Professor Martin Pera

University of Melbourne

Melbourne

Stem Cells and Regenerative medicine- the Future is Now - Main Session (Workshop options scheduled)

Friday, 21 June 2013 Start 5:50pm Duration: 20mins Baytrust

Human Pluripotent Stem Cells: The Future is Now

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

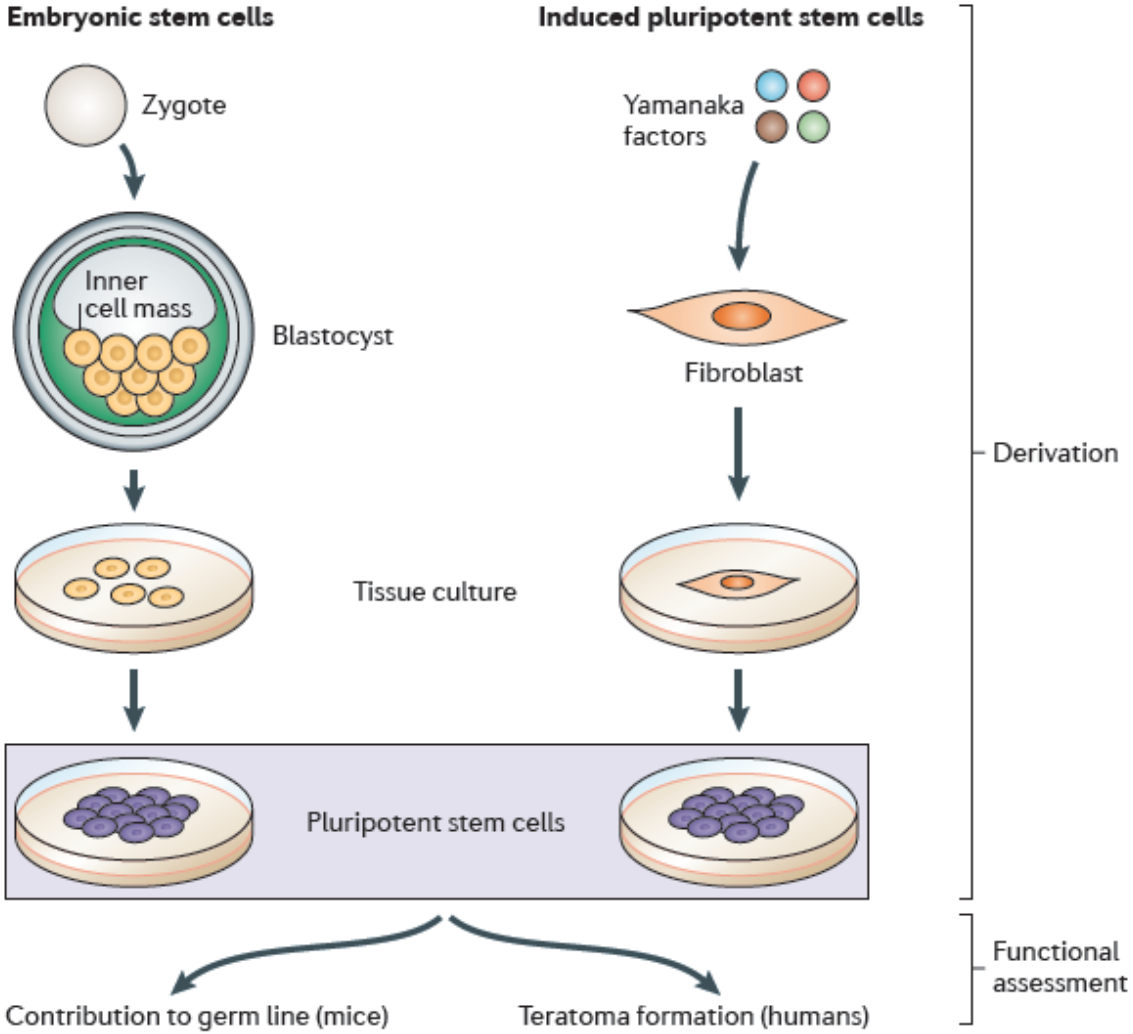
STEMCELLS AUSTRALIA

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

Properties of pluripotent stem cells

- Grow indefinitely in vitro
- Maintain normal genetic makeup
- Cloned lines capable of differentiation into a wide range of somatic and extraembryonic tissues in vivo and in vitro-at high frequency and under a range of conditions
- Capable of colonising all tissues including germ line after blastocyst injection to give chimeric offspring



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

Two types of human pluripotent stem cell

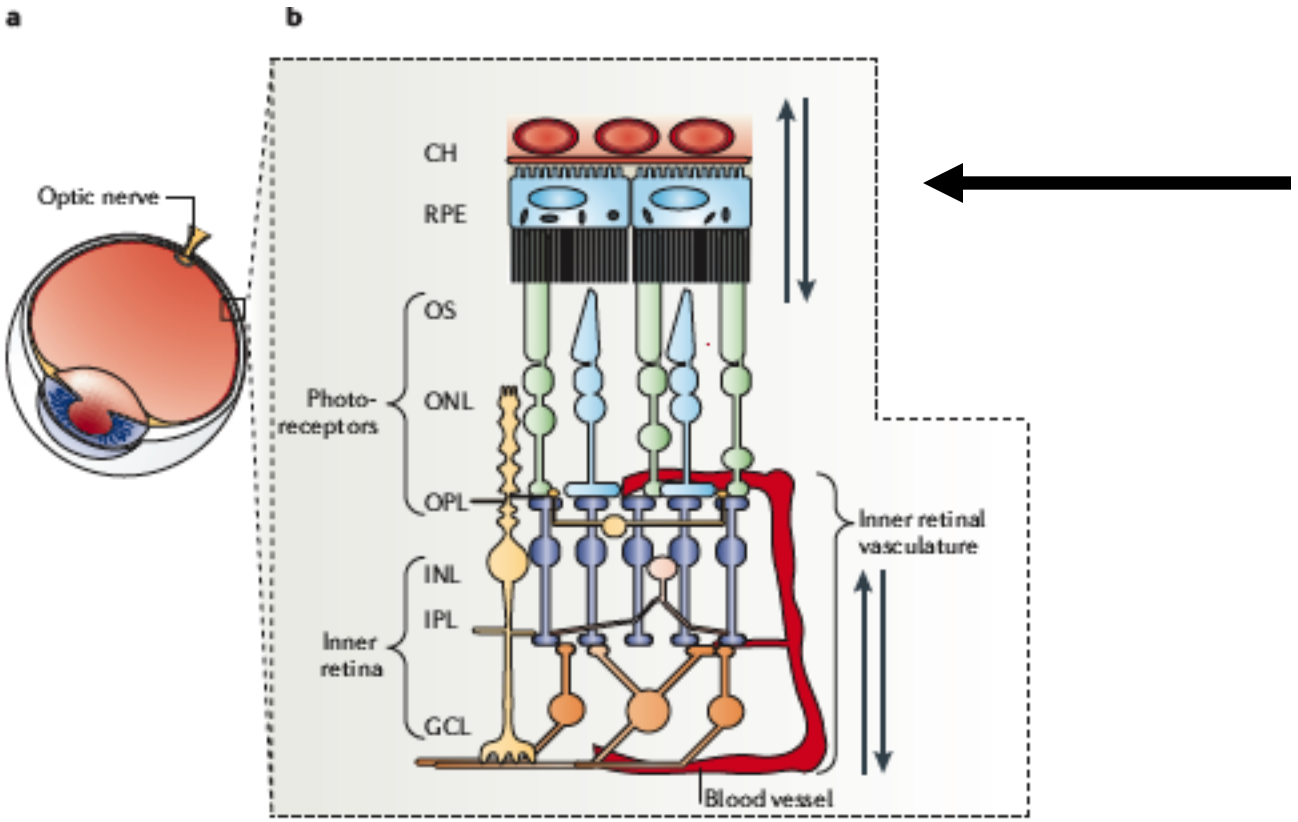
Embryonic stem cells

- Derived from spare embryos before specialised tissue of the body begin to form
- Can multiply indefinitely in laboratory cultures
- Retain the ability of embryonic cells to turn into any type of tissue

Blindness

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

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



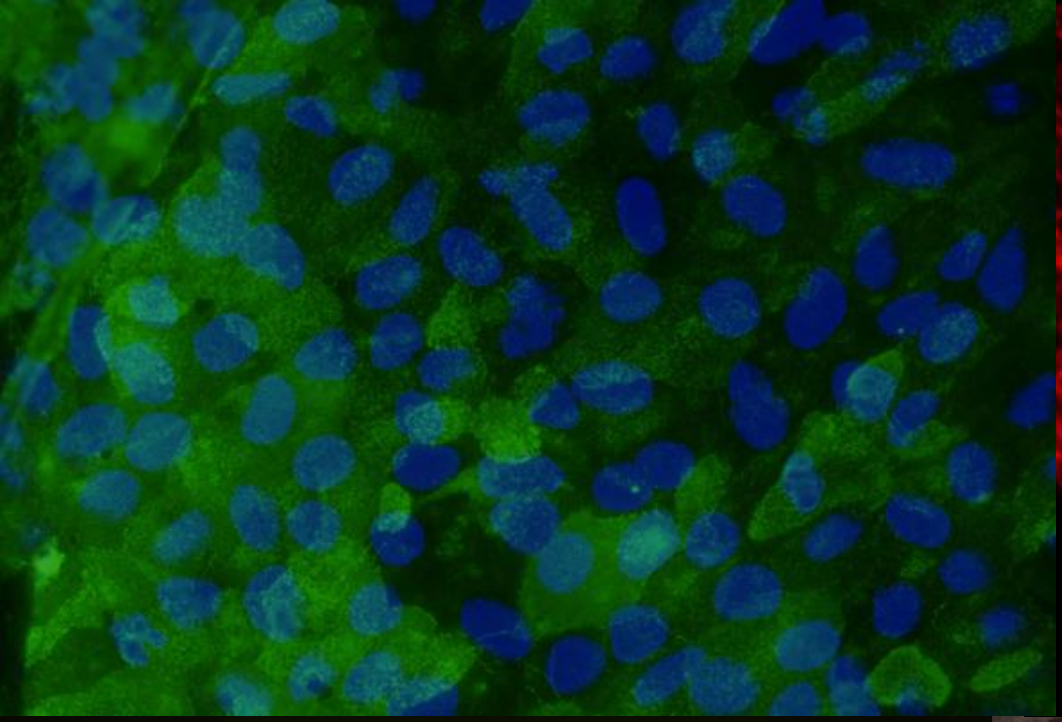
Nov 98- human embryonic stem cells discovered

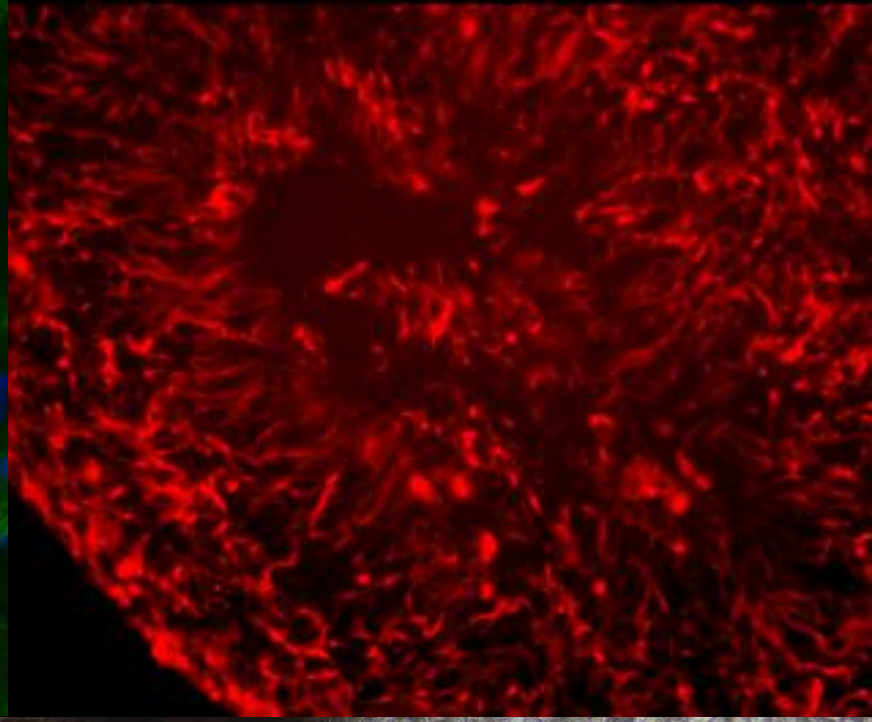
2012-First human trials of human embryonic stem cell therapeutics

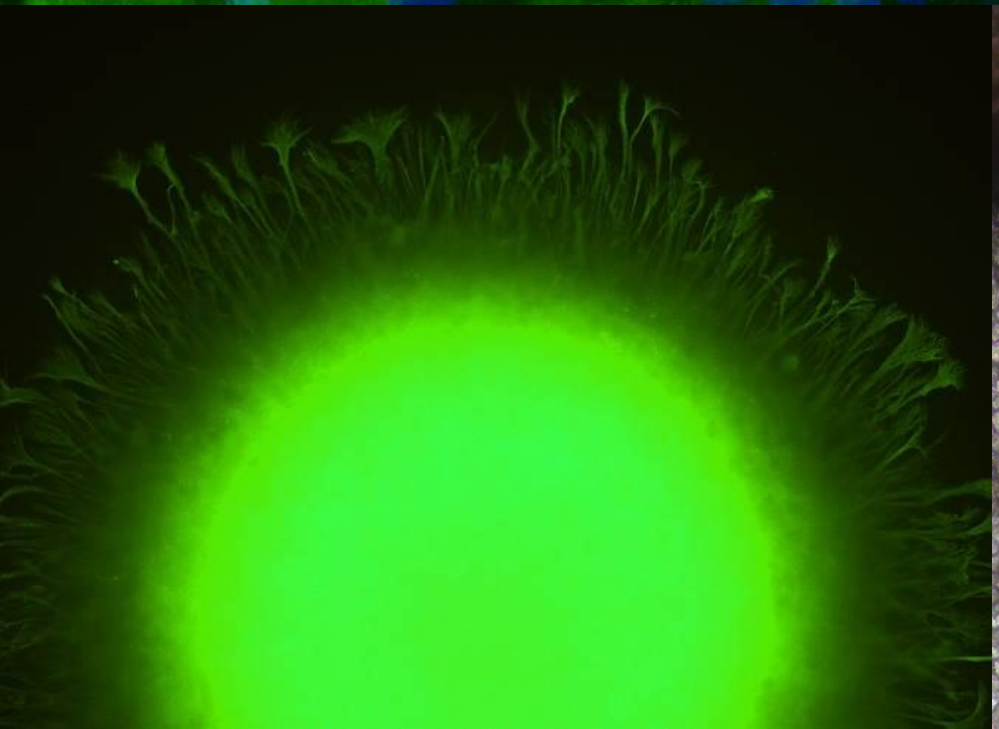
Embryonic Stem Cell Lines Derived from Human Blastocysts

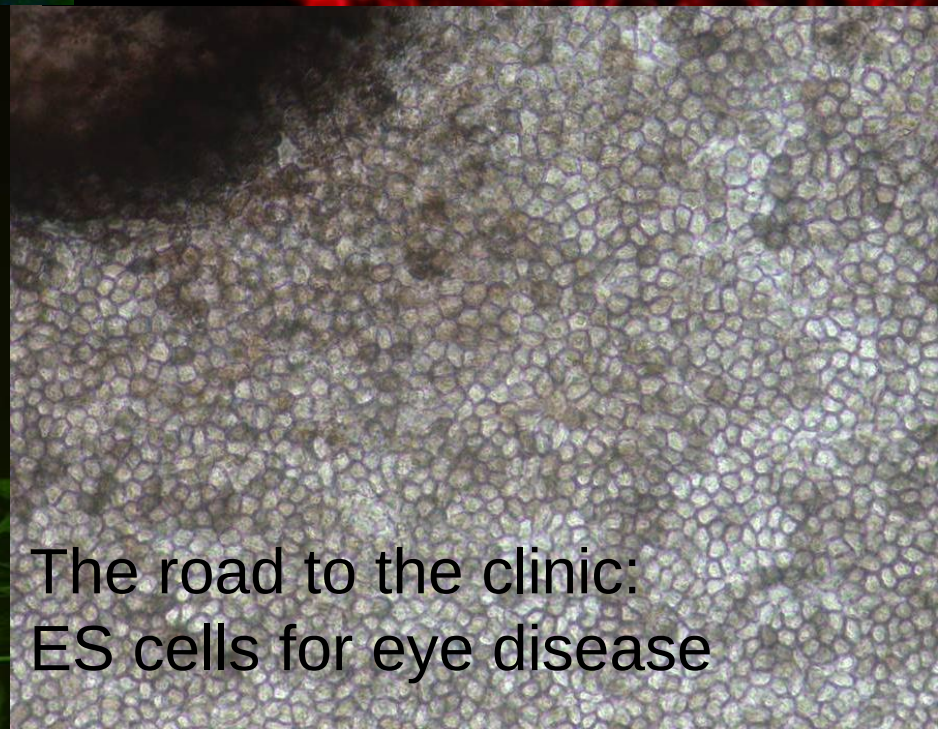
James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

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.



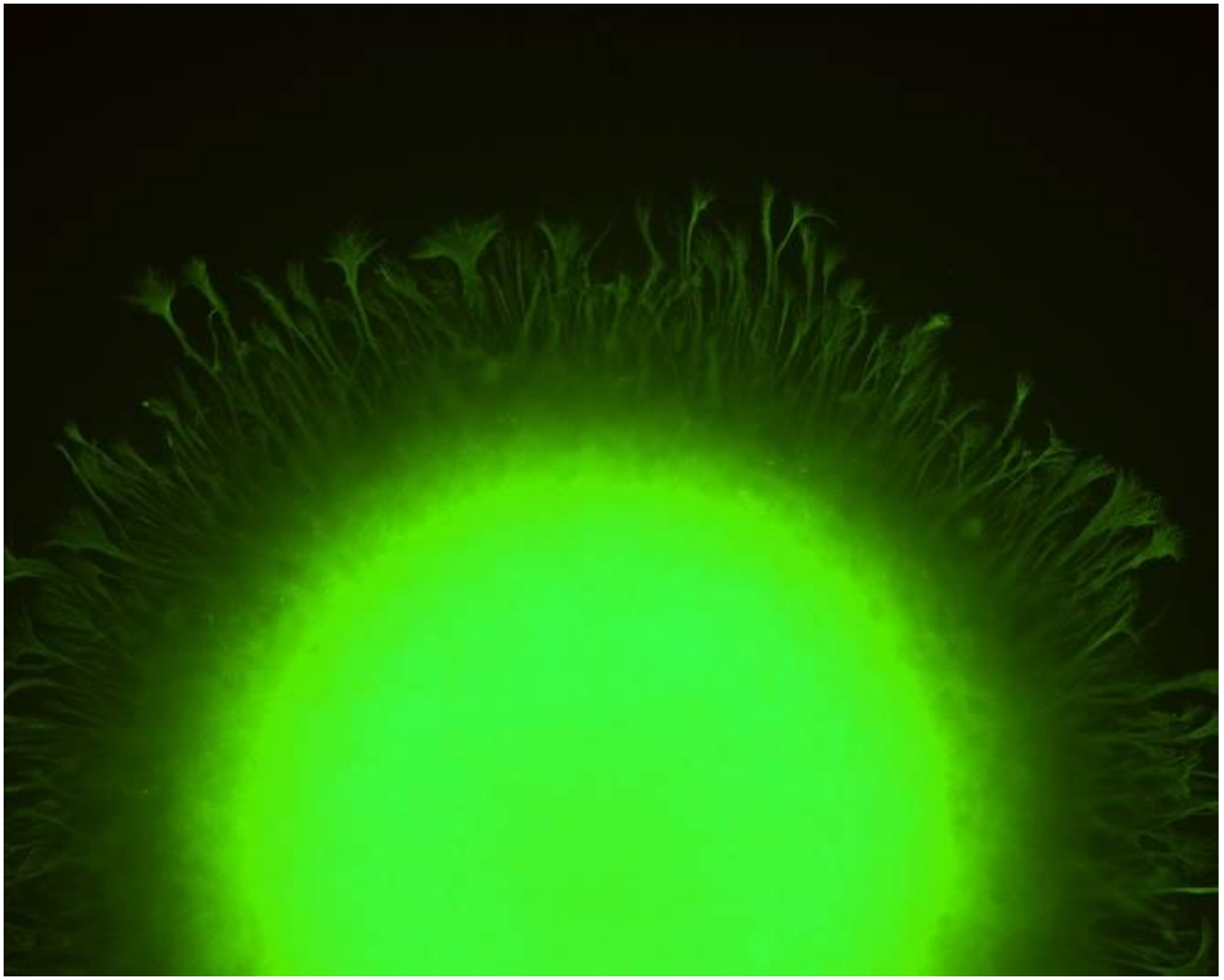




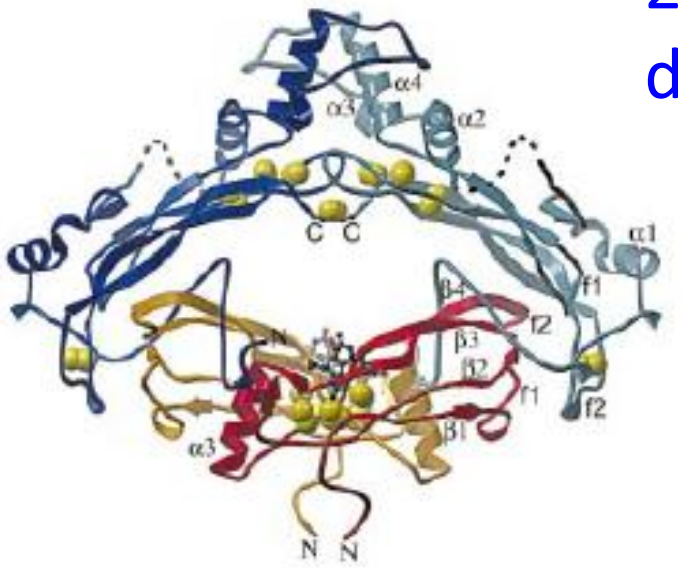


The road to the clinic:
ES cells for eye disease

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

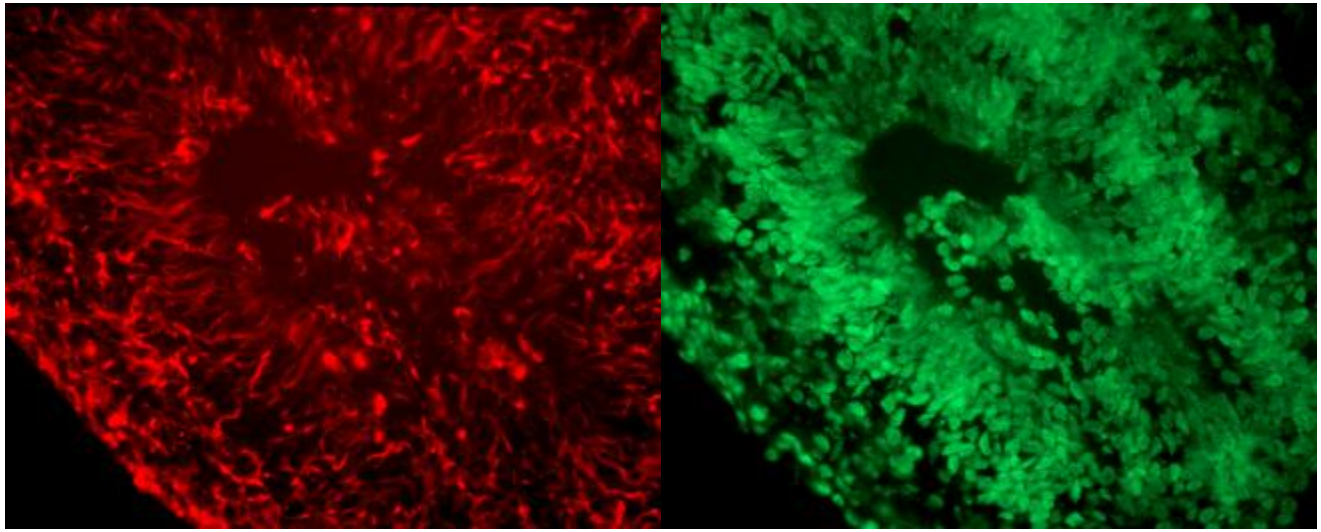


2004-directed neural differentiation

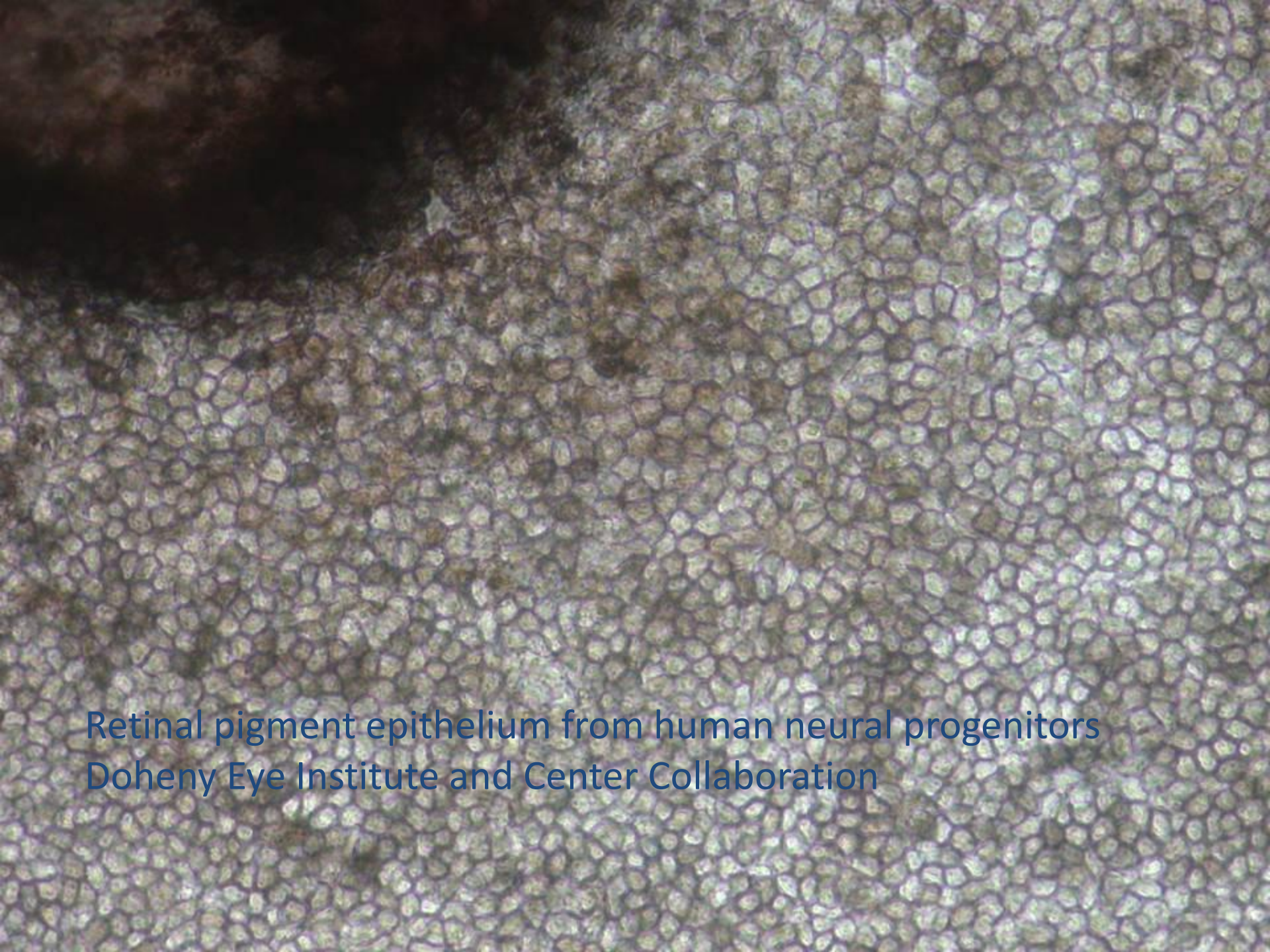


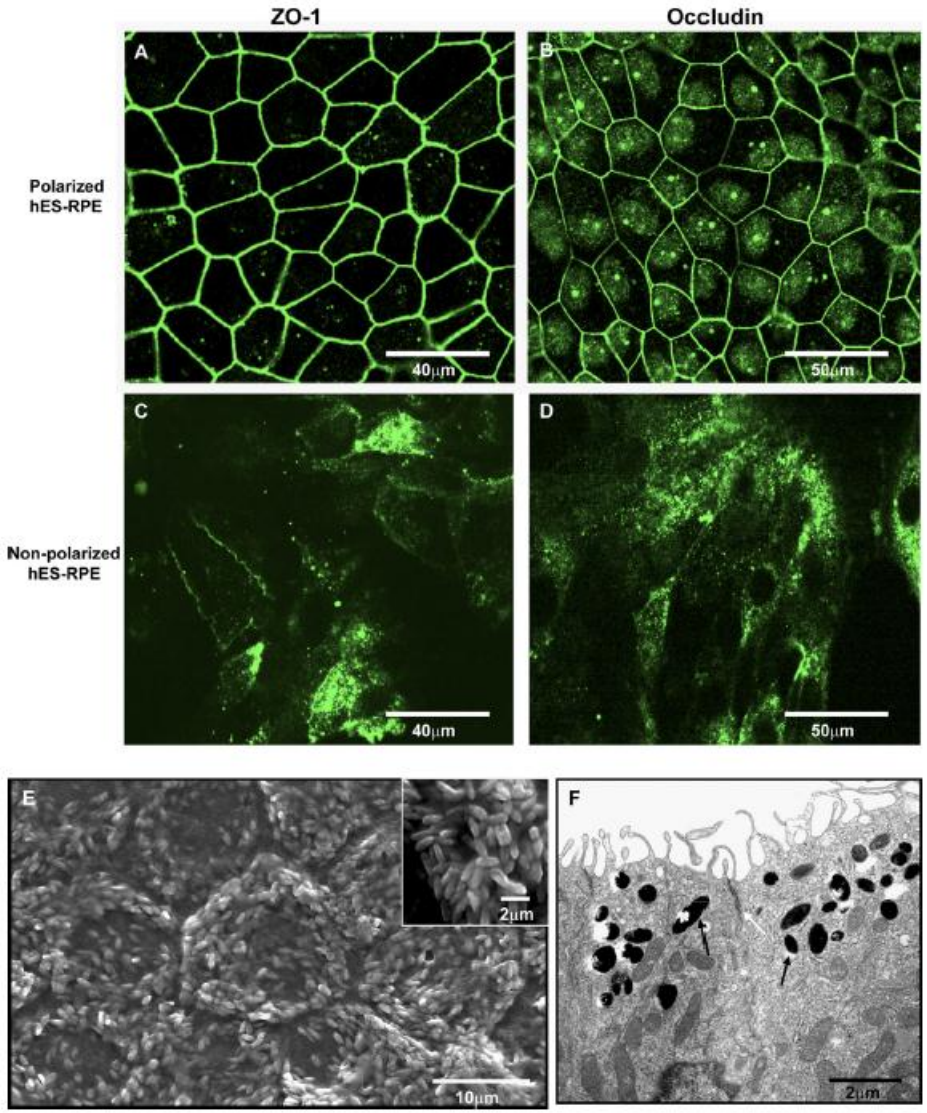
Treatment with the embryonic head inducer noggin
induces differentiation of human ES cells into primitive neural tissue
Nestin and Sox-2, markers of early neurogenesis

Groppe et al. Nature 420: 636, 2002



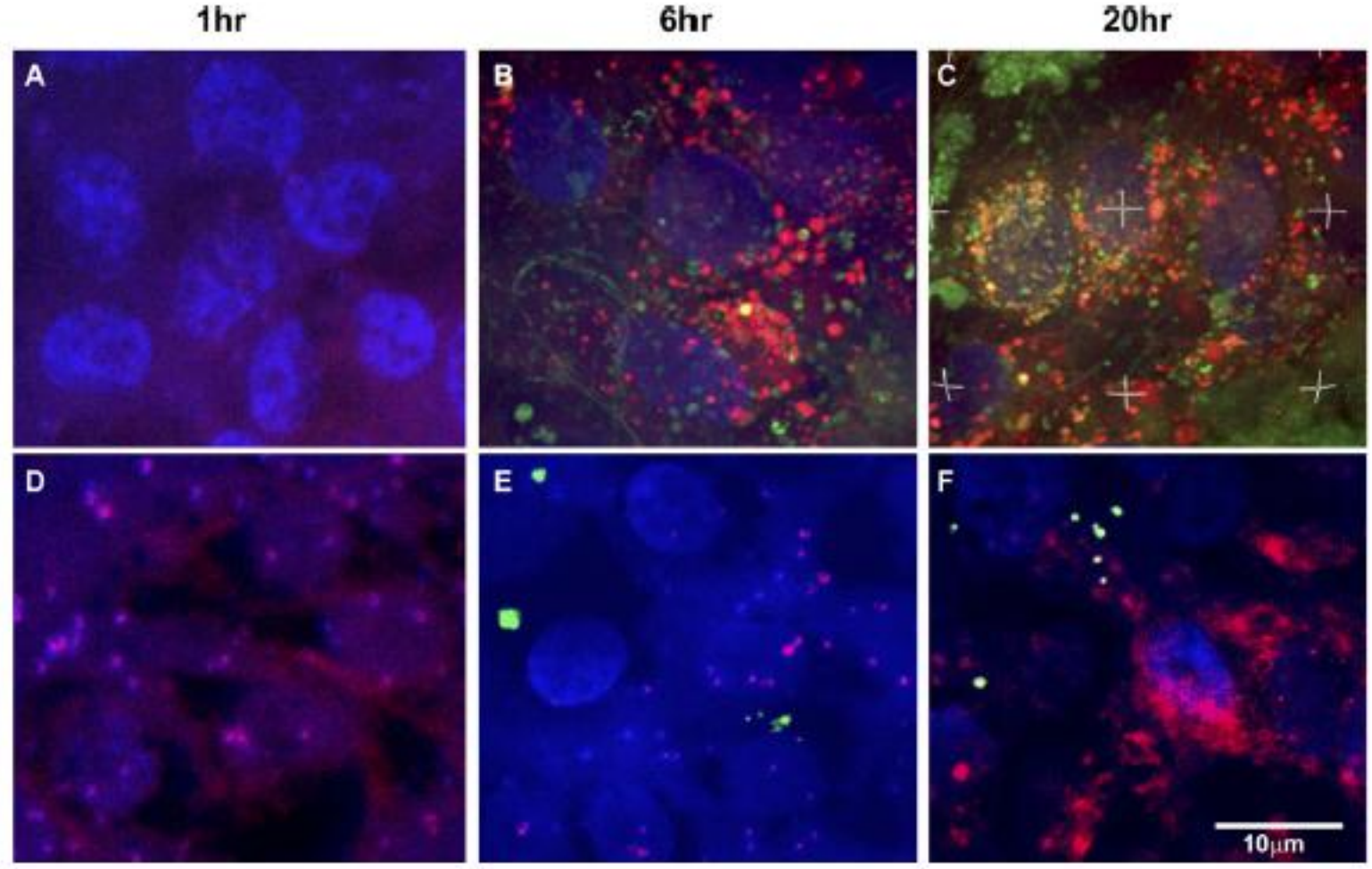
Conservation of developmental mechanisms


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



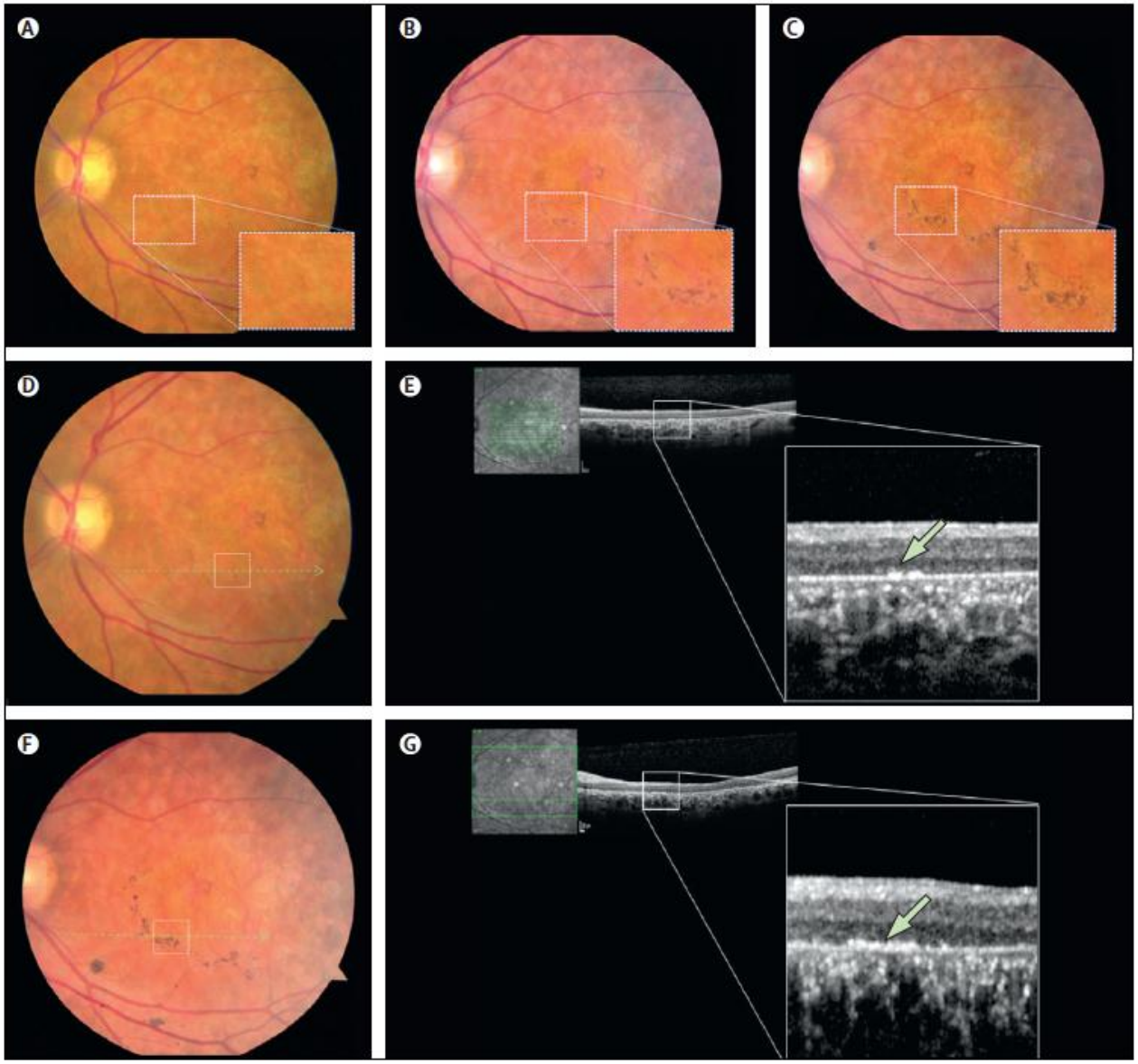
Macular Degeneration is a Promising Early Target

- 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

Induced Pluripotent Stem Cells

Induced pluripotent stem cells provide a new approach to tissue matching for transplantation and powerful research tools

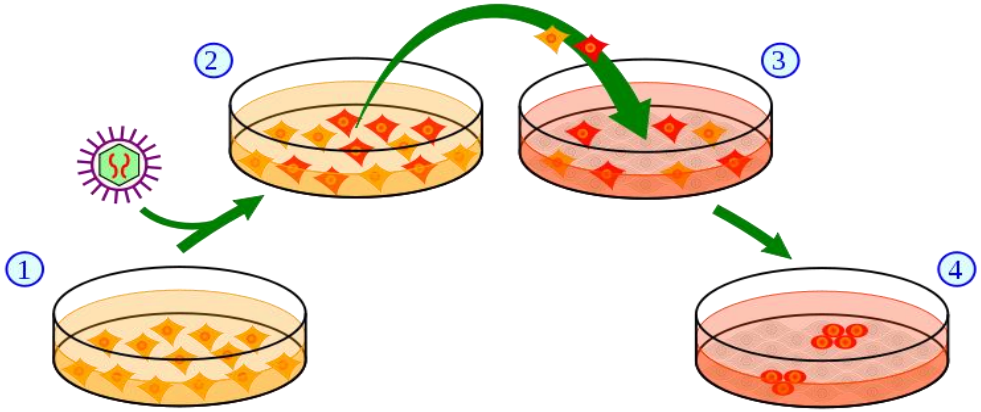
Reprogramming to Pluripotency

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

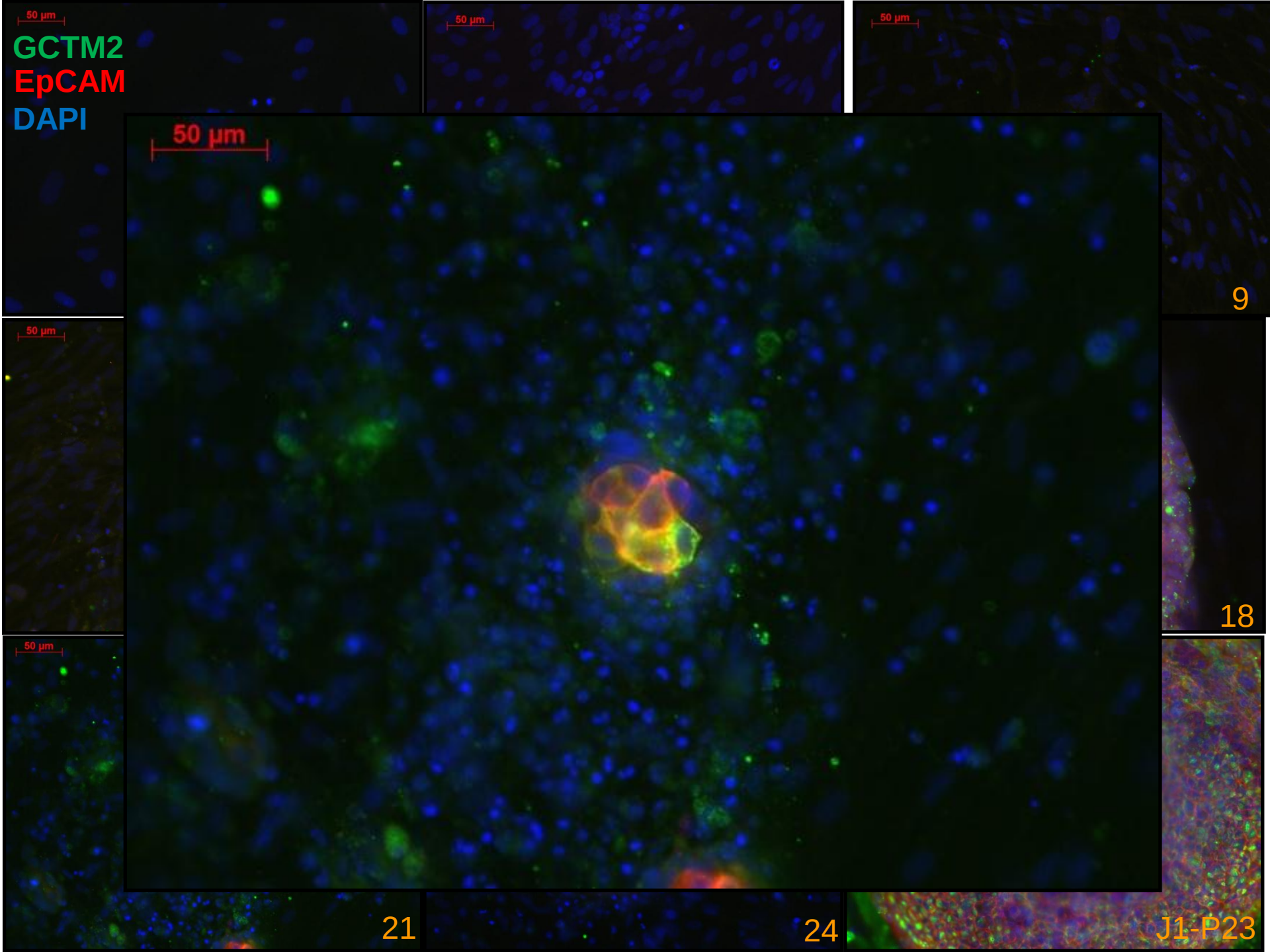
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

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

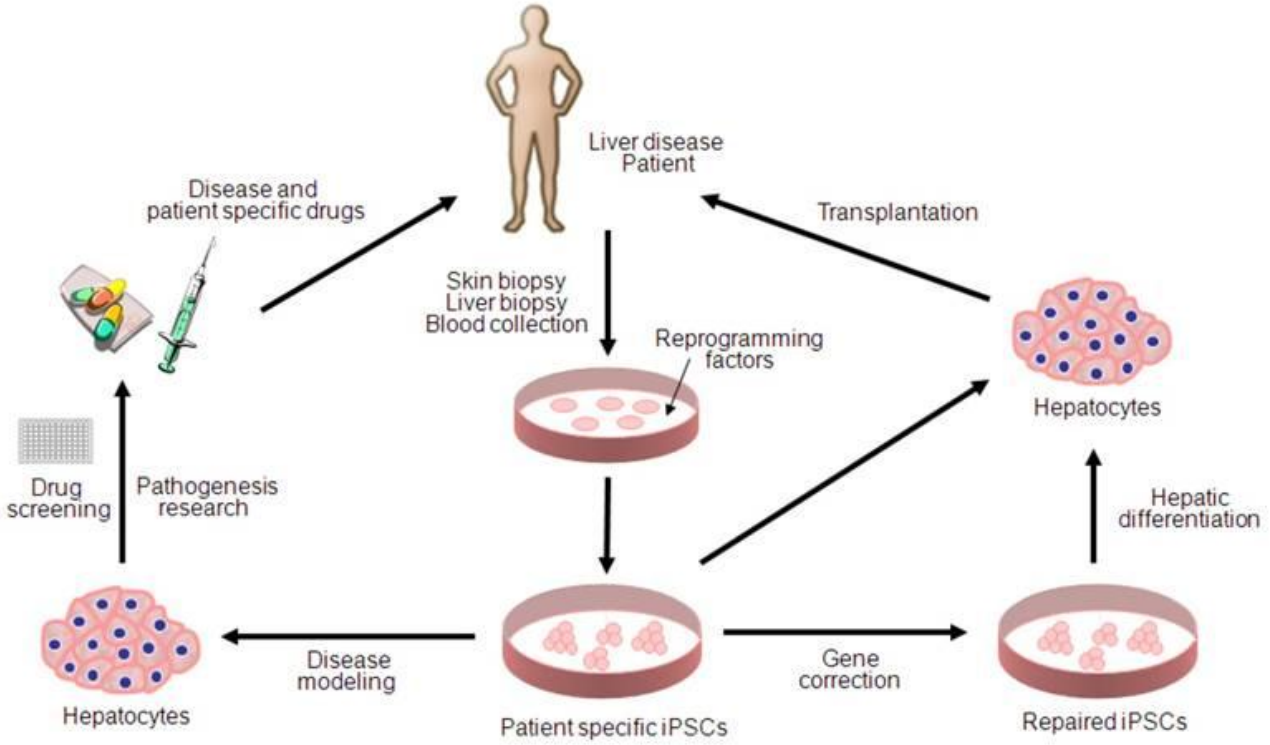


Yamanaka S et al *Cell* 2006 (mouse) and 2007 (human)


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

iPSC: applications

- Research: Disease Modeling
- Therapy: Tissue Matching



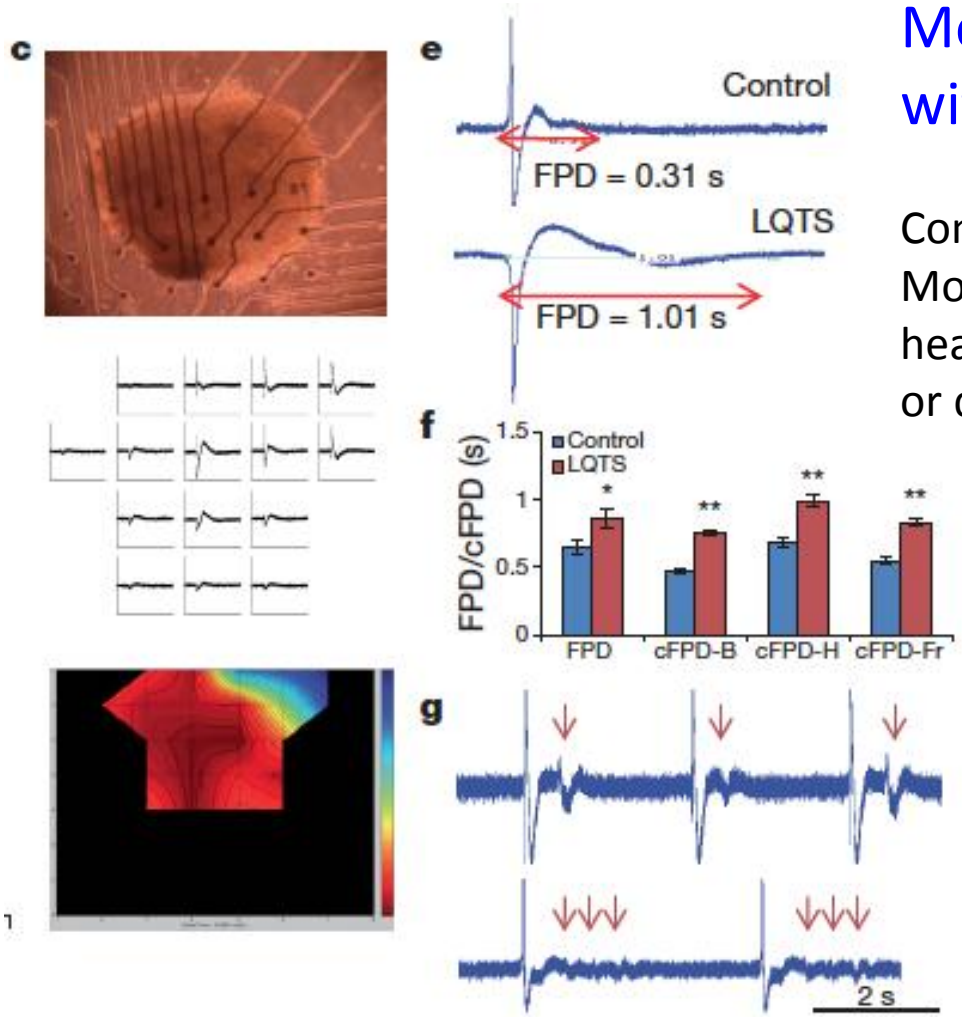
Yamanaka S et al *Cell* 2007
Chun YS et al *Int J Biol Sci* 2010

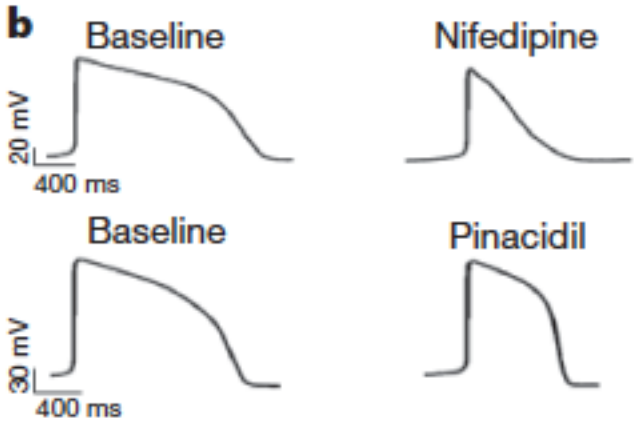
Pluripotent stem cells have important applications in biomedical research

- Basic studies of early human development and its disorders-birth defects, childhood cancers
- Functional genomics in human cells
- Discovery of novel factors controlling tissue regeneration and repair
- In vitro models for drug discovery and toxicology

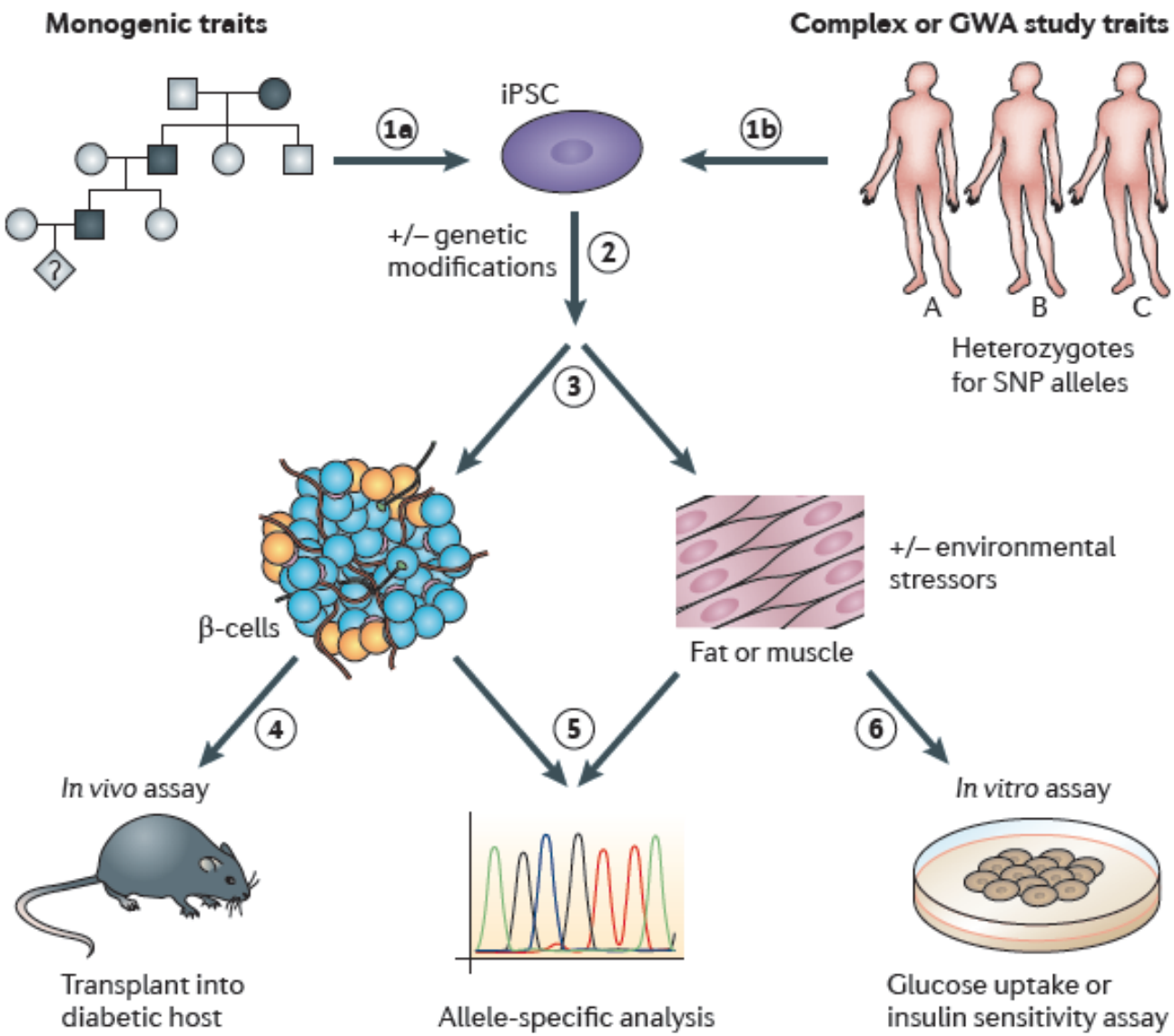
Modeling the long Q-T syndrome with human iPSC

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





Itzhaki et al. Nature 471: 225, 2011



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

Approaches to human functional genomics

Human Pluripotent cells: Functional Genomics Revolution

- There are differences between mice and humans
- We can make targeted genetic modifications in human ES cells to create disease models. We can study the effects of the mutations on development and physiology of specific cell types
- We can use the differentiated cells to develop and screen new medicines

iPSC advantages

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

Stem Cell Ethics

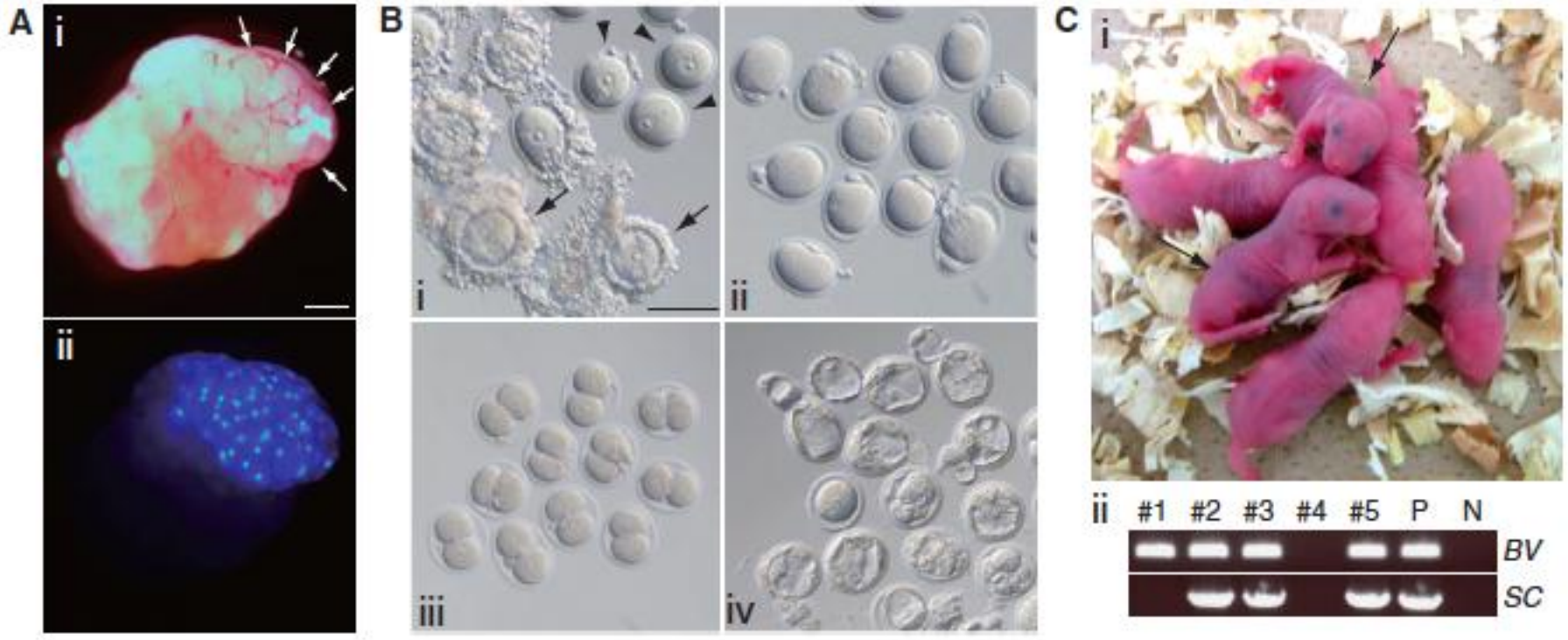
- The debate over the use of human embryos in research is not over, but it is of diminishing relevance to the field
- The availability of over 1000 ES cell lines and iPSC technology means that arguments for the use of embryos to achieve a new advance (that cannot be achieved by other means) must be very 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]*



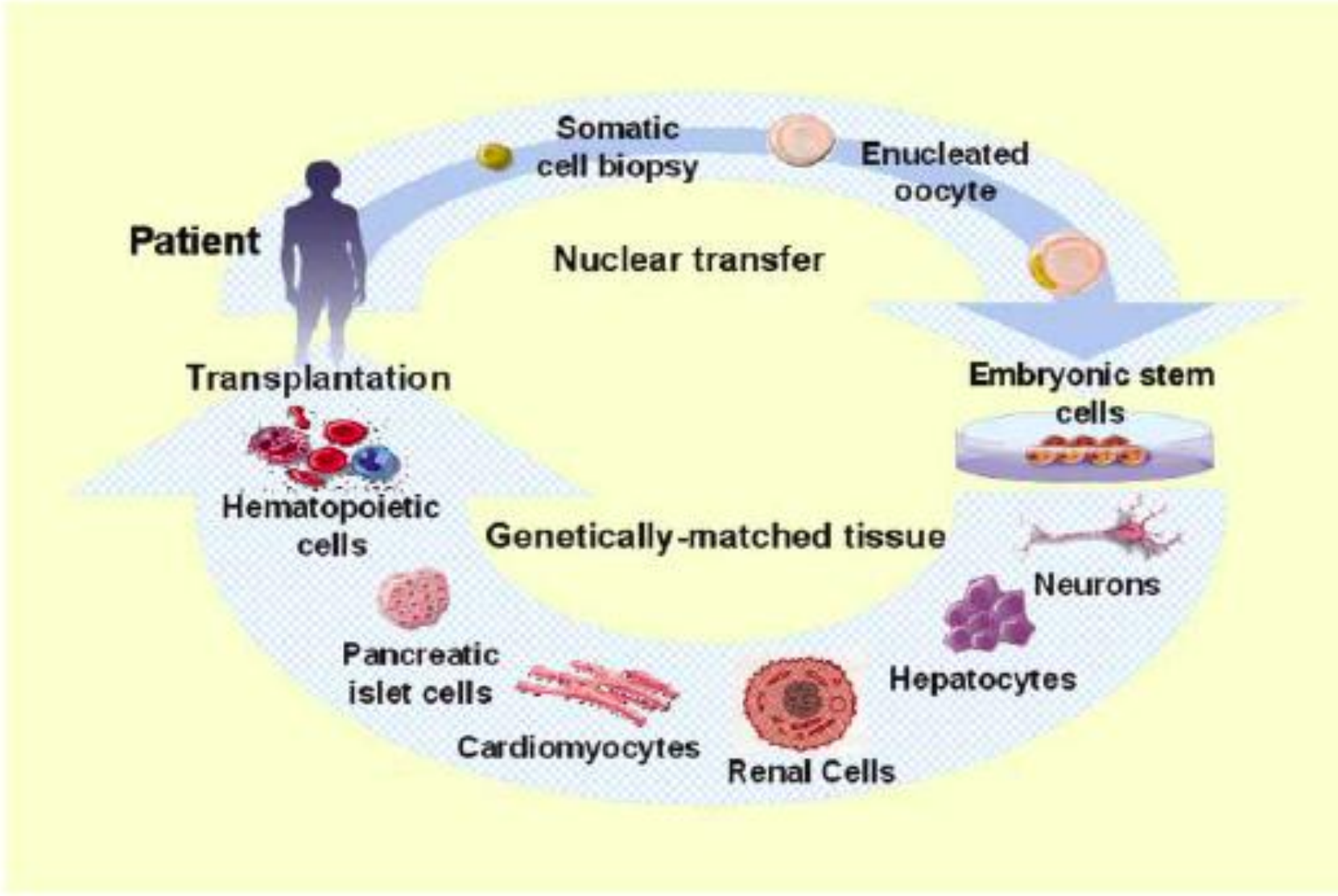
Differentiation of human iPS cells into gametes

- 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

Somatic cell nuclear transfer and patient specific therapy



A cat cloned by nuclear transplantation

This kitten's coat-coloration pattern is not a carbon copy of its genome donor's.

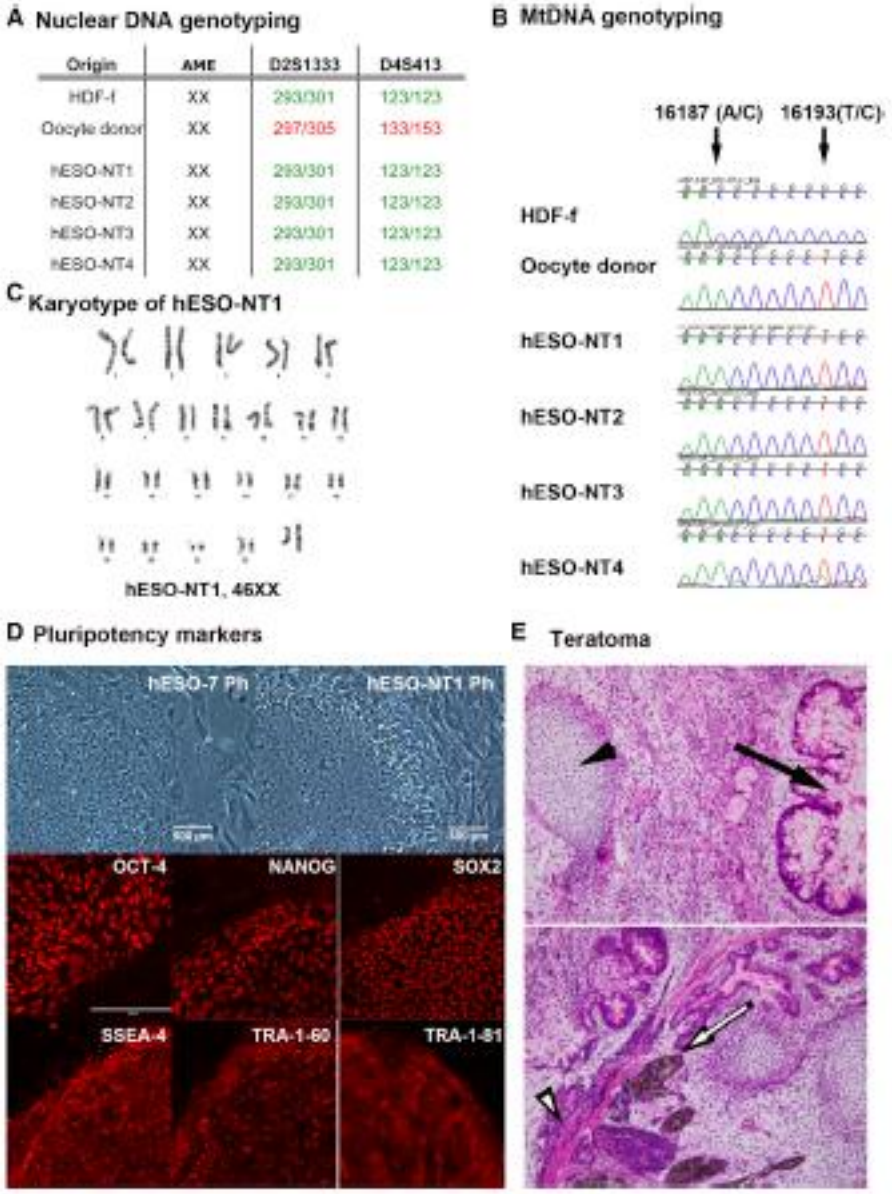
Sheep, mice, cattle, goats and pigs have all been cloned by transfer of a donor cell nucleus into an enucleated ovum[1–5], and now we add the successful cloning of a cat (*Felis domesticus*) to this list. However, this cloning technology may not be readily extendable to other mammalian species if our understanding of their reproductive processes is limited or if there are species-specific obstacles.

As a first attempt, we isolated adult fibroblast cells from oral mucosa obtained from an adult male cat, passaged them three to seven times for expansion in culture, and then froze and stored them in liquid nitrogen. For nuclear transfer, we thawed these cells and fused them with cat ova that had matured *in vitro* and which had had their metaphase chromosomes removed by micromanipulation (see supplementary information). We then transferred cloned embryos into synchronized recipient queens for development.

We carried out 188 such nuclear-transfer procedures, which resulted in 82 cloned

Figure 1 Nuclear-donor cat, and cloned kitten with its surrogate mother. **a**, Adult female cat that supplied cumulus cells for nuclear transplantation. The cells were cultured in DMEM/F12 medium for 5 days at 37 °C until confluent; micromanipulation was used to enucleate ova obtained after routine ovariohysterectomy and to transfer the donor's cumulus cells into the perivitelline space. Enucleated ova and cumulus cells were fused by electrofusion; a second electropulse was applied to activate the oocytes. **b**, The surrogate mother, a synchronized recipient of three cloned embryos, produced one cloned kitten, which was delivered by caesarian section 66 days after embryonic transfer. For further details, see supplementary information.

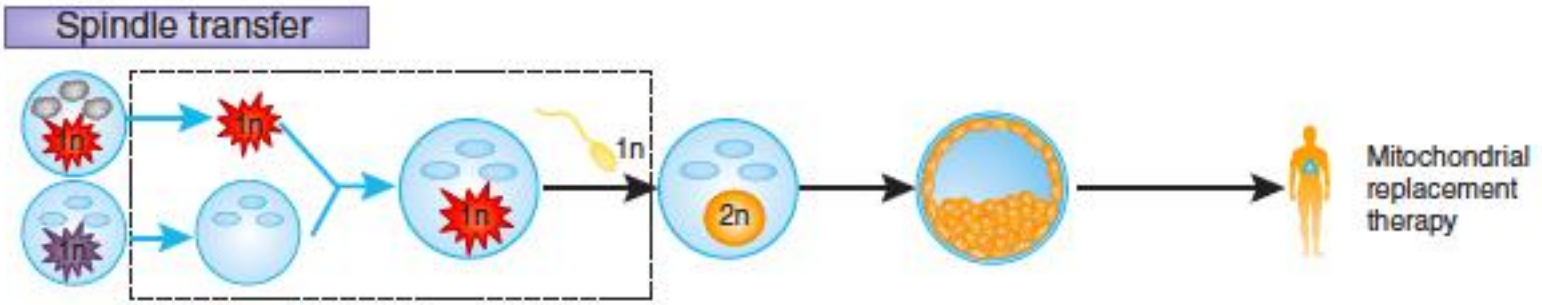
Many species of mammal have now been cloned. A cloned kitten costs $50K US.



Human SCNT: Multiple Refinements to the procedure enabled ES generation from a small number of oocytes.

Tachibana et al.
Cell 153: 1, 2013

Prevention of Mitochondrial Disease



Transfer of Maternal DNA from a patient with mitochondrial disease (spindle transfer) into a Healthy donor egg followed by fertilization by father's sperm

Unproven and Unfounded Stem Cell Treatments

GROW NEW BREASTS

Grant McArthur

MELBOURNE scientists are poised to begin revolutionary surgery to help cancer victims regrow their breasts.
The world-first trial could also change the cosmetic surgery industry by allowing women to grow bigger natural breasts.
The experimental stem cell breast-growing technique — called Neopec — could replace breast reconstructions and implants within years. The trial offers hope to more than 5000 Australian women who lose their breasts to cancer each year.
Continued Page 10

Stem cell cancer cure hope

Dental & Medical News: Stem Cells Grow Replacement Tooth in Mouse

Cloned puppies glow-in-the-dark

heraldsun.com.au

Stem cell method offers new hope

Grow your own heart

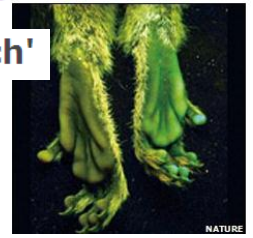
Mail Online

No men OR women needed: Scientists create sperm and eggs from stem cells

Rabbit penis grown in laboratory

Stem-Cell-Coated Contact Lenses Are Curing the Blind

Glowing monkeys 'to aid research'



Breakthrough in diabetes research

mX News

FLABBY LIFESAVER

Lipo fat turns to stem cells

HEALTH

Raelian leader says cloning first step to immortality

Weird True Freaky

Artificial sperm takes men out of equation

The Courier-MailApril 09, 2008 01:08am

Alzheimer's hope

MELBOURNE scientists are hoping to find a treatment for Alzheimer's disease through stem-cell research.

so in a world first they wo the neurons and implant
The team would use

Daily Telegraph

Scientists create 'monstrous' human-cow hybrid

STEM CELL CRYSTAL BALL

Stem cells turned into glow-in-the-dark blood cells

Grow your own organs

Unproven, Unfounded and Unethical Stem Cell Therapies

- Costly
- Claim widespread benefits for many conditions
- Based on anecdotal evidence, patient testimonials
- Poorly defined product
- No scientific rationale
- Take advantage of misinformation concerning stem cells and patients looking for hope

Adult stem cell therapies walk the line

Tension between practitioners who believe autologous stem cells should be considered a service and the FDA, which considers some of them biologics, has come to a head in recent months. Laura DeFrancesco investigates.

Almost a year after Texas governor Rick Perry received injections of his own stem cells for a back ailment, a US Food and Drug Administration (FDA) inspection of the company that prepared the cells—Sugar Land, Texas–based Celltex—has raised red flags. The FDA's inspection report, a so-called form 483, details myriad problems with facilities and sample preparation. As *Nature Biotechnology* went to press, Celltex is still operating and claims to be cooperating with the FDA. But Celltex's problems are indicative of those at other adult stem cell companies that have fallen afoul of FDA oversight. For example, a legal battle that dragged on for four years between the FDA and Broomfield, Colorado–based Regenerative Sciences, which offers mesenchymal stem cell treatments for orthopedic indications. The company claims that it has altered the procedures that originally infringed FDA rules and that it is now compliant. Meanwhile, it has filed suit against the agency for interfering with what its management (and others) consider a legitimate use of autologous stem cells[1]. As *Nature Biotechnology* was going to press, the US District Court for the District of Columbia ruled in favor of the FDA's position.

Celltex and other such outfits that prepare autologous stem cells for medical uses claim that they provide a medical product, not a drug, and hence lie outside the FDA's jurisdiction. The FDA begs to differ (**Box 1**). It has designated Celltex a biological drug manufacturer, which must, as such, follow good manufacturing practices. But groups of stem cell practitioners in both the US and Europe continue to push back against regulators. The issues involved are varied and complex—from states' and patients' rights to the mechanism of stem cell action. The field is waiting to see whether the resulting wrangle will mean regulators will clamp down or give it more freedom.

The proven pathway

Numerous autologous cell therapies are being taken down the established regulatory pathway for cellular products, with a small number having received approval. According to data compiled by Lee Buckler of the Cell Therapy Group, a consultancy group located in Bellingham, Washington, 47 industry-sponsored clinical trials of autologous cells are in pivotal or late stages involving 37 companies. (http://celltherapyblog.blogspot.ca/2011/12/active-phase-iii-or-iiiii-cel-therapy.html). The majority of these trials, however, originate not in companies but in academia or hospital settings, many of which are being conducted outside of the US. Commercial entities, although active in the space, face considerable hurdles with autologous stem cells, due to the difficulty and expense of creating individualized drugs. Seattle-based Dendreon's epic problems with both the manufacture and the uptake of its autologous prostate cancer vaccine (Provenge, sipuleucel-T) typify the difficulty with this approach.

Coming to a state near you. Celltex Therapeutics celebrates its new 15,000-square-foot facility in Sugar Land, Texas, and its new partner, the Korean stem cell outfit RNL BIO, in this picture from last December.

However, a few autologous cell therapies have passed regulatory muster in various locales. In the US, in addition to Provenge, an autologous fibroblast product for filling in wrinkles was approved this year (laViv, manufactured by Fibrocell; Exton, PA, USA) and autologous chondrocytes were approved in the mid-nineties for cartilage repair (Carticel, Genzyme/Sanofi, Paris). In South Korea, two autologous programs have received approval in the past few years—an autologous bone marrow–derived cell therapy for myocardial infarction (Hearticellgram, manufactured by Pharmacell of Seoul.) and an adipose tissue–derived cell therapy for anal fistulas (Cupistem, manufactured by Anterogen of Seoul). Clinical trials results have never been published for the products approved in Korea nor have the claims been replicated in laboratories outside of the companies.

Elsewhere, a small cadre of companies is trying to make a go of it with autologous stem cells (**Table 1**). Aastrom Biosciences, based in Ann Arbor, Michigan, may be the farthest along; the company reported positive phase 2 results with its autologous bone marrow–derived product (ixmyelocel-T) for critical limb ischemia last November[2] and has initiated phase 3 studies, which are expected to be completed in 2014, according to Aastrom CEO Tim Mayleben.

Going rogue

Other groups, both within the US and outside of it, are treating patients and collecting payments—rather substantial ones, in the tens of thousands of dollars—for procedures that have not been thoroughly vetted, from breast augmentation and other cosmetic procedures to orthopedic conditions and a host of hard-to-treat disorders, such as Alzheimer's disease and amyotrophic lateral sclerosis. In the past, these clinics operated largely outside the US, but increasingly they are popping up within US borders.

Whereas certain autologous cell-based therapies are regulated by the FDA's Center for Biological Evaluation and Research (CBER), those posing minimal risk and fulfilling other requirements, are not (**Box 2**), enabling companies to go straight to the market if they feel they do not qualify for oversight. This ostensibly puts the onus on the FDA to find firms that have flouted rules and stop them if they continue to do business outside the law. The ability to operate under the FDA's radar provides some unscrupulous companies with a window of opportunity to profit from products that are of dubious efficacy at best or, at worst, unsafe for patients.

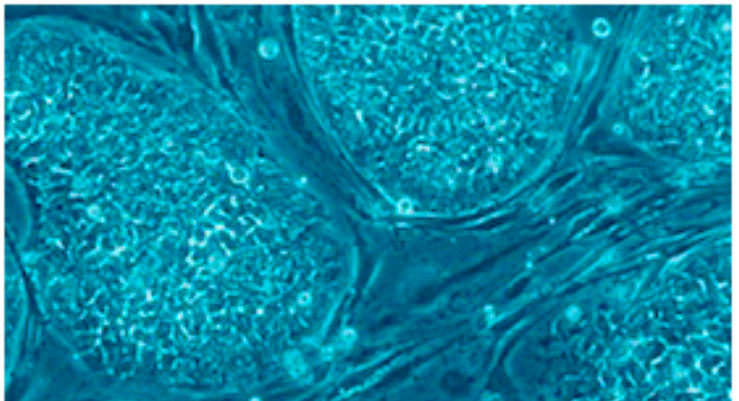
Another strategy for commercializing cell-based therapies that companies like Celltex are using is to position themselves as producers of cells, which they then provide to physicians who can decide, based on their medical expertise, how best use to use the preparation. Celltex believes this puts the therapy in the realm of medical practice, which the FDA does not regulate.

In the Flesh: The Embedded Dangers of Untested Stem Cell Cosmetics

Unapproved procedures and skin care products endanger consumers and clinical research

By Ferris Jabr

When cosmetic surgeon Allan Wu first heard the woman's complaint, he wondered if she was imagining things or making it up. A resident of Los Angeles in her late sixties, she explained that she could not open her right eye without considerable pain and that



Clinical Trials of Stem Cells

- Well characterized product and manufacturing process
- Preclinical evidence for efficacy and safety
- Mode of action defined
- Approved by regulatory body eg FDA
- Approved by local IRB
- Appropriate risk/benefit ratio