Recerca en cèl.lules mare pluripotents

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Pluripotència i cèl.lules mare pluripotents

Cèl.lules mare embrionàries (ESC)

Reprogramació nuclear

- Transferència nuclear
- Cèl.lules mare de Reprogramació induïda (iPS)

Caracterització i Diferenciació

Terapia Cel.lular

ESSAY

30 years: from IVF to stem cells

Ruth Deech, former chair of Britain's Human Fertilisation and Embryology Authority, reflects on how the science that gave an infertile couple a baby has been extended to saving lives.

Evening News

LATE SPECIAL

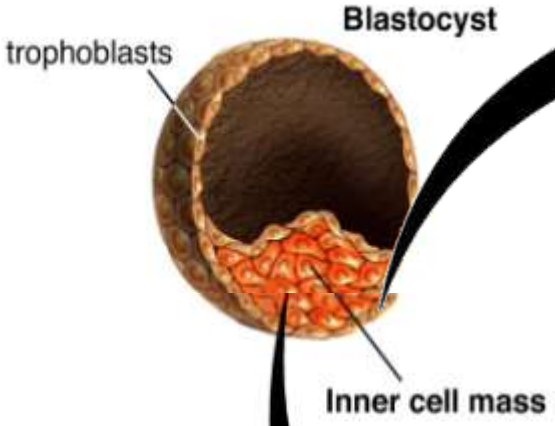
Meet Louise, the world's first test-tube arrival

SUPERBABE

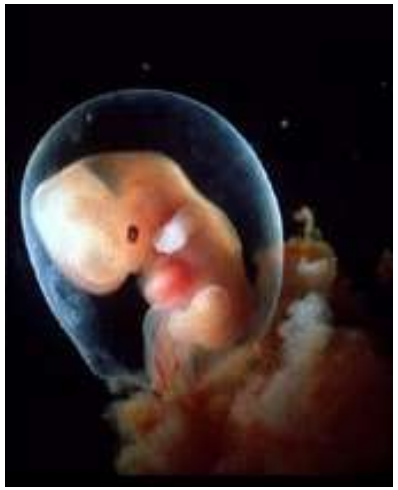
Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

The Nobel Prize in Physiology or Medicine 2010

Robert G. Edwards

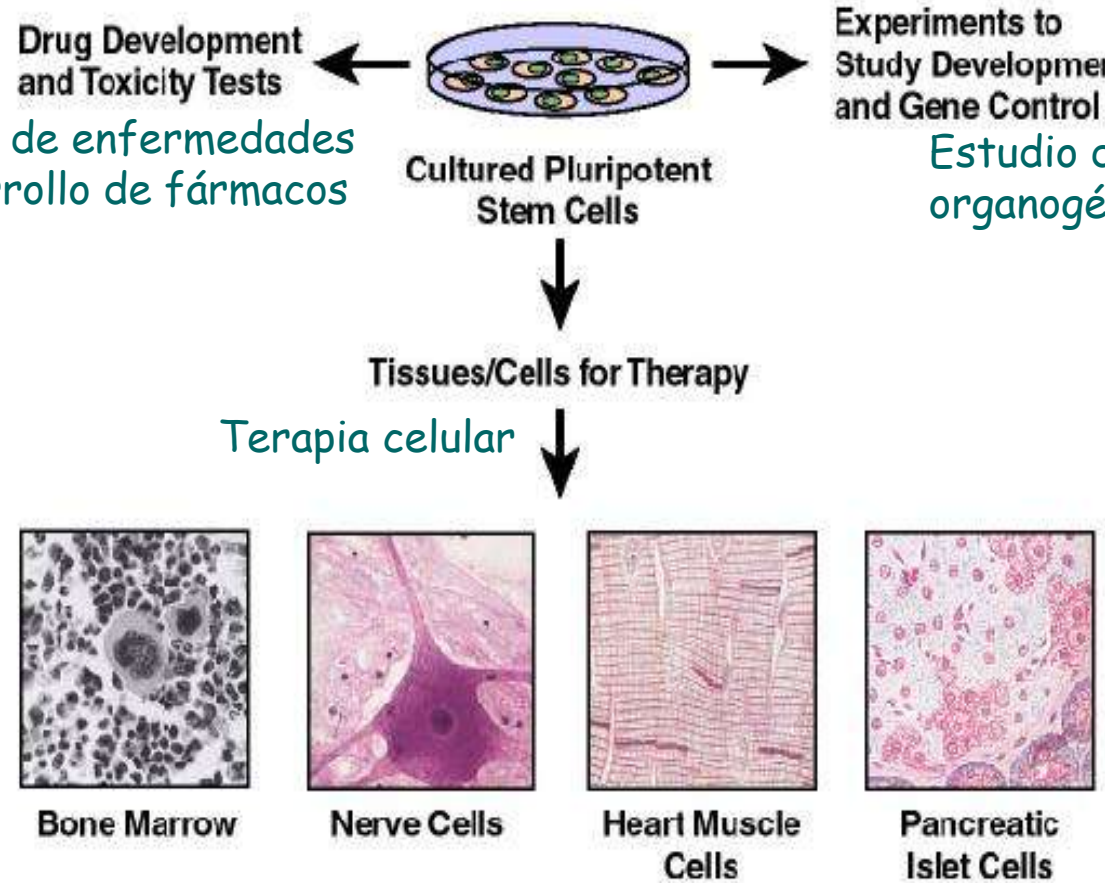


In vivo



In vitro

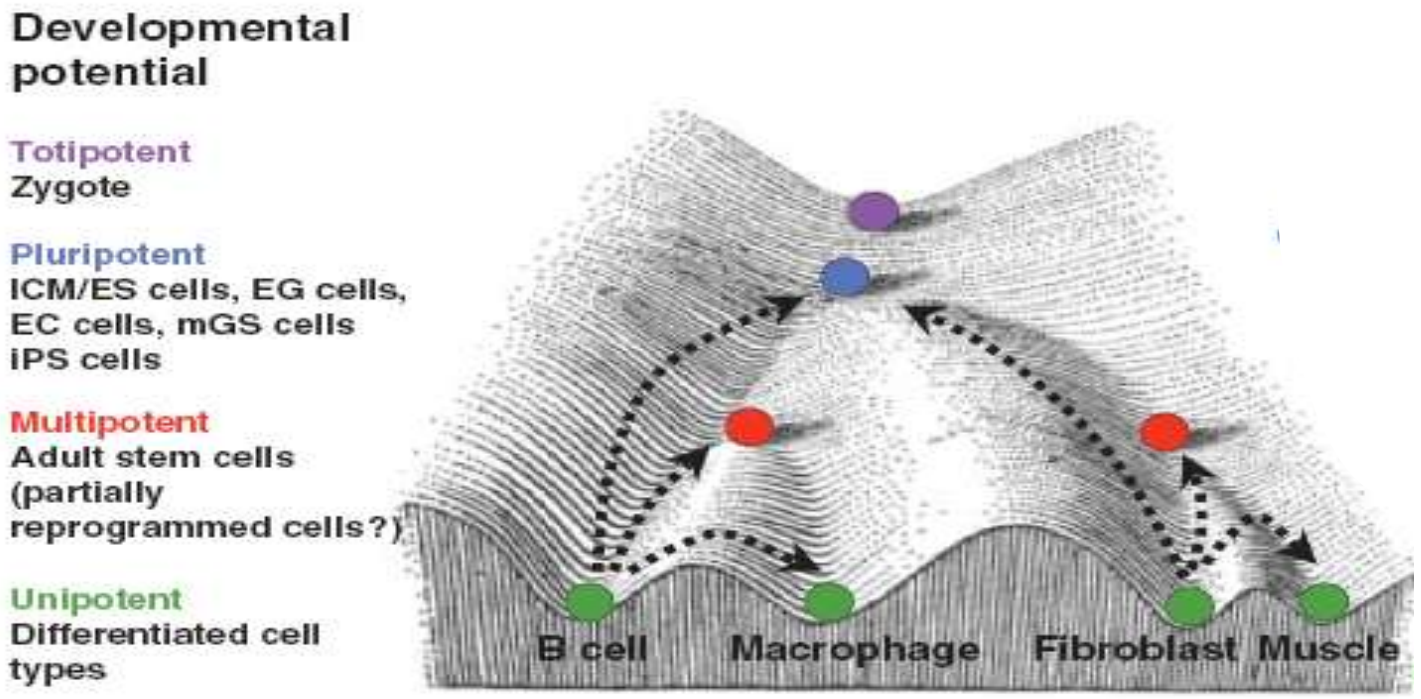
The Promise of Stem Cell Research



Modelo de enfermedades y desarrollo de fármacos

Estudio del desarrollo y organogénesis

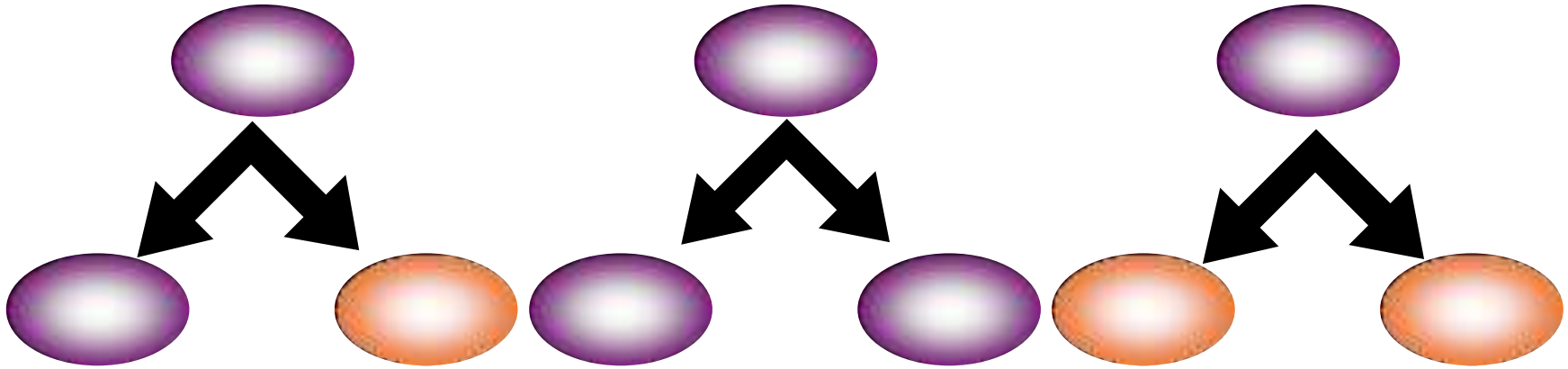
Terapia celular



Hochedlinger, Development 2009 (adapted from Waddington, 1957)

- Pluripotent Stem Cells can be obtained from cells located in the inner cell mass of blastocysts, early embryos and isolated blastomeres **(hESC)** and from nuclear reprogramming (Somatic Cell Nuclear Transfer - **SCNT** and induced Pluripotent Stem Cells - **iPS)**

Pluripotent stem cells: Self renewal/Differentiation



Asymmetric division Self renewal Differentiation

human Embryonic Stem Cells (hESC)

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

EMBRYONIC STEM CELLS

- Derived from the inner cell mass of the blastocyst (ICM) (day 5-7 of development,

  ± 150-200 cells). They give rise to the 3 germ layers: ectoderm, mesoderm and endoderm.

Nuclear Reprogramming

- Somatic Cell Nuclear Transfer (SCNT)
- Induced Pluripotent Stem Cells (iPS)

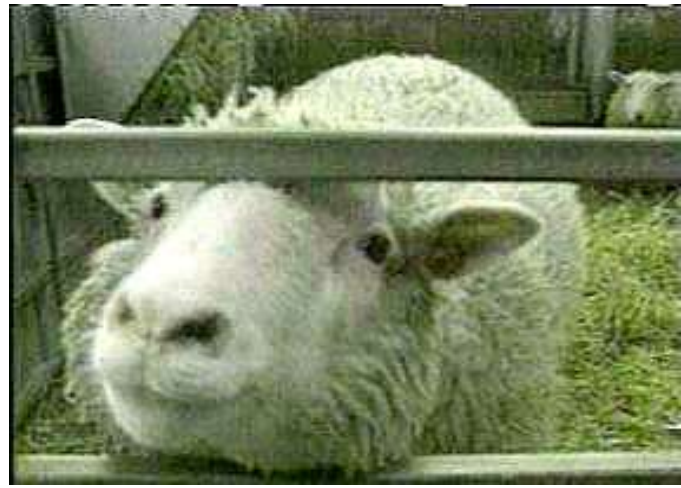
- Somatic Cell Nuclear Transfer (SCNT)


12-12-00 2H
14:19:47
Oocyte Enucleation

CLONACIÓN
TRANSFERENCIA DE NUCLEO (SCNT)

célula de glándula mamaria

Transferencia a ovocito enucleado

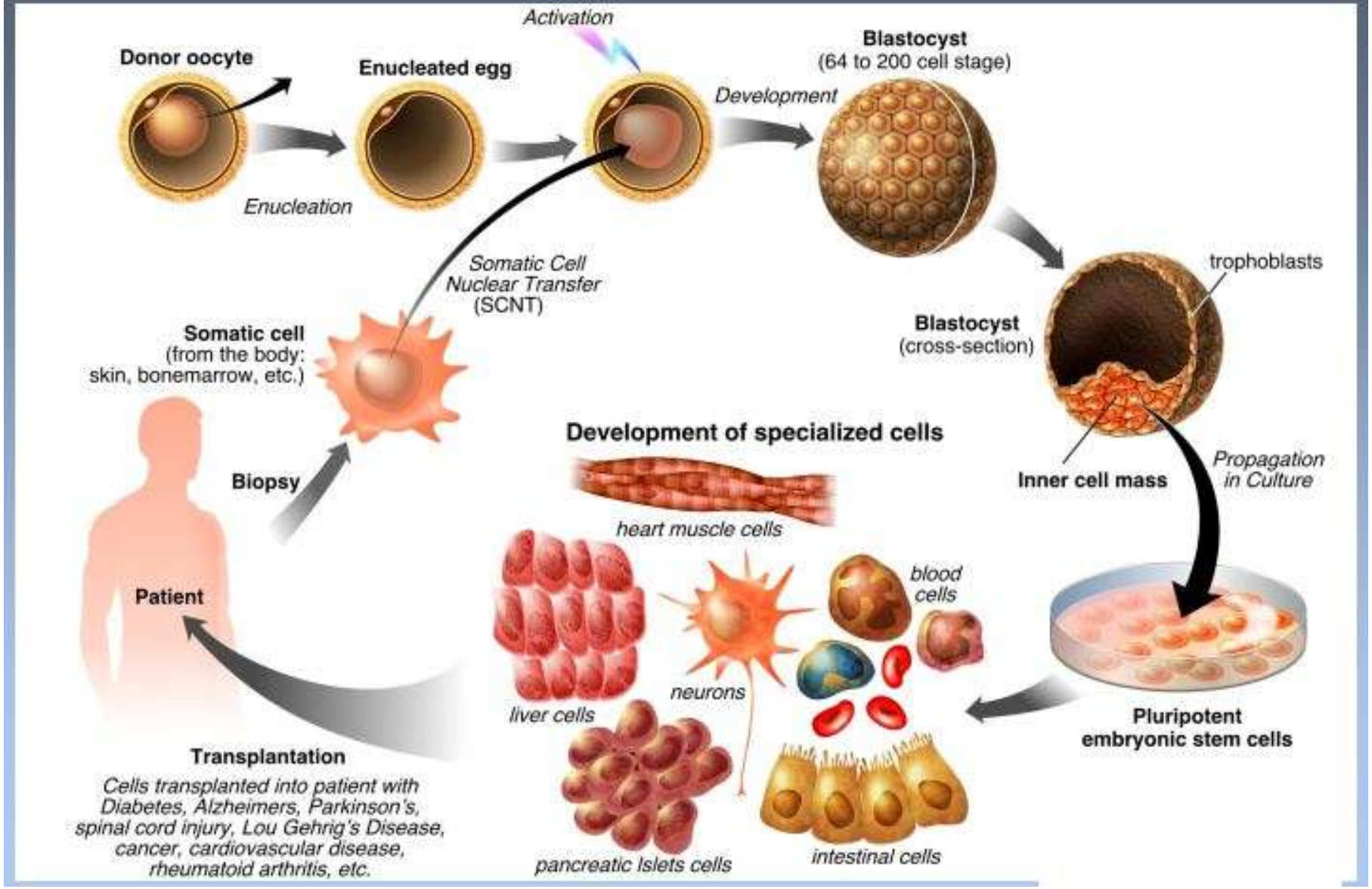


embrión

Transferencia a oveja portadora

Dolly

Nuclear transplantation



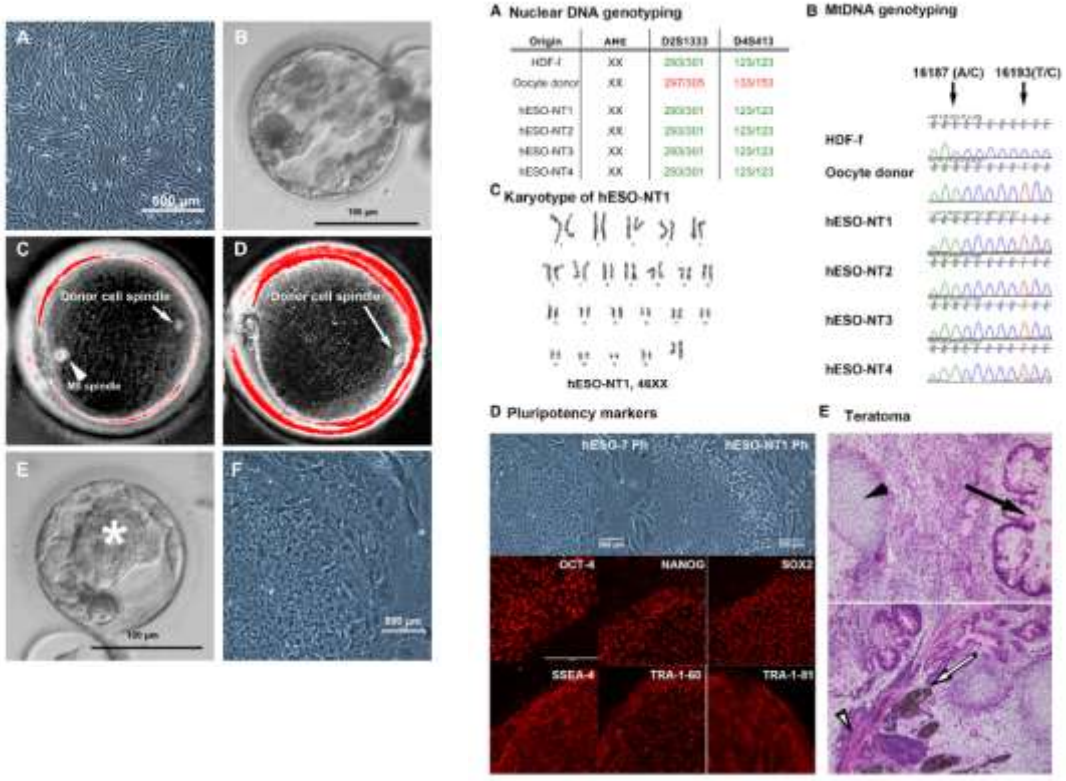
Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer

http://dx.doi.org/10.1016/j.cell.2013.05.006

Masahito Tachibana,[1] Paula Amato,[2] Michelle Sparman,[1] Nuria Marti Gutierrez,[1] Rebecca Tippner-Hedges,[1] Hong Ma,[1] Eunju Kang,[1] Alimujiang Fulati,[1] Hyo-Sang Lee,[1,6] Hathaitip Sritanaudomchai,[3] Keith Masterson,[2] Janine Larson,[2] Deborah Eaton,[2] Karen Sadler-Fredd,[2] David Battaglia,[2] David Lee,[2] Diana Wu,[2] Jeffrey Jensen,[1,4] Phillip Patton,[2] Sumita Gokhale,[5] Richard L. Stouffer,[1,2] Don Wolf,[1] and Shoukhrat Mitalipov[1,2,*]

2013

Cell



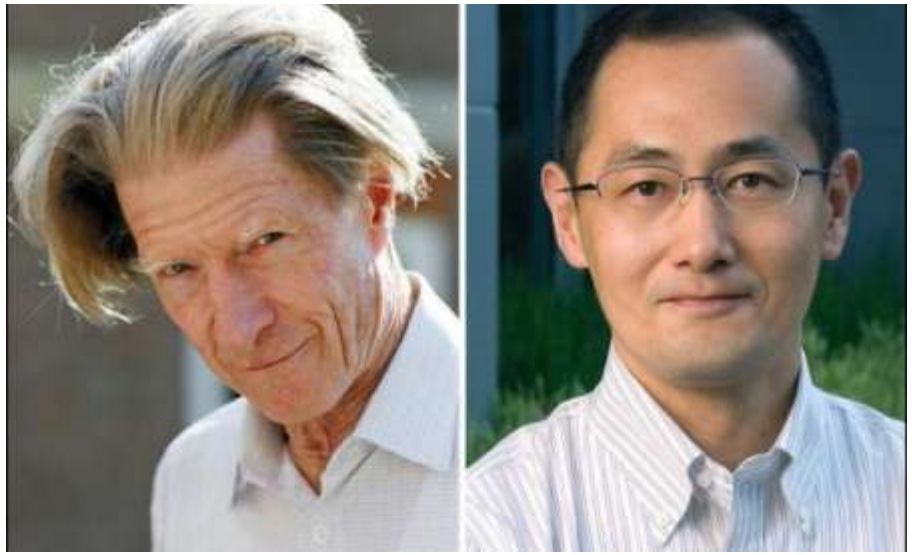
- 4 NT hESC were obtained with optimized SCNT approaches.
- Key factors for the success of SCNT:
  - Oocyte quality
  - Adequate exit from meiosis
  - Oocyte activation by electroporation
  - Improved embryo development with caffeine

- NT hESC displayed normal diploid karyotypes and inherited their nuclar genome from parental somatic cells
- Gene expression and differentiation profiles are similar to embryo derived hESC.

Nuclear Reprogramming

- Induced Pluripotent Stem Cells (iPS)

The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka *"for the discovery that mature cells can be reprogrammed to become pluripotent"*



SCNT iPS

Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.

Takahashi K, Yamanaka S.

Cell. 2006.

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

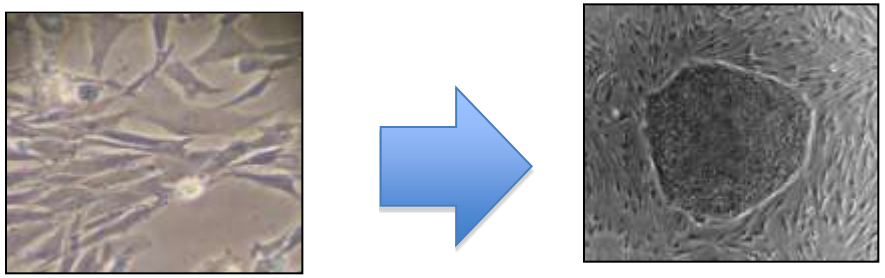
Cell, 2007

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,[1,2*] Maxim A. Vodyanik,[2] Kim Smuga-Otto,[1,2] Jessica Antosiewicz-Bourget,[1,2] Jennifer L. Frane,[1] Shulan Tian,[3] Jeff Nie,[3] Gudrun A. Jonsdottir,[3] Victor Ruotti,[3] Ron Stewart,[3] Igor I. Slukvin,[2,4] James A. Thomson[1,2,5*]

Science, 2007

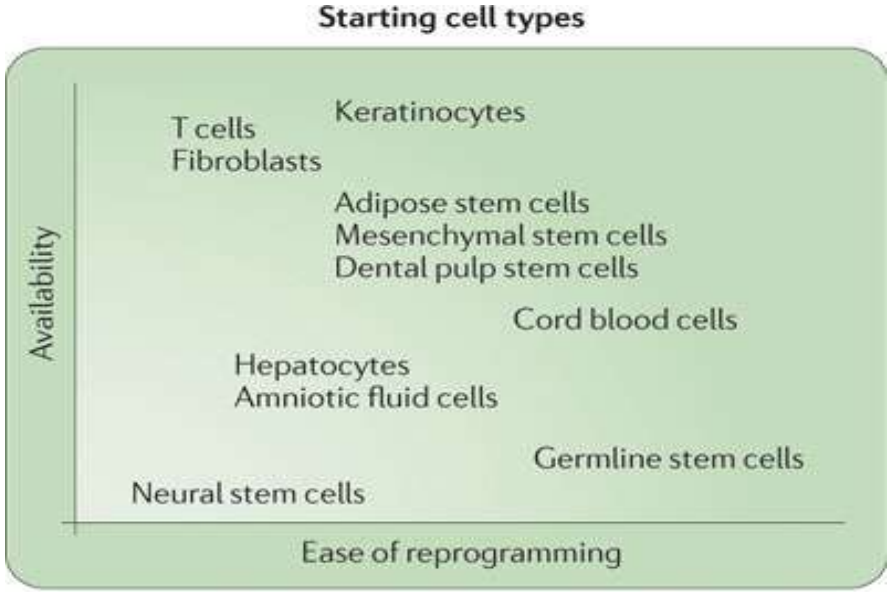
Induced pluripotent stem cells (iPS) generation

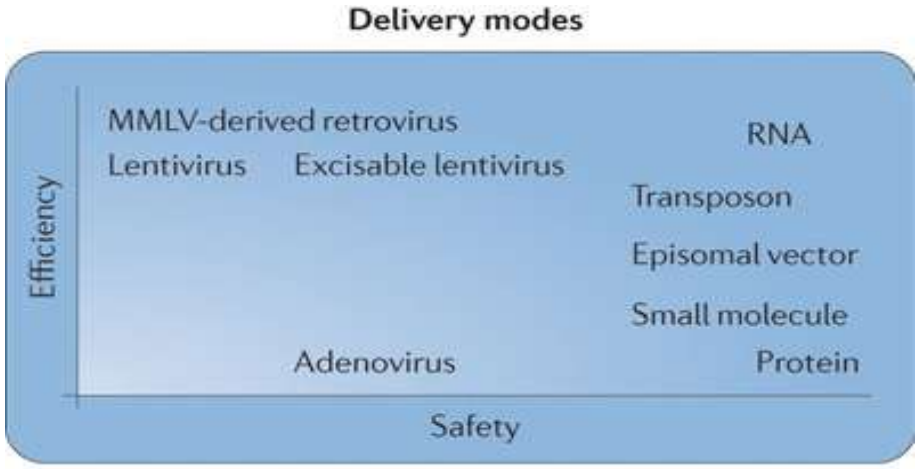


- The first iPS cell line generated with 24 factors. (Takahashi K & Yamanaka S. Cell 2006)

- The Classical 4 factors cocktail: **Oct4/3, Sox2, c- myc & KlF4 or Oct4/3, Sox2, Lin28 & Nanog** (Takahashi K & Yamanaka S. Cell 2006, Takahashi K et al, Cell 2007, Yu et al, Science NY, 2007Park et al, Nature 2008)

Stable Karyotype
Methylation of Nanog/Oct4 promoters
Transgene expression silencing
Expression of endogenous pluripotent associated markers
In vitro/In vivo differentiation
Chimera contribution*





F. González et al *2011*

Nature Reviews | Genetics

iPS GENERATION

- Choice of starting cell types
- Choice of methods of factor delivery
- Choice of factors

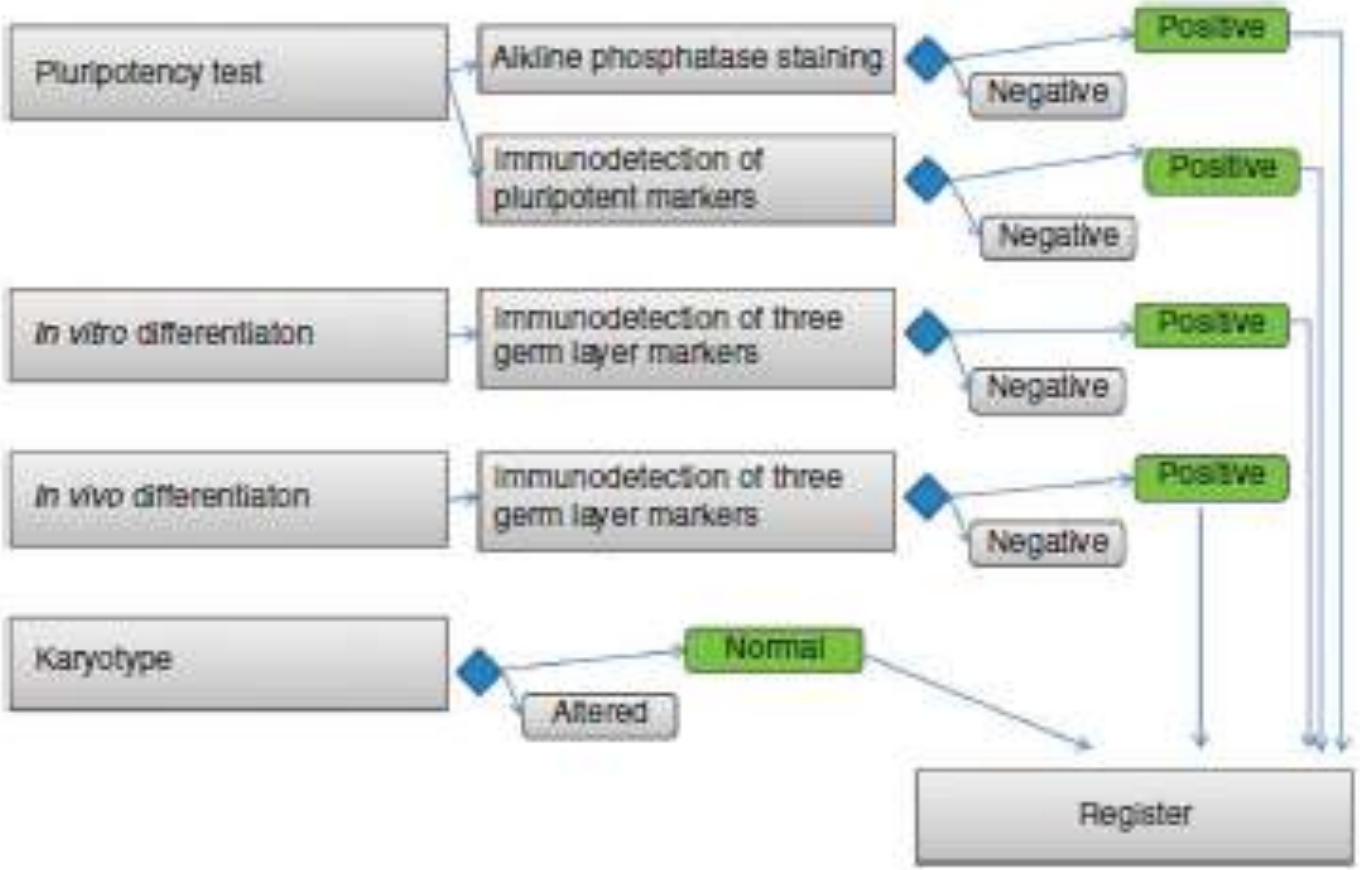
Factors

To express/overexpress	To repress
Important for embryonic development: OCT4, SOX2, NANOG, UTF1, LIN28, SALL4, NR5A2, TBX3, ESSRB, DPPA4	Apoptosis, cell cycle and senescence: $p16^{INK4A‡}$, p53‡, microRNA, p21
Proliferation and cell cycle: MYC*, KLF4*, SV40LT*, REM2, MDM2*, cyclin D1*	Epigenetic regulators: histone deacetylase, histone demethylase, G9a, DNMT1*
Epigenetic regulators: CHD1, PRC2	Signalling pathways: TGFβ, WNT, ERK–MAPK
Others: vitamin C, hypoxia, E-cadherin, miR-294, TERT*	*Potential oncogene ‡Potential tumour suppressor gene

- Lentivirus/retrovirus mediated reprogramming methods are still major approaches for generation of iPS

Characterization of pluripotent stem cells

Mercè Martí[1], Lola Mulero[1], Cristina Pardo[1], Cristina Morera[1], Meritxell Carrió[1], Leopoldo Laricchia-Robbio[1], Concepcion Rodriguez Esteban[1,2] & Juan Carlos Izpisua Belmonte[1,2]

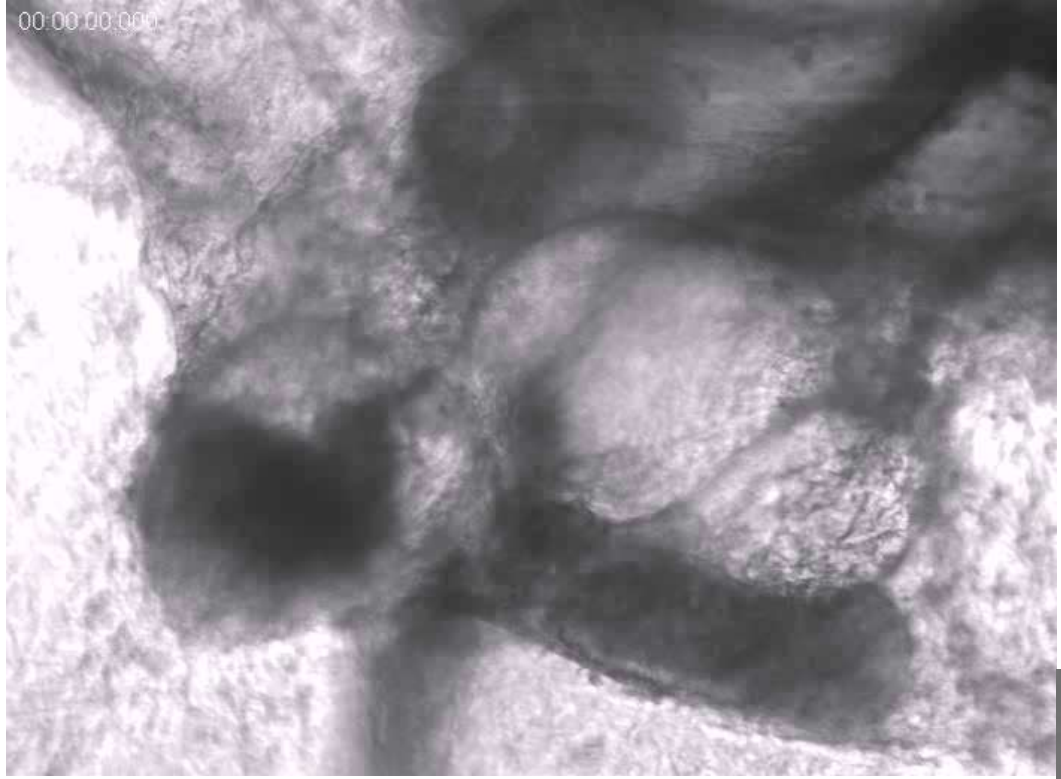


DIFFERENTIATION

Different cell types have been obtained

- Cardiomyocytes
- Neuronal cells
- Hematopoyetic cells
- Pancreatic cells
- Hepatocytes
- Trophectoderm

- Gametes: oocytes and sperm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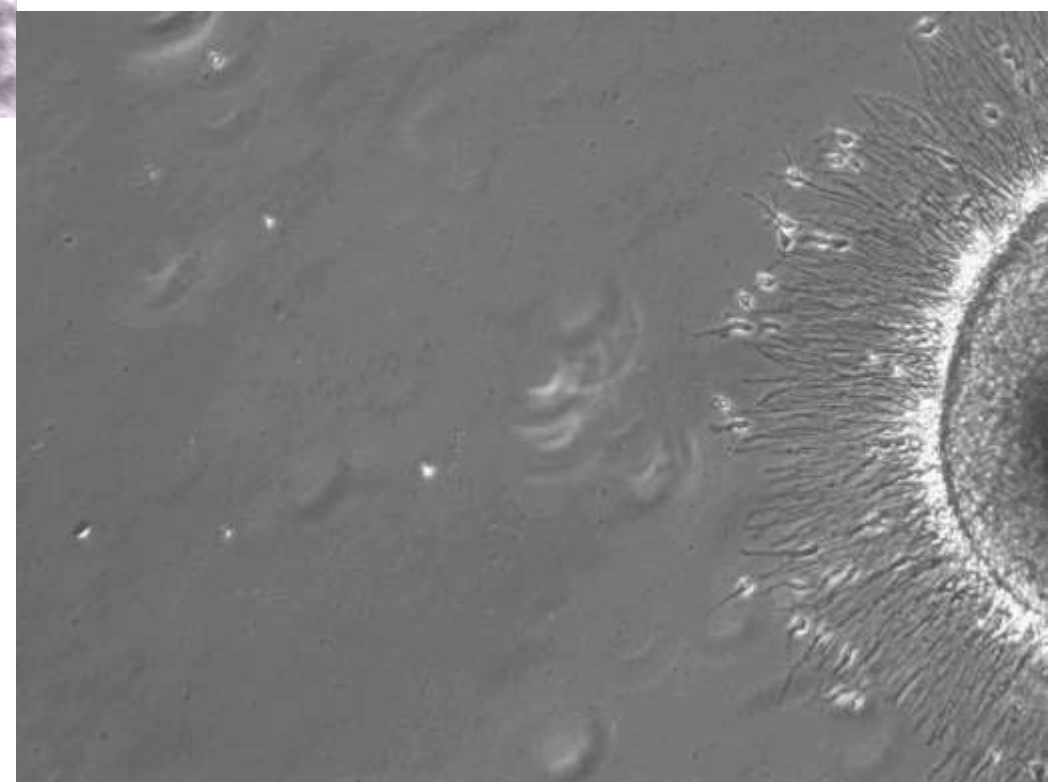
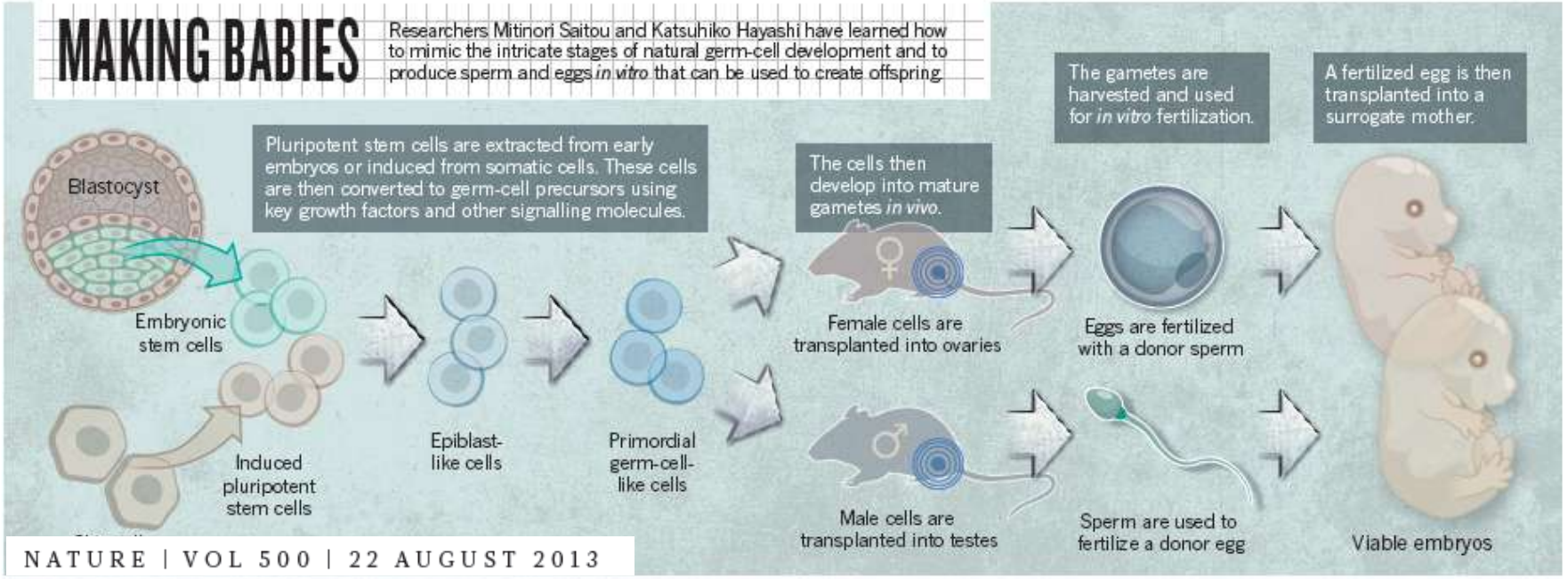
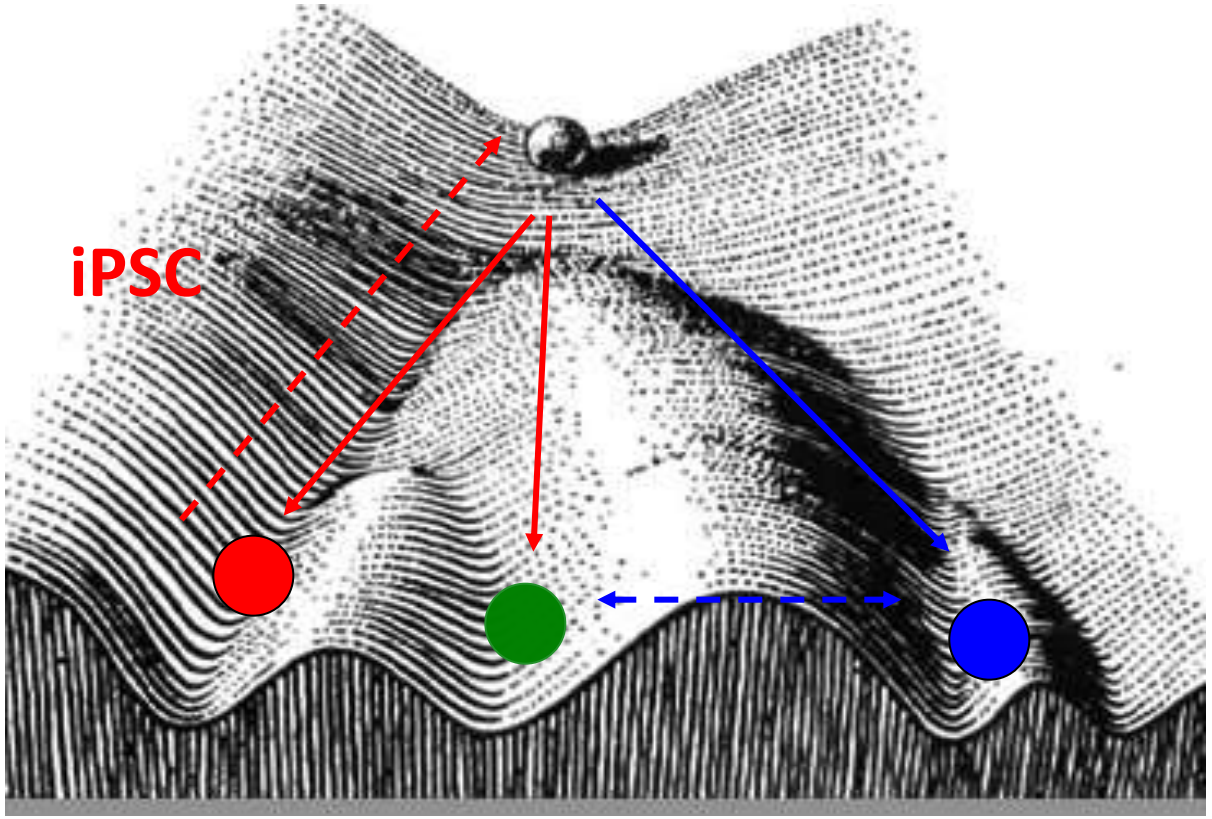


GAMETES FROM PLURIPOTENT STEM CELLS



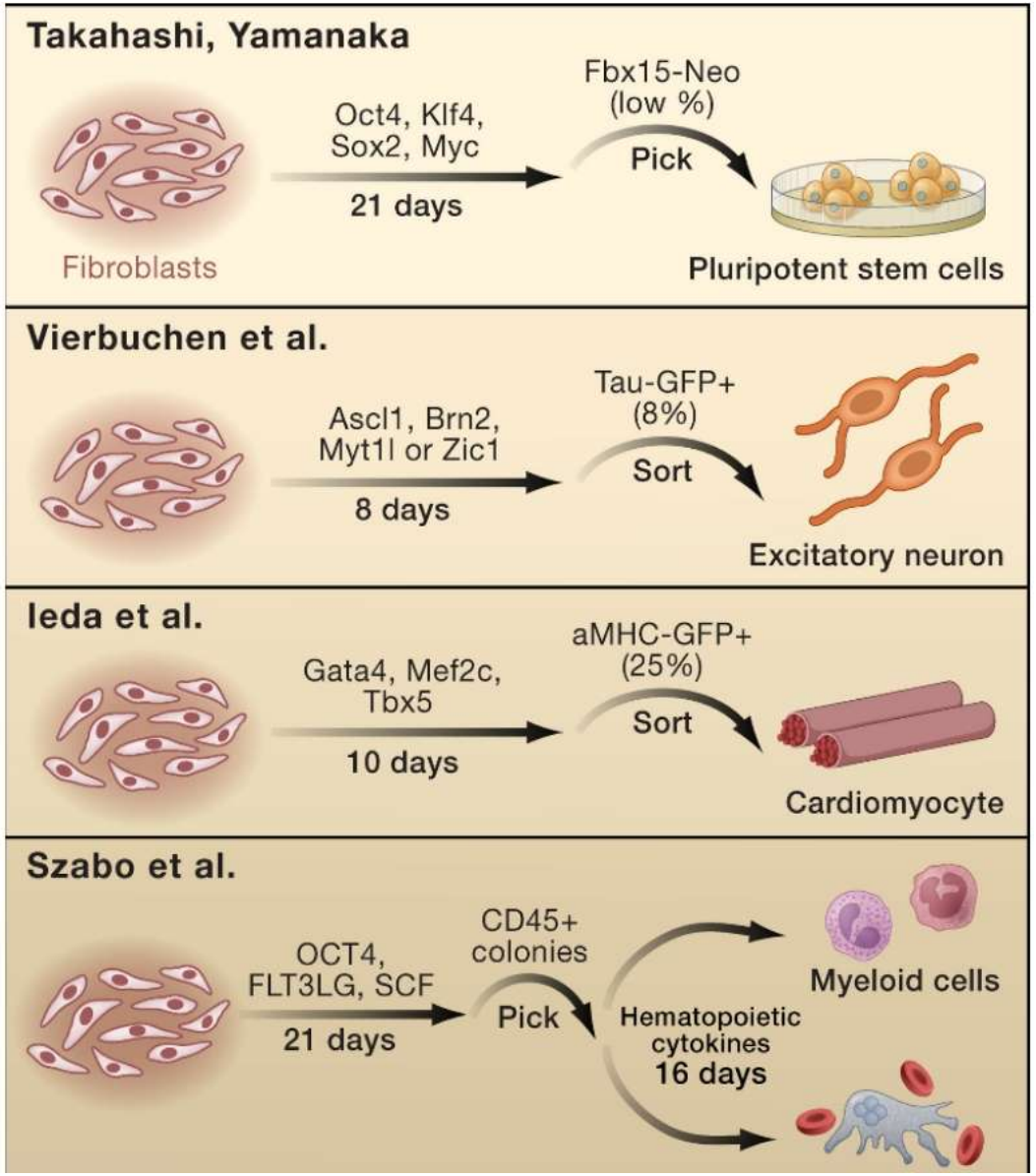
- Reconstitution of the essential first steps of pluripotent stem cell-based gamete production *in vitro* has been established (male and female)
- Abnormal methylation patterns and offspring when the process is completed in *in vitro* conditions
- Normal healthy offspring with normal methylation patterns of imprinted genes if gametogenesis is resumed in *in vivo* conditions (mouse)
- The use of such gametes in ART remains a “distant prospect"
- Stem cell derived gametes can become a valuable resource for research: germ cell development , epigenetic reprogramming and germline gene modification

Transdifferentiation

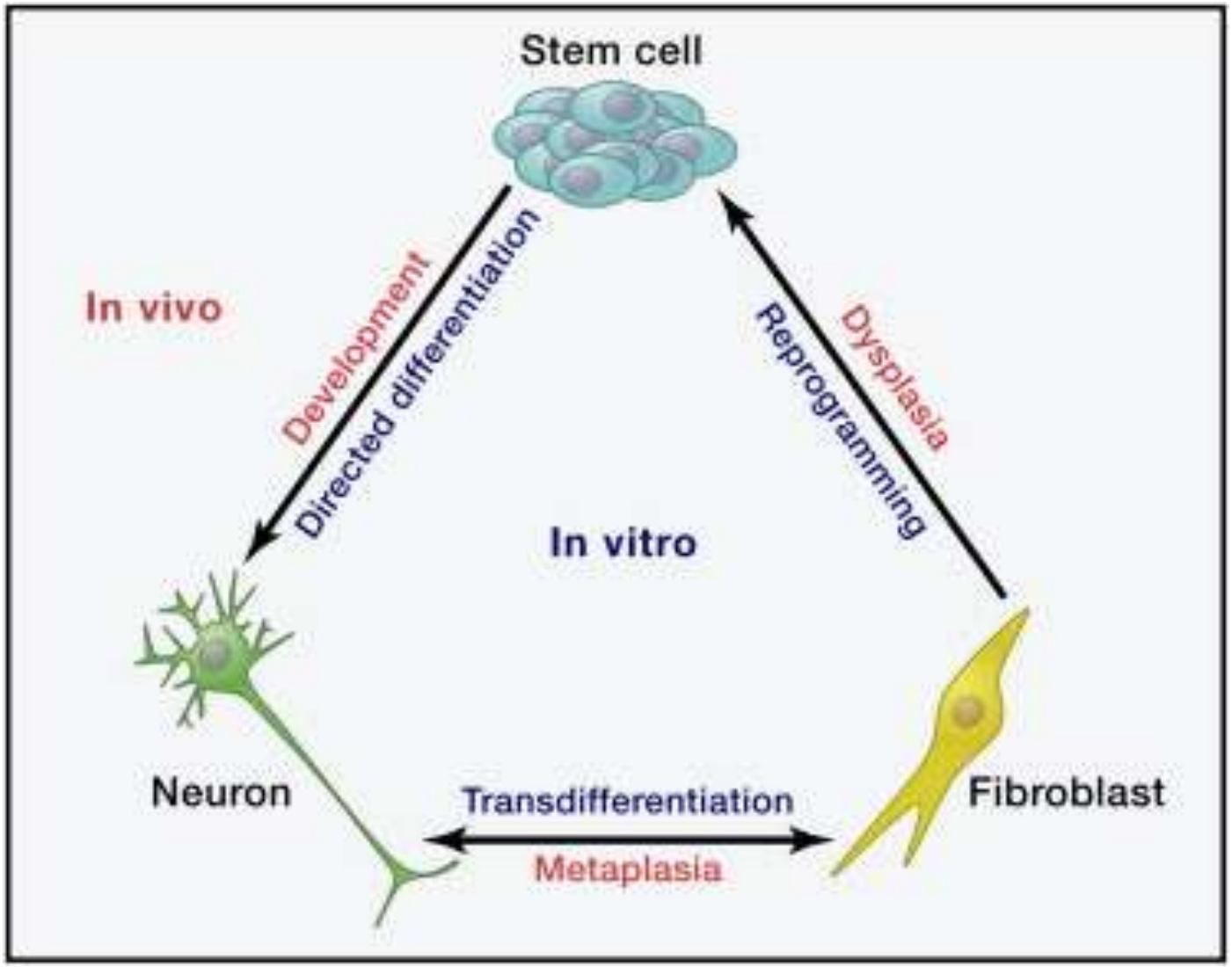


It is possible to convert one differentiated cell type into another without having to reverse differentiation all the way back to a pluripotent state by using methods broadly similar to that used to induce complete reprogramming .

TRANSDIFERENTIATION



Stuart M. Chambers Cell 2011



Cell 2012 *Cherry et al.*

Policy and Regulatory Framework

Countries can be grouped under five broad categories:

Very permissive allowing even the creation of embryos, for research purposes: Belgium, Sweden, UK.

Permissive with restrictions allowing research only on surplus IVF embryos and prohibiting the creation of embryos solely for research purposes: Cyrus, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Iceland, The Netherlands, Norways, Portugal, Slovenia, Spain, Switzerland, Bulgaria.

Restrictive by default where the legislation is not explicit but national practices are quite restrictive in practice : Romania, Turkey.

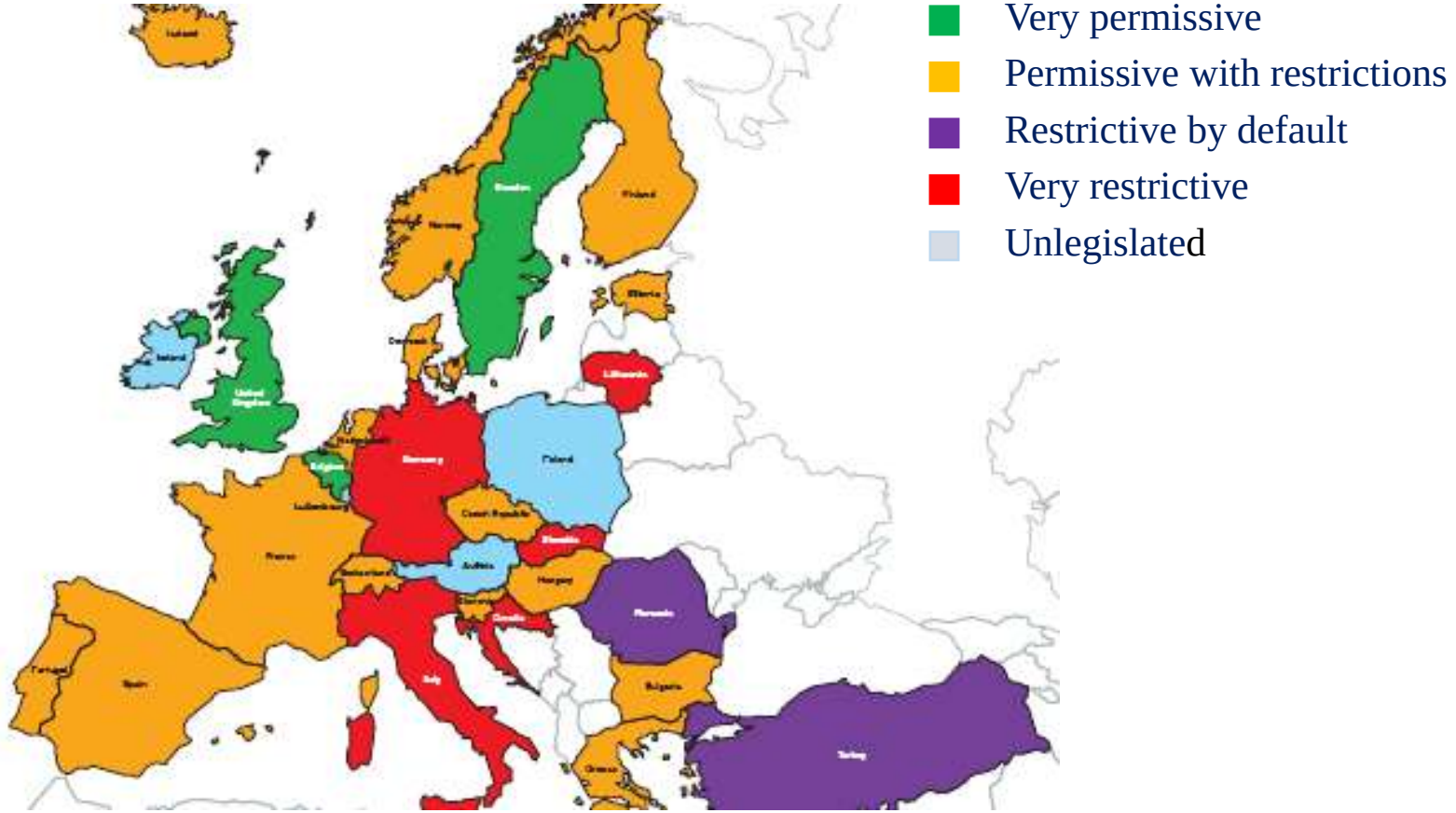
Very restrictive where legislation explicitly bans research on hESCs: Croatia, Germany, Italy, Lithuania, Slovakia

Unlegislated where there is no legislation on hESCs: Austria, Ireland, Luxembourg, Poland.

Human Stem Cell Research and Regenerative Medicine

Focus on European policy and scientific contributions

EUROPEAN SCIENCE FOUNDATION



National positions on human embryonic stem cell research policy and regulatory framework in Europe

Spanish Stem Cell Bank

- 36 hESC lines
  - 2 monogenic disease
  - 3 from isolated blastomeres
  - 2 parthenogenetic
- 45 iPS
  - 17 disease iPS

Banco Andaluz de Células Madre

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

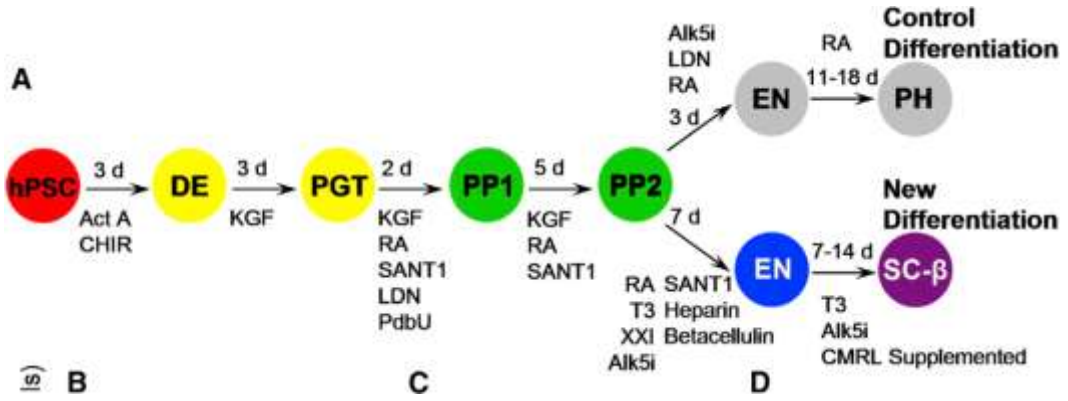
CMR[B]

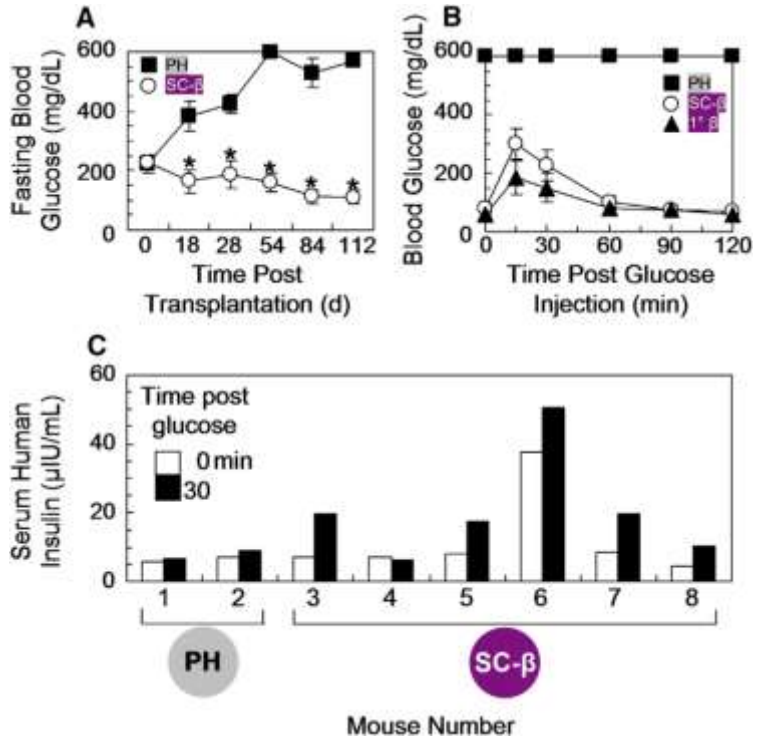
Centre de Medicina Regenerativa de Barcelona
Centro de Medicina Regenerativa de Barcelona
Center of Regenerative Medicine in Barcelona

Generation of Functional Human Pancreatic β Cells In Vitro

Cell, 2014; Pagliuca et al.

- Scalable differentiation protocol that generates hundreds of milions of glucose-responsive ß cells from hPSCs in vitro.
- Stem cell derived ß cells express markers found in mature ß cells, flux Ca^{2+} in response to glucose, package insulin into secretory granules, and secrete quantities of insulin comparable to adult ß cells in response to multiple sequential glucose challenges in vitro.
- Cells secrete human insulin into the serum of mice shortly after transplantation in a glucose-regulated manner.
- Transplantation ameliorates hyperglycemia in diabetic mice.

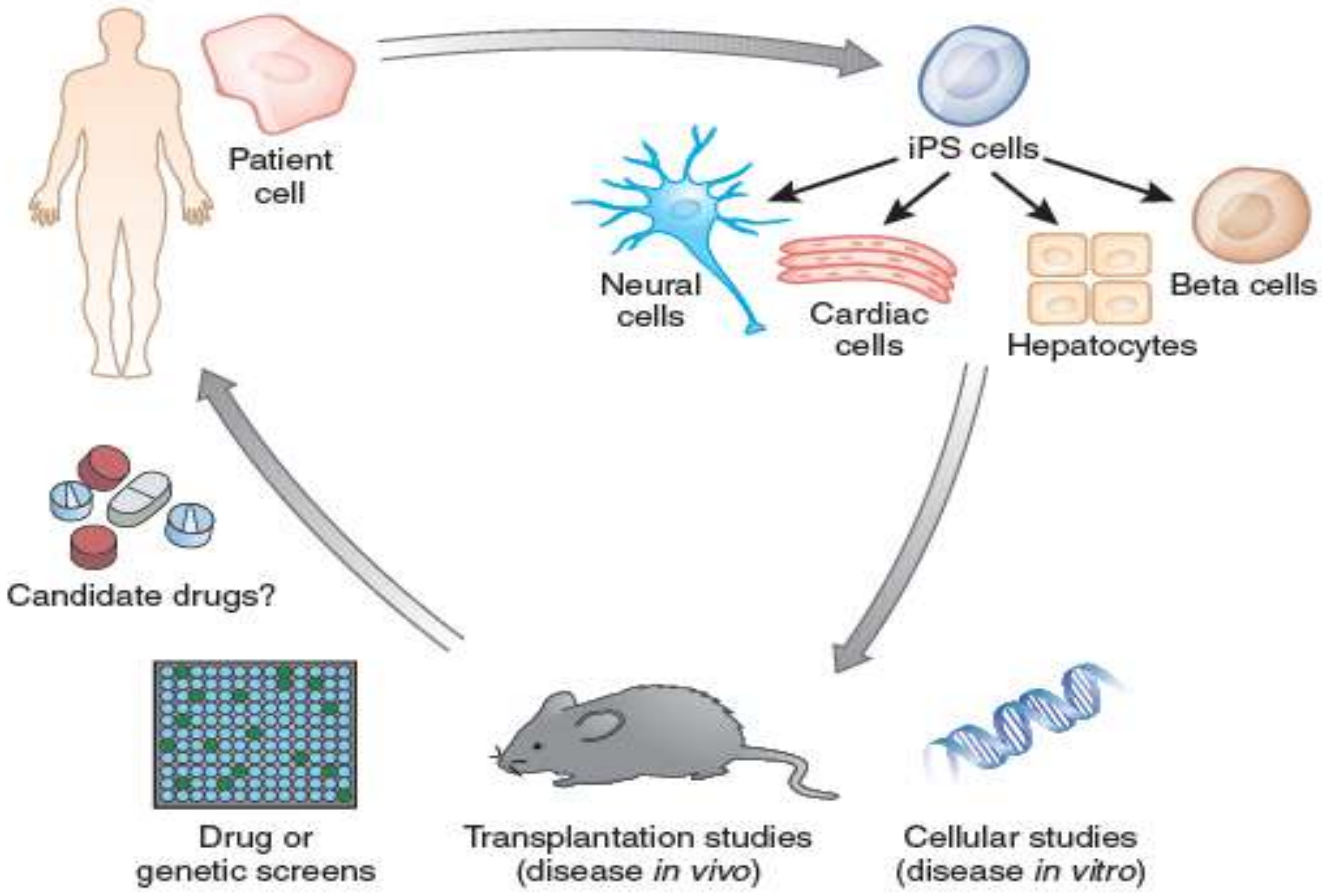




Disease models

Cell Therapy

IPS cells as model for human disease



Lee G. Nature Methods 2010

- Disease-Specific iPS cells could represent a substrate for experimental analysis of disease pathophysiology , a tool for drug screening and a target for gene repair

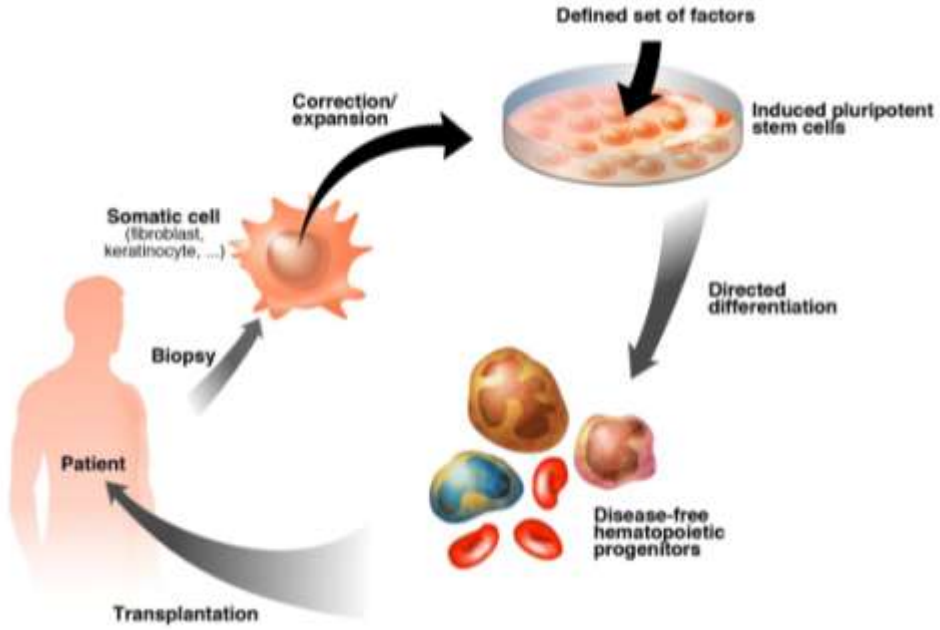
doi:10.1038/nature08129 nature

ARTICLES

Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells

Ángel Raya[1,2,3], Ignasi Rodríguez-Pizà[1], Guillermo Guenechea[4,5], Rita Vassena[1], Susana Navarro[4,5], María José Barrero[1], Antonella Consiglio[1,6], Maria Castellà[5,7], Paula Río[4,5], Eduard Sleep[1,3], Federico González[1], Gustavo Tiscornia[1], Elena Garreta[1,3], Trond Aasen[1,3], Anna Veiga[1], Inder M. Verma[8], Jordi Surrallés[5,7], Juan Bueren[4,5] & Juan Carlos Izpisúa Belmonte[1,9]

Nature, 2009



Cell Therapy: problems to be solved

- Inmunologic rejection
- Tumor formation (Teratomas hESC and iPS)
- Oncogenesis – Insertional mutagenesis iPS
- Differentiation efficiency – Purity of cell populations
- Large scale cell production
- GMP production

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

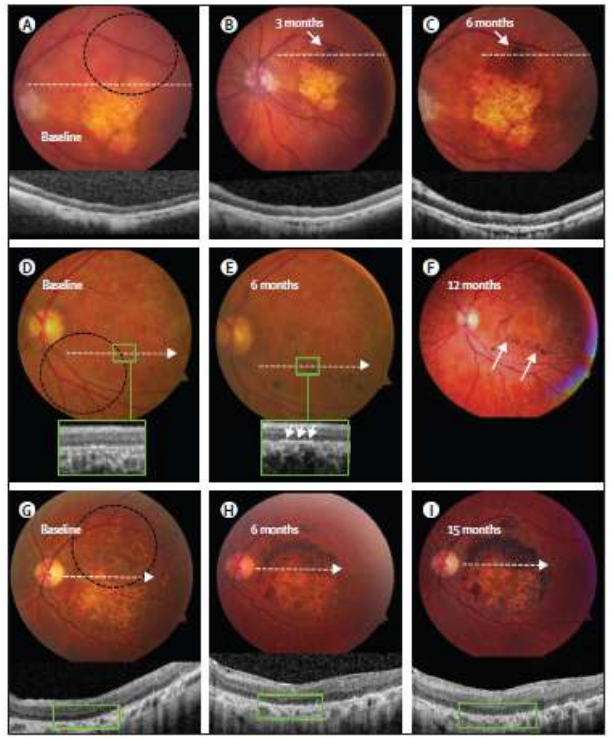
- Safety Study of GRNOPC1 in Spinal Cord Injury
- Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (hESC-RPE) Cells in Patients With Stargardt's Macular Dystrophy (SMD)
- Safety and Tolerability of Sub-retinal Transplantation of hESC Derived RPE (MA09-hRPE) Cells in Patients With Advanced Dry Age Related Macular Degeneration (Dry AMD)
- Sub-retinal Transplantation of hESC Derived RPE(MA09-hRPE)Cells in Patients With Stargardt's Macular Dystrophy

Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies

Schwartz et al, The Lancet, Sept 2014

Advanced Cell Technology. Retina Division, Jules Stein Eye Institute

- Two prospective phase 1/2 studies to assess the primary endpoints **safety** and **tolerability** of subretinal transplantation of hESC-derived retinal pigment epithelium
- 9 patients with Stargardt's macular dystrophy and 9 with atrophic age-related macular degeneration.
- 3 dose cohorts (50 000, 100 000, and 150 000 cells) were treated for each eye disorder.
- Transplanted patients were followed up for a median of 22 months by use of serial systemic, ophthalmic, and imaging examinations

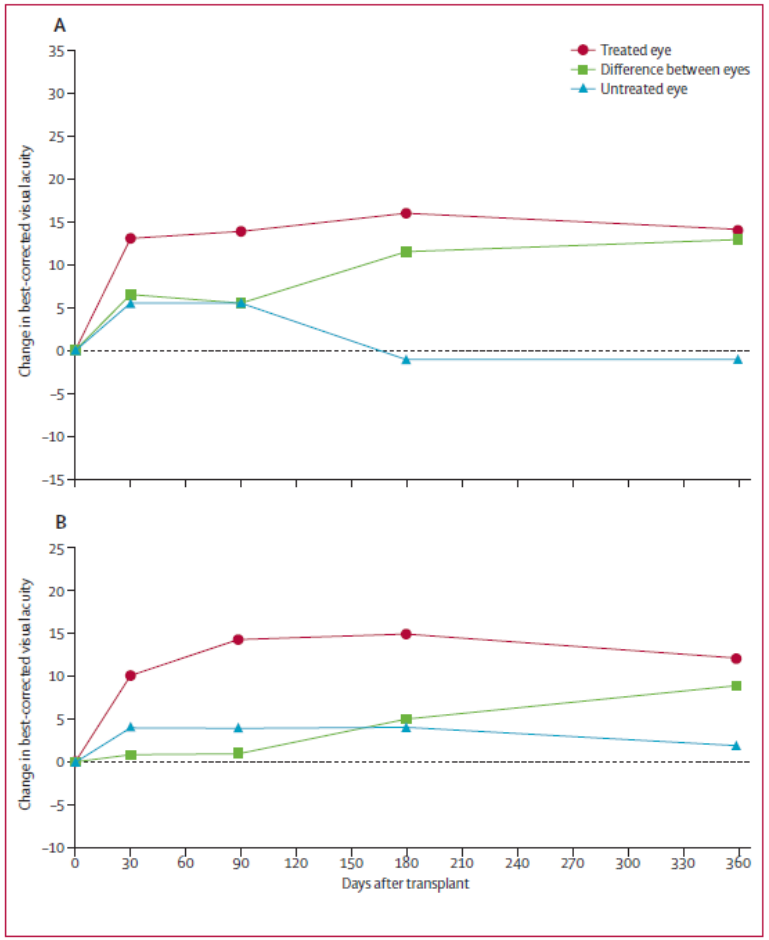


Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies

Schwartz et al, The Lancet, Sept 2014

Advanced Cell Technology. Retina Division, Jules Stein Eye Institu

- No evidence of adverse proliferation, rejection, or serious ocular or systemic safety issues related to the transplanted tissue.
- 13 (72%) of 18 patients had patches of **increasing subretinal pigmentation** consistent with transplanted retinal pigment epithelium.
- Best-corrected visual acuity, monitored as part of the safety protocol, improved in 10 eyes, improved or remained the same in 7 eyes, and decreased by more than ten letters in 1 eye.
- Vision-related quality-of-life measures increased for general and peripheral vision.



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