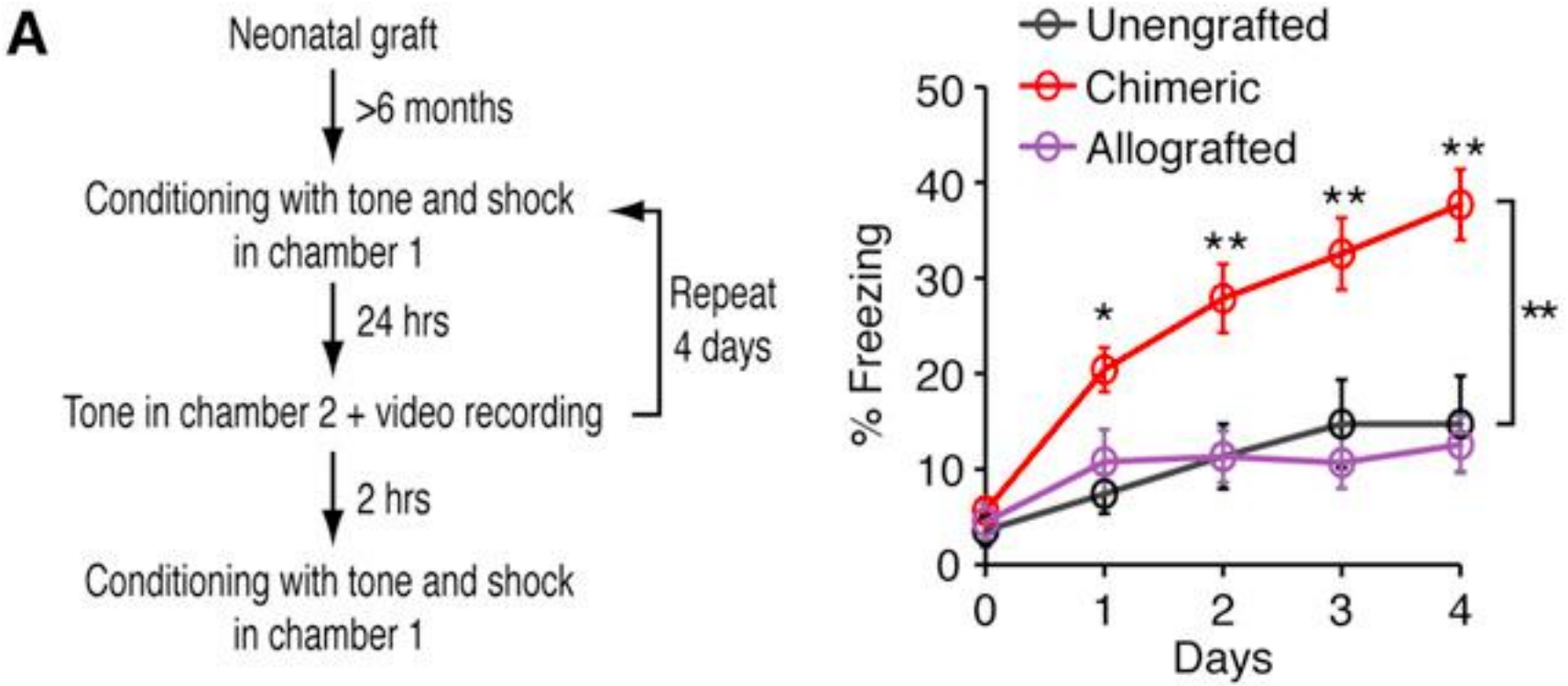


Figure 1. Neural Stem Cell Transplantation for the Repair of Spinal Cord Injury





Transplanted stem cells grow across lesion and restore communication. Functional relays are reestablished.



Forebrain Engraftment by Human Glial Progenitor Cells Enhances Synaptic Plasticity and Learning in Adult Mice

Xiaoning Han,[1,2] Michael Chen,[1,2] Fushun Wang,[1,2] Martha Windrem,[1,3] Su Wang,[1,3] Steven Shanz,[1,3] Qiwu Xu,[1,2] Nancy Ann Oberheim,[1,2] Lane Bekar,[1,2] Sarah Betstadt,[4] Alcino J. Silva,[5] Takahiro Takano,[1,2] Steven A. Goldman,[1,2,3,*] and Maiken Nedergaard[1,2,3,*]

The Future is Now

- Strong basic science and translational capacity in Australia
- Interdisciplinary strength
- Great opportunity in this field
- Time to explore urgently new models for funding and managing research

16 & 17 MAY 2013
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PLENARY SPEAKERS

Professor Allan Robins, ViaCyte Inc, San Diego ***'Developing an Encapsulated Stem Cell Therapy for Diabetes'***
Professor Chris Mason, University College London ***'Winners Take All in the Race to be a Cell Therapy Nation'***
Professor Paul Simmons, Mesoblast Ltd, Melbourne ***'Mesenchymal Precursor Cells (MPC): a Platform Technology for Multiple Therapeutic Applications'***
Professor Bob Graham, Victor Chang Cardiac Research Institute and UNSW, Sydney ***'The Structural, Physiological and Cellular Ontogeny of the Post-Natal Heart'***