Theretical Fundamentals of Clinical Medicine

Significance and Perspectives of the Stem Cells in Clinical Medicine I

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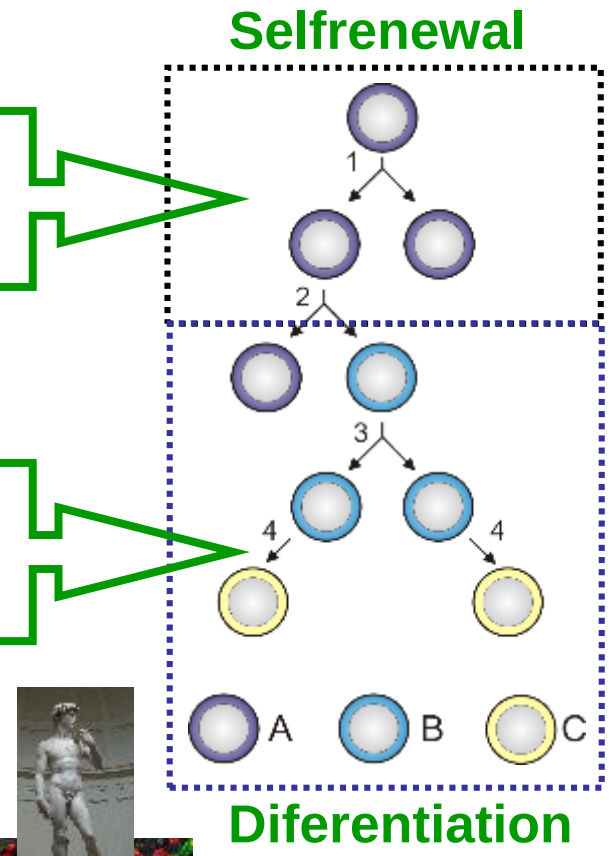
Department of Biology
Faculty of Medicine • Masaryk University

Stem Cell (SC): criteria and definition

Ability to create its copies

Ability to change its properties and differentiate



Clonal capacity
- Symetrical division
- Asymetrical division

Toti
Pluri
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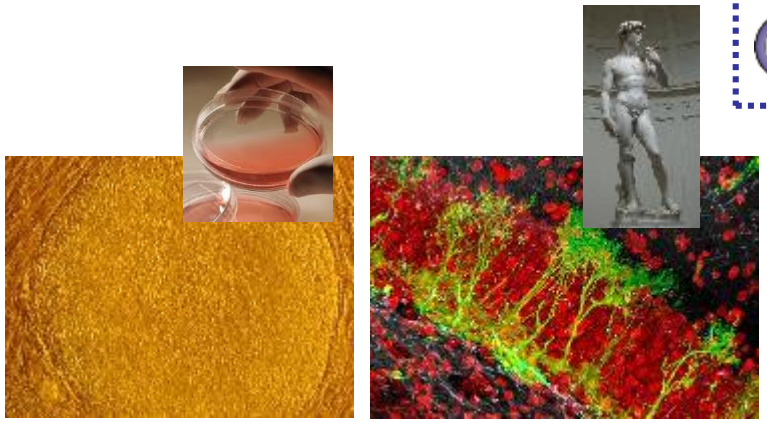
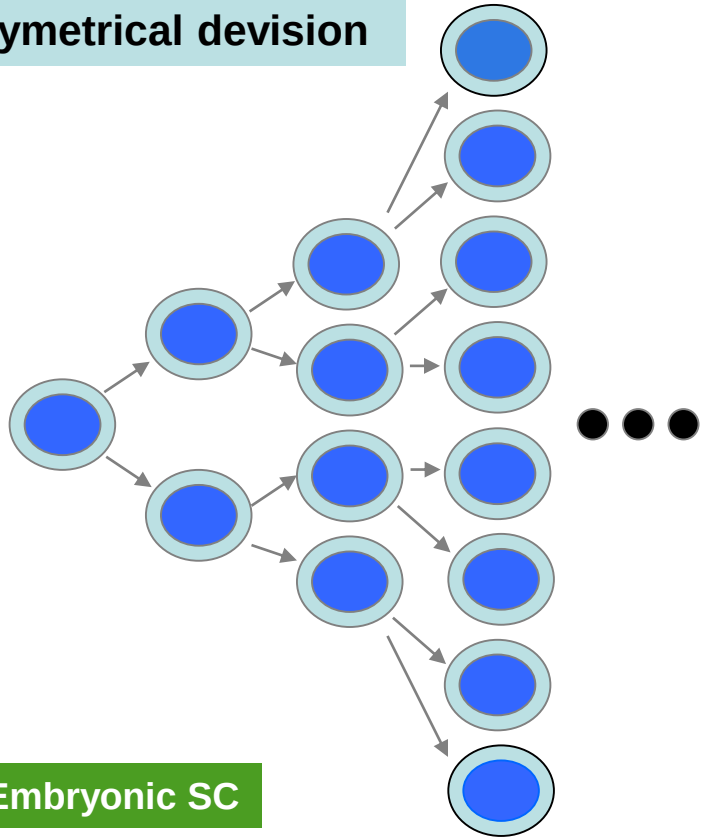
Oligo
Uni
} potent



Stem cells self-renew, proliferate

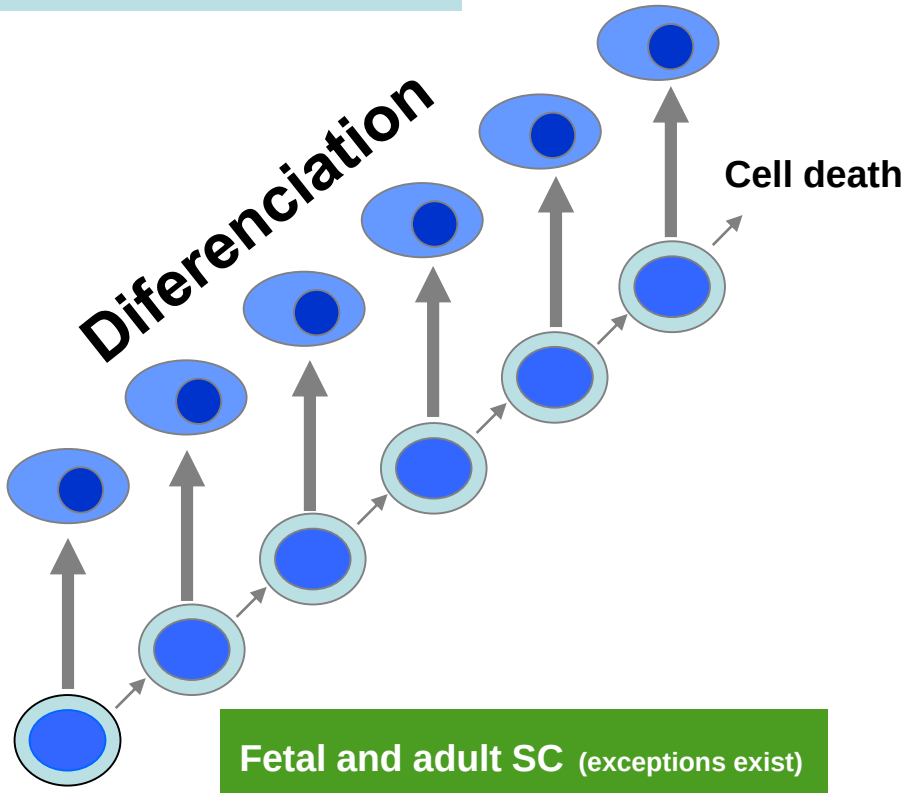
Self-renewal = the most important property of stem cells; ability to produce identical copies

Symetrical devision



Embryonic SC

Asymetrical devision



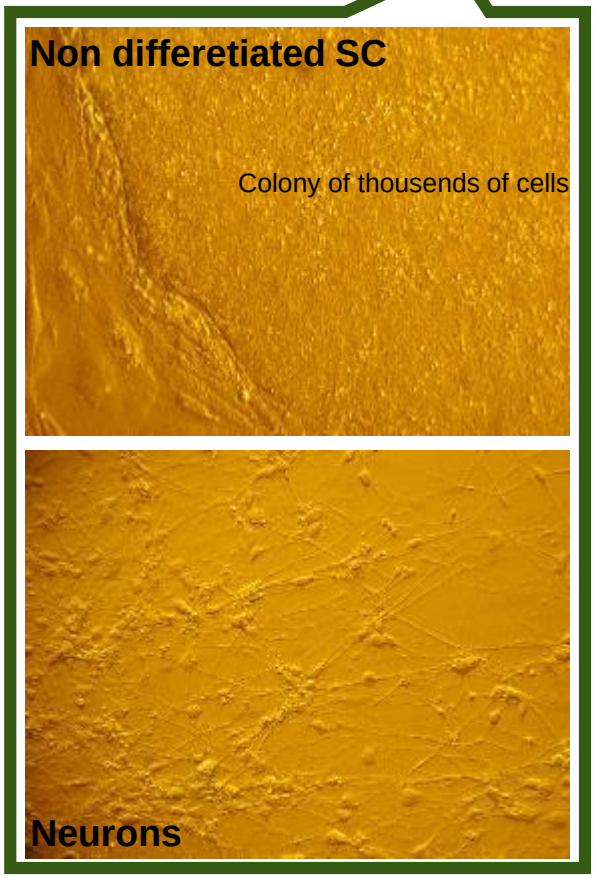
Fetal and adult SC (exceptions exist)

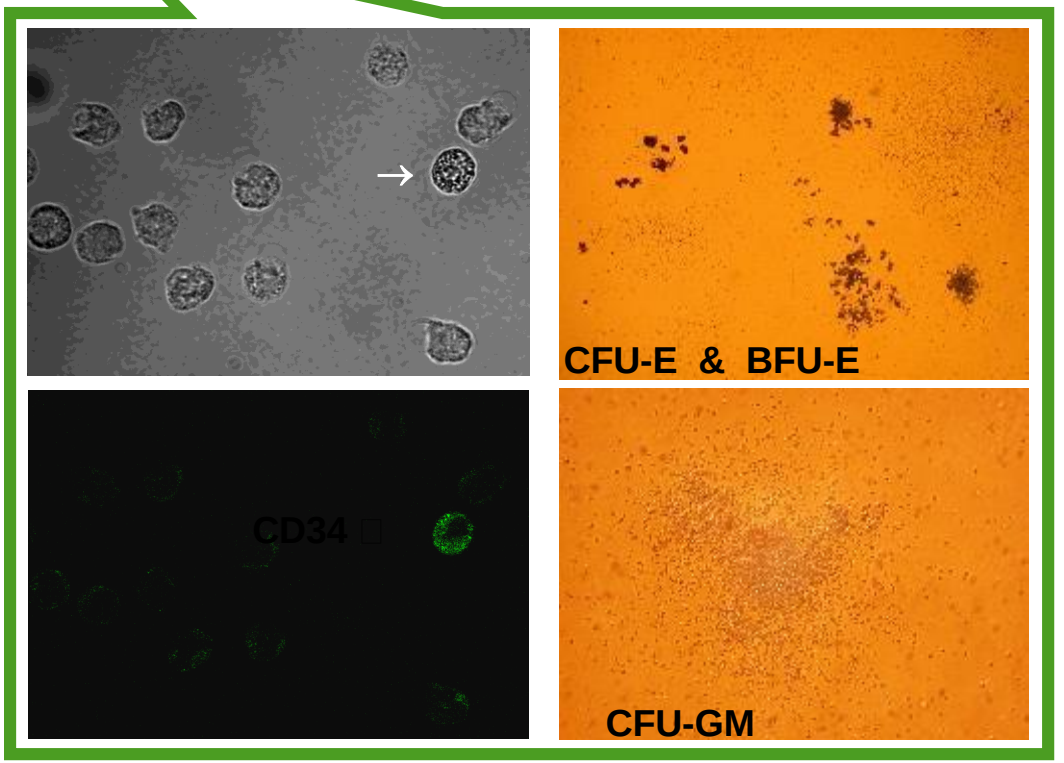
Combination of both mechanisms = neural SC

.... SC differentiate and regenerate tissues

totipotent ➡ pluripotent ➡ multipotent ➡ oligopotent ➡ unipotent

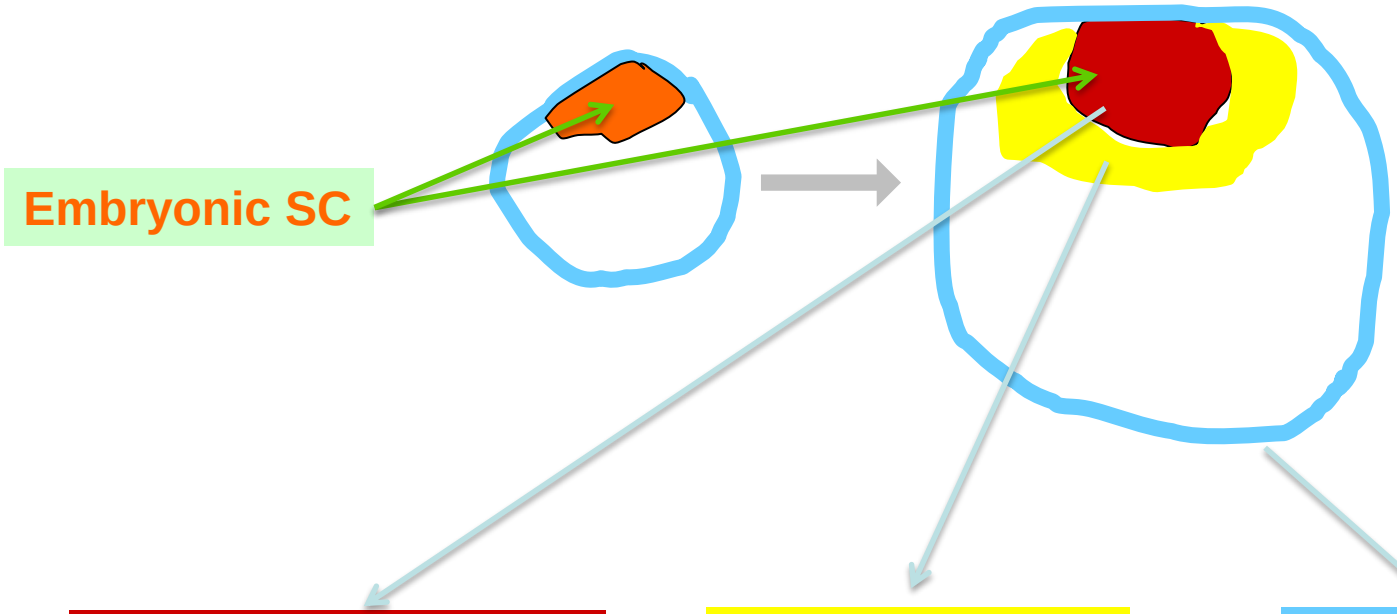
zygote | Embryonic SC | Hematopoetic SC | Gastrointestinal SC | Prostate SC





Just examples

Origin and developmental ontogenesis of stem cells (SC)



Embryonic SC

Primitive ectoderm/epiblast

Primitive entoderm

Trofectoderm

Primordial stem cells
- **gametes**

Ectoderm
- **neural tissue– neural SC**
- **skin – skin SC**

Mesoderm
- **bone marrow and blood– hematopoetic and mesenchymal SC**
- **muscules and bones – tissue specificSC**

Entoderm
- **lungs, liver, pancreas – organ/tissue specific SC**
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Polar trofectoderm
Extraembryonal ectoderm
Chorionic ectoderm
Trofoblast placenty
} placenta

Mural trofectoderm
Ectoplacentálnal cone
Giant cells of trophoblast
} Parietal yolk sac

Stem Cells in Medicine

- Pluripotent
  embryonic and induced…
- Mesenchymal (Dr. Pešl)
  clinical trials and stem cell turism
- Hematopoeitic (prof. Krejčí)
  mostly hematooncologic and imune disorders

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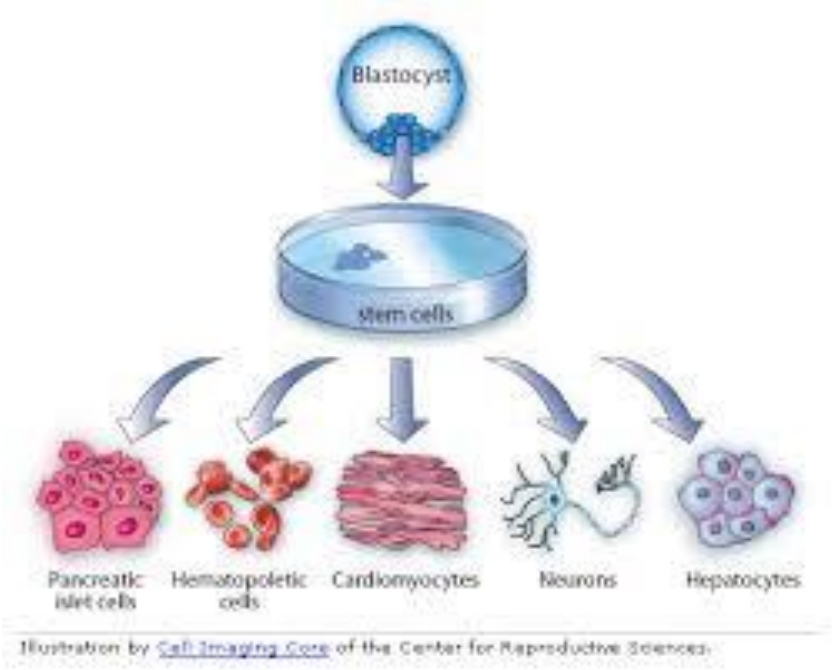
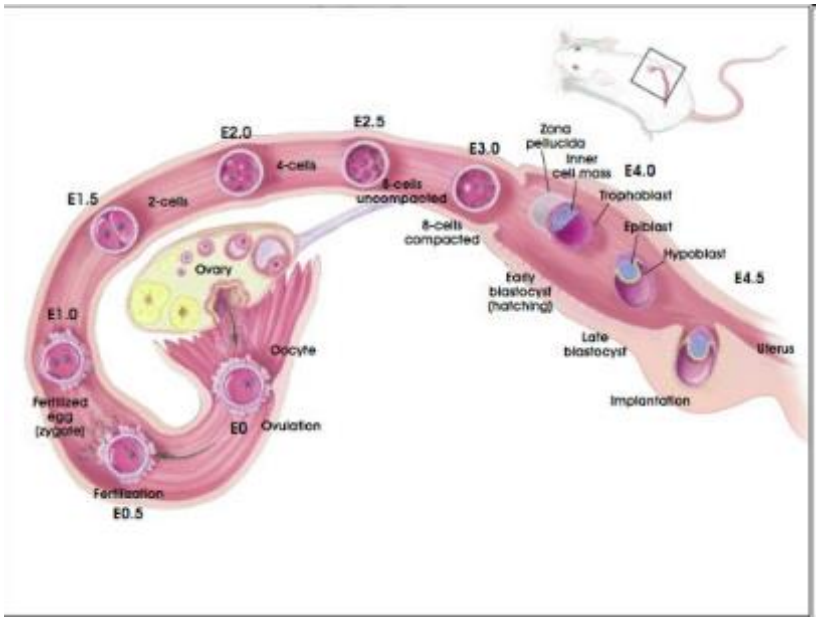
Pluripotent stem cells



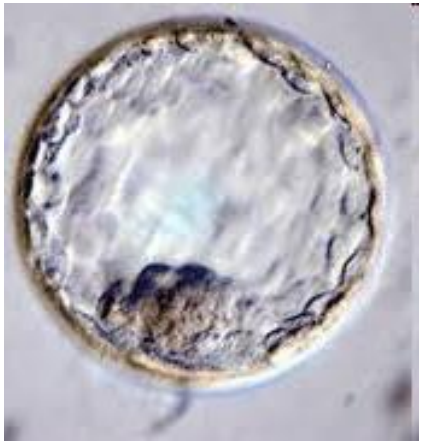
One Ring to Rule Them All…

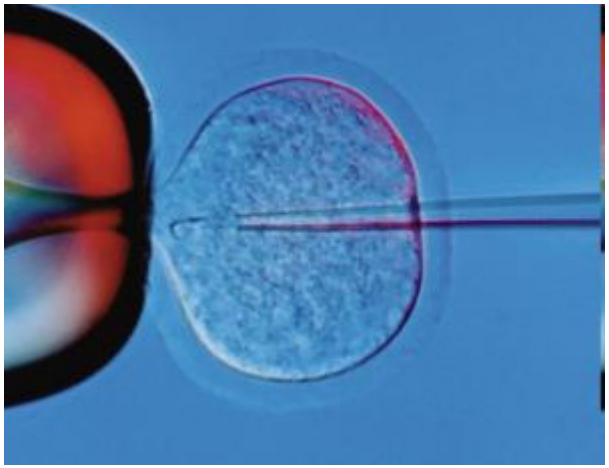
…or shall it be:
One cell to rise them all…?



What do we mean by EMBRYO, when talking Stem Cells

- preimplantation stage
- blastocyst 4 days old
- composed of several dozens of cells
- inner cell mas





~9000 embryos implanted (typicaly 3 at the time) anualy in CR
rest >50% remains frozen …

Benedict XIV

The destruction of human embryos to harvest stem cells is "not only devoid of the light of God but is also devoid of humanity" and "does not truly serve humanity."

James Thompson

"[T]he bottom line is that there are 400,000 frozen embryos in the United States, and a large percentage of those are going to be thrown out. Regardless of what you think the moral status of those embryos is, it makes sense to me that it's a better moral decision to use them to help people than just to throw them out. It's a very complex issue, but to me it boils down to that one thing."

Act 227/2006 Sb

Act on the human embryonic stem cell research and related issues

- Governmental registry of the hESC lines
- Research only with the aproval of Ministry of Youth and Schools
- Research must not lead to human being creation (cloning)

Act 273/2011 Sb

- Embryo storage for infinite time (EU usually 5 yrs)
- Embryos discarded after 10 yrs, if the pair does not wish othervise

Act 227/2006 Sb

Act on the human embryonic stem cell research and related issues

Possible CATCH 22…?

„Frozen generation“ – circa ½ million frozen embryos in US

CR: embryo storage (ČR) …. ~ 10 000 CZK (single payment - usually)

in 2007 – 3400 IVFs

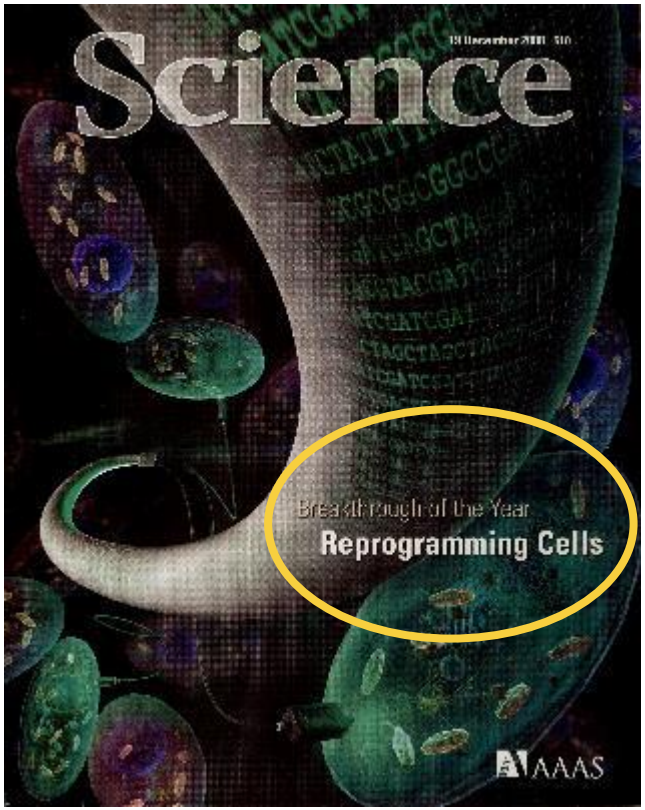
§9 par 1: a) extra embryo may be used for research only with written consent from both parents signed before embryonic stem cell derivation.

Blok I - discussion

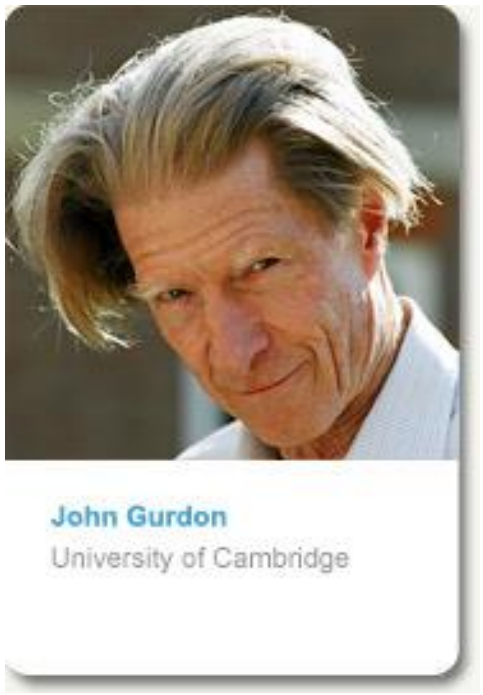
- Is this topic relevant to med students?
- Are the arguments about human existence from conception till birth relevant and are the arguments about the absence of certain abilities of the embryo relevant (e.g. Ability to think ad feel pain/stress)?
- (in)sufficient legislature concerning hESC in CR?

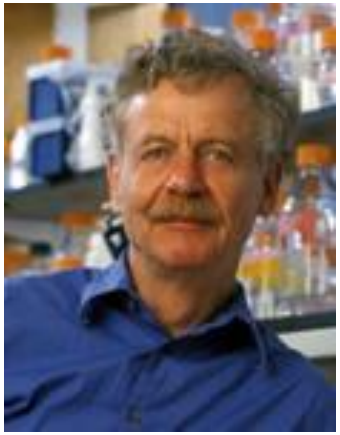
Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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



Albert Lasker basic medical research award 2009





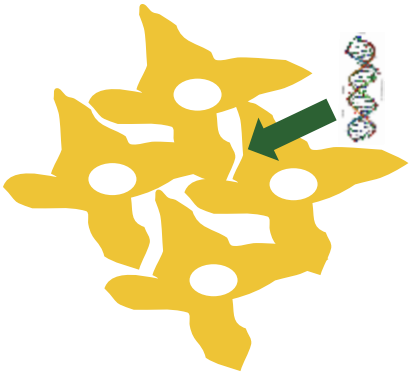
Induced Pluripotent Stem Cells IPSC

(Yamanaka 2006)

Alternative source of pluripotency - iPS cells

- SC created from somatic (i.e. differentiated) cells using genetic manipulation

Kinetics of fibroblast reprogramming into the pluripotentnt SC

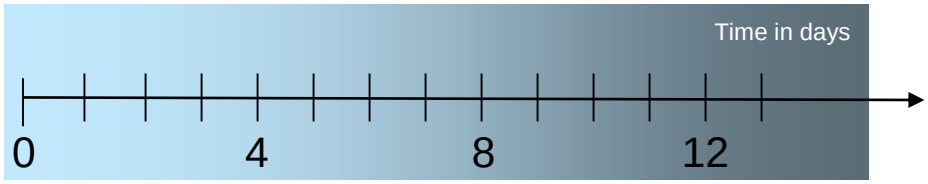


Somatic cell

i.e. skin fibroblast

Oct4
Sox2
c-Myc
Klf4

Stable reprogramming to stemness



Thy1 (and other genes typical for fibroblasts))

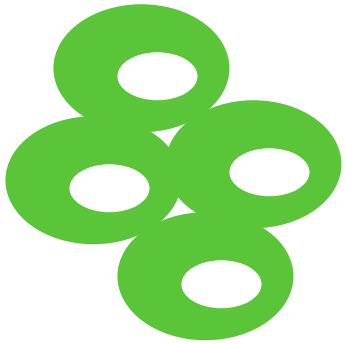
Pluripotency markers

retroviral activity

Pluripotency genes

Telomerase activity

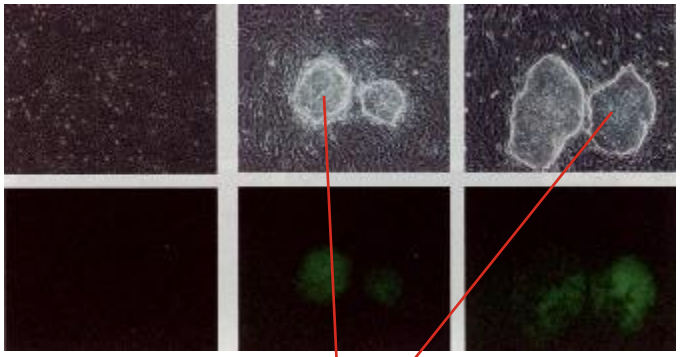
Inactivation of X chromosome



iPS cell

iPS are indeed capable of chimera formation

Oct-4 GFP fibroblasts | Oct-4 GFP iPS | Nanog GFP iPS



Injection of chimera (white and gray hair)

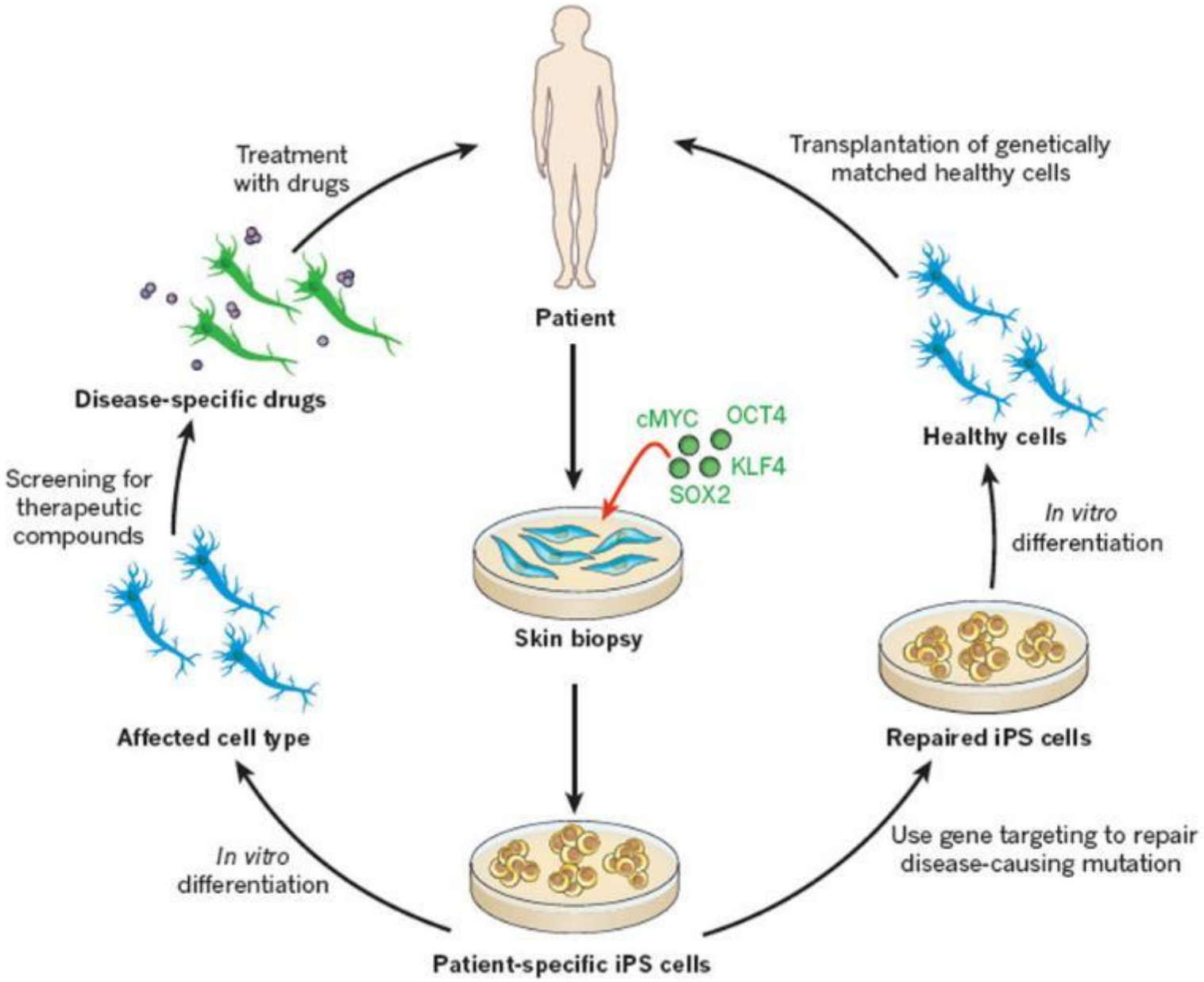
Nanog GFP iPS chimera

Oct-4 GFP iPS chimera

Brambrink et al. Cell Stem Cell, February 2008

Induced pluripotent stem cells

Promissing in future medicine - patient-specific cells

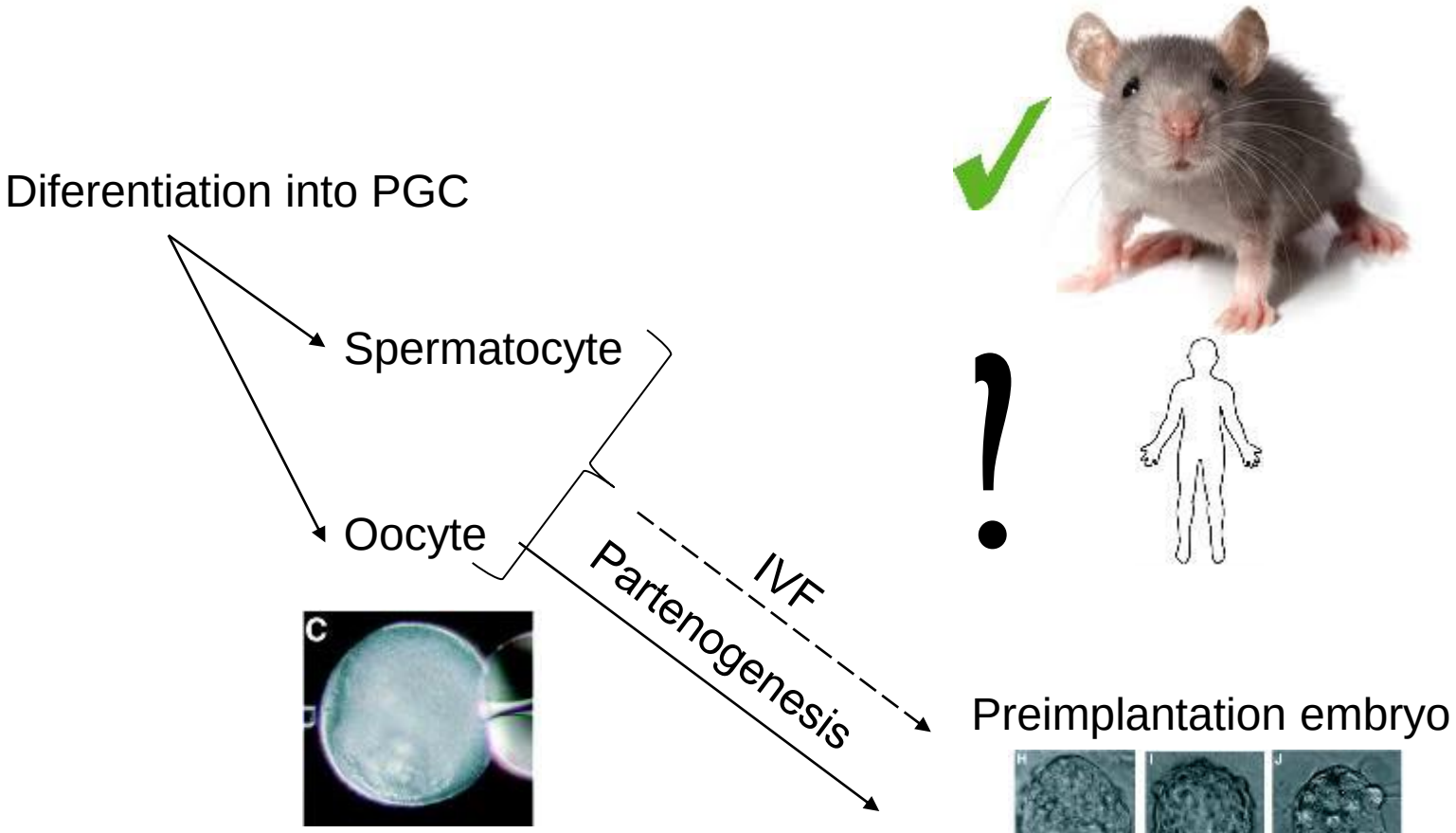


Human Induced Pluripotent Stem Cells

- **No need to destry human embryo**
- **Patient specific cells as the source for reprogramming – limits the risc of GVH**

Ethical problem with hiPSC?

Germ cells out of hiPSC



Hubner. Science. 2003

Ethical problem with hiPSC …. II

Cell Stem Cell
Forum

Cell PRESS

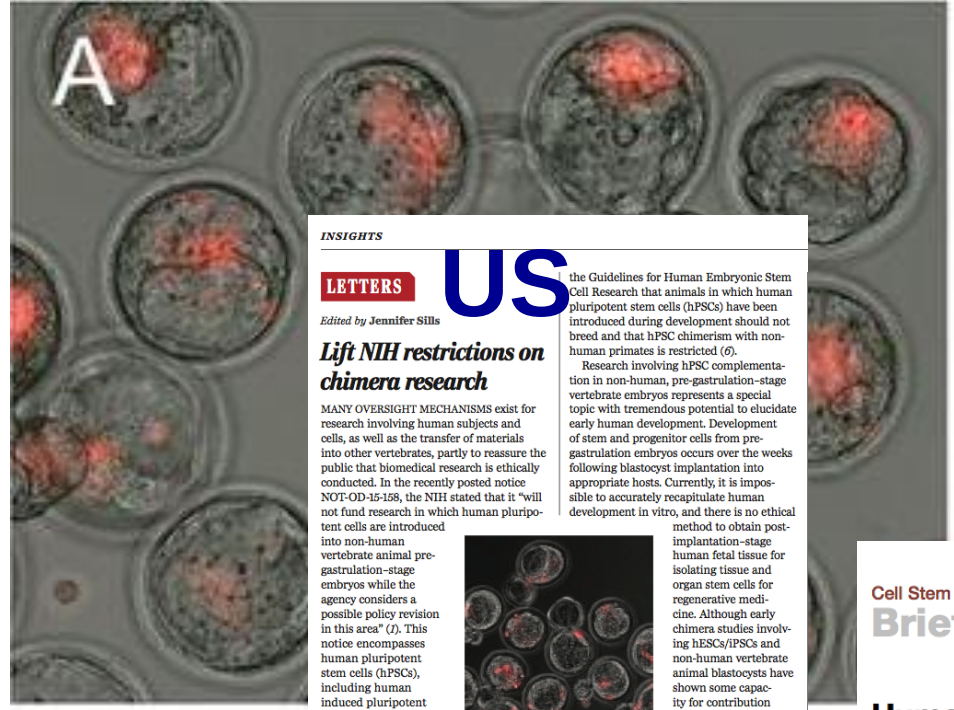
Patients' Attitudes toward the Donation of Biological Materials for the Derivation of Induced Pluripotent Stem Cells

Ishan Dasgupta,[1] Juli Bollinger,[1,2] Debra J.H. Mathews,[1,3] Neil M. Neumann,[3,4] Abbas Rattani,[1] and Jeremy Sugarman[1,3,*]
[1]Berman Institute of Bioethics, Johns Hopkins University, Baltimore, MD, 21205 USA
[2]Genetics and Public Policy Center, Johns Hopkins University, Washington, D.C., 20036 USA
[3]School of Medicine, Johns Hopkins University, Baltimore, MD 21205, USA
[4]Medical Scientist Training Program, Johns Hopkins University, Baltimore, MD 212105, USA
*Correspondence: jsugarman@jhu.edu
http://dx.doi.org/10.1016/j.stem.2013.12.006

- **Altruistic sentiment and benefit expectation**

versus

- **Privacy protection**
- **Establishment of permanent (immortal) cell line (i.e.HeLa)**
- **Human cells and tissues comercionalisation**
- **Possibility to produce gametes/partenogenesis (germ cells)**
- **Genome editting before returning to the patients**



US

LETTERS

Edited by **Jennifer Sills**

Lift NIH restrictions on chimera research

MANY OVERSIGHT MECHANISMS exist for research involving human subjects and cells, as well as the transfer of materials into other vertebrates, partly to reassure the public that biomedical research is ethically conducted. In the recently posted notice NOT-OD-15-158, the NIH stated that it "will not fund research in which human pluripotent cells are introduced into non-human vertebrate animal pre-gastrulation–stage embryos while the agency considers a possible policy revision in this area" (*1*). This notice encompasses human pluripotent stem cells (hPSCs), including human induced pluripotent stem cell (hiPSC)–based human/non-human chimera studies. We believe that this notice poses a threat to progress in stem cell biology, developmental biology, and regenerative medicine. We hope the guideline recommendations that emerge from the NIH Workshop on 6 November will accelerate the decision to reinstate NIH funding for this research area, which has tremendous promise. We strongly believe that a continued dialogue between scientists and bioethicists regarding human/non-human chimera studies is critical for advancing human health through basic science.

Much of the bioethical concern in regard to human/non-human chimerism arises from the possibility of chimeric animals harboring human neurons and germ cells. Can human neural cells coexist with those from animals and establish "humanized" cerebral anatomy and circuitries? Furthermore, would such chimeras be elevated to a higher metaphysical state and "think" more like us (*2*)? Current scientific data have not supported such possibilities, despite hundreds of xenotransplant studies introducing human neurons into the mouse brain (*3*–*5*). With regard to germline transmission, the National Academy of Medicine and the National Research Council have stated in the Guidelines for Human Embryonic Stem Cell Research that animals in which human pluripotent stem cells (hPSCs) have been introduced during development should not breed and that hPSC chimerism with non-human primates is restricted (*6*).

Research involving hPSC complementation in non-human, pre-gastrulation–stage vertebrate embryos represents a special topic with tremendous potential to elucidate early human development. Development of stem and progenitor cells from pre-gastrulation embryos occurs over the weeks following blastocyst implantation into appropriate hosts. Currently, it is impossible to accurately recapitulate human development in vitro, and there is no ethical method to obtain post-implantation–stage human fetal tissue for isolating tissue and organ stem cells for regenerative medicine. Although early chimera studies involving hESCs/iPSCs and non-human vertebrate animal blastocysts have shown some capacity for contribution to host tissues (*7*–*9*), much work remains to unravel key differences in early development between humans and other vertebrates. If we succeed in inducing significant chimerism between hPSCs and pre-gastrulation–stage embryos from non-human vertebrates, tremendous potential exists to develop humanized disease models for studying drug pharmacology. Similarly, implantation of hPSCs derived from patients with heritable diseases could illuminate genetic disease pathogeneses in an appropriate in vivo context. It may even be possible to generate an unlimited supply of therapeutic replacement organs using porcine or sheep models, an effort that we (H.N.) have undertaken with support from the California Institute for Regenerative Medicine. By eliminating federal funding for this research, the NIH casts a shadow of negativity towards all chimerism studies regardless of whether human cells are involved.

Ultimately, we believe that human/non-human chimerism studies in pre-gastrulation embryos hold tremendous potential to improve our understanding of early development, enhance disease modeling, and promote therapeutic discovery. Given that the objective of the NIH is to enable discoveries that advance human health, the restrictions presented

Engraftment of hiPSC (red) into mouse blastocyst-stage embryo

Cell Stem Cell
Brief Report

UK

CellPress

Human-Mouse Chimerism Validates Human Stem Cell Pluripotency

Victoria L. Mascetti[1,*] and Roger A. Pedersen[1]
[1]The Anne McLaren Laboratory, Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, Department of Surgery and British Heart Foundation Centre of Regenerative Medicine, University of Cambridge, Cambridge, CB2 0SZ, UK
*Correspondence: vlm37@cam.ac.uk
http://dx.doi.org/10.1016/j.stem.2015.11.017

Interspecies chimeras for human organ transplantations

Blok II - discussion

- Is reprogramming relevant to med students?
- Arguments about the human uniquness in relation to cloning..
- (in)sufficient legislature concerning hiPS in CR?

Rational design of the novel pluripotent stem cell

applications in clinics requires

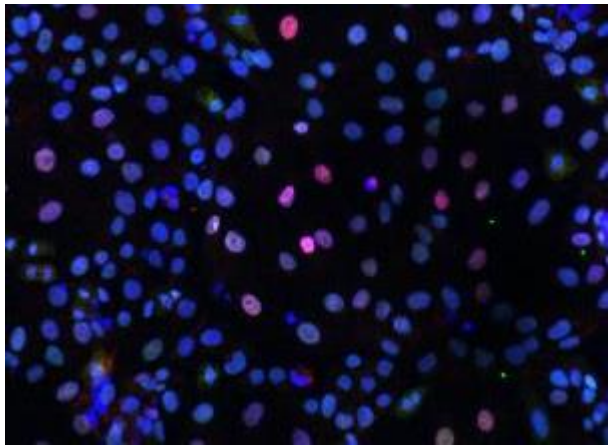
- **Opened and truthfull communication with public and stakeholders**
- **Buletproof patient consent protecting both the researchers as well as the patient**
- **Absolute transparency of the scientific results and preclinical tests**
- **Confidence in the systém and individuals (scientists and MDs)**

What is this for?

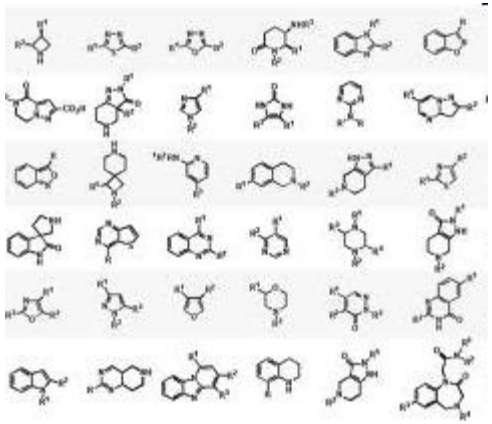
Disease modelling

Regenerative and reconstructive medicine

Understanding pathological processes



Drug development



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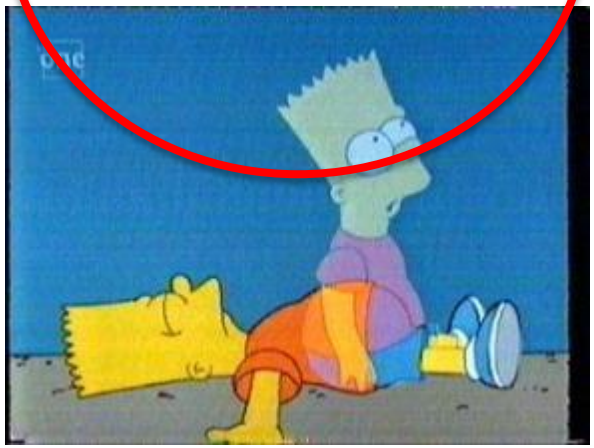

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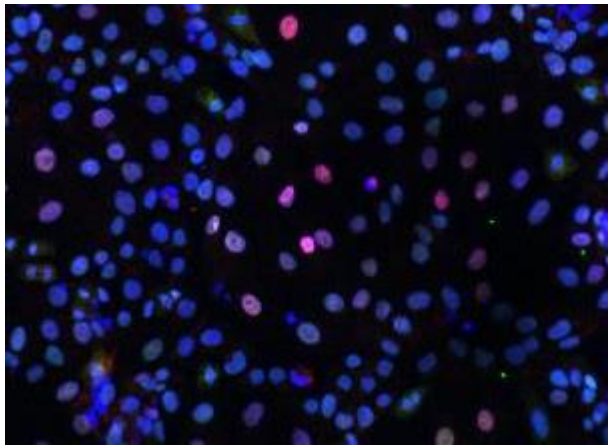

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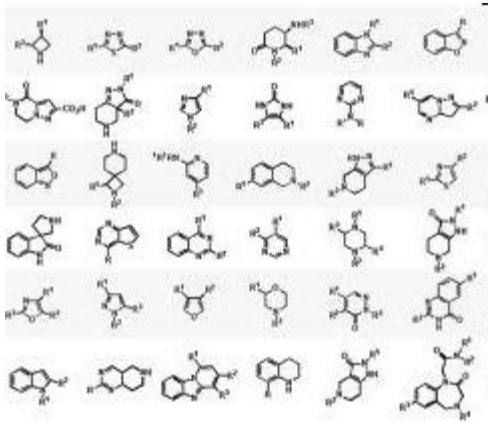
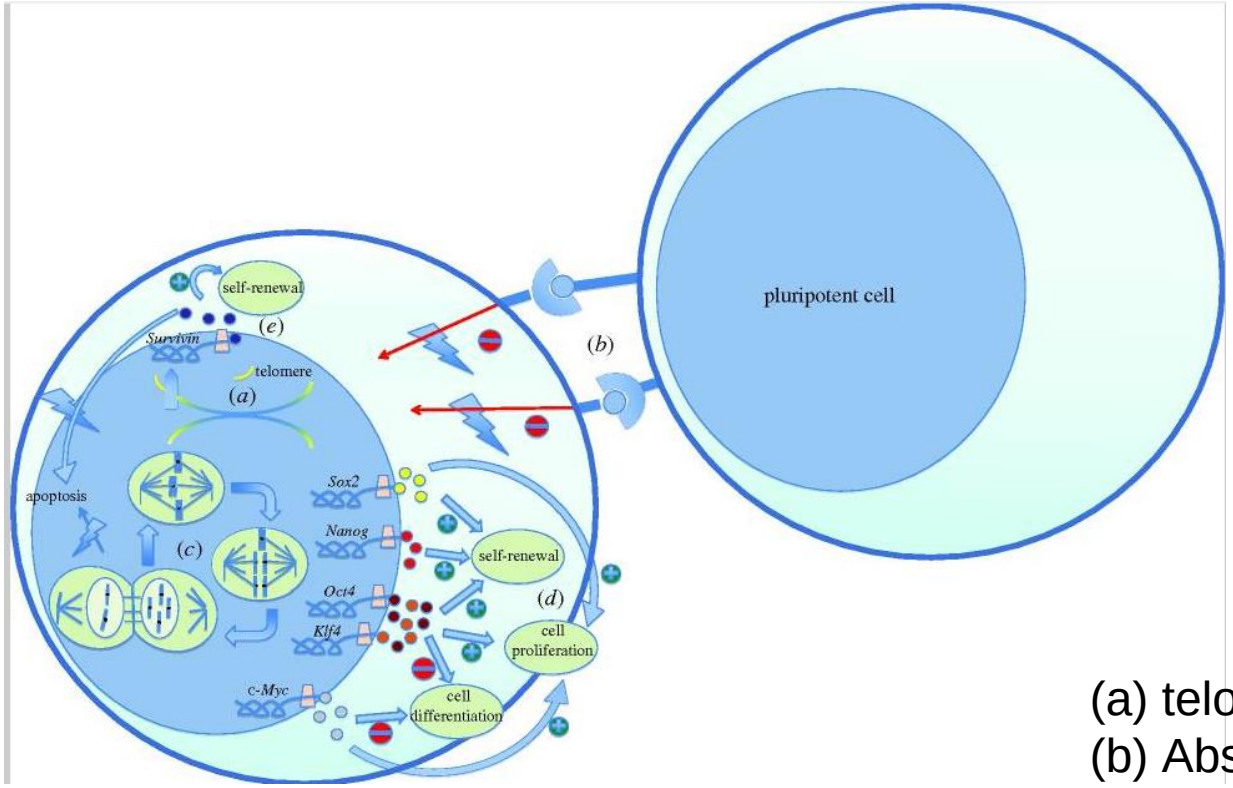


Any PSC aplication requires quantitative differenciation into the target cell type

Relation between tumorogenicity and pluripotency

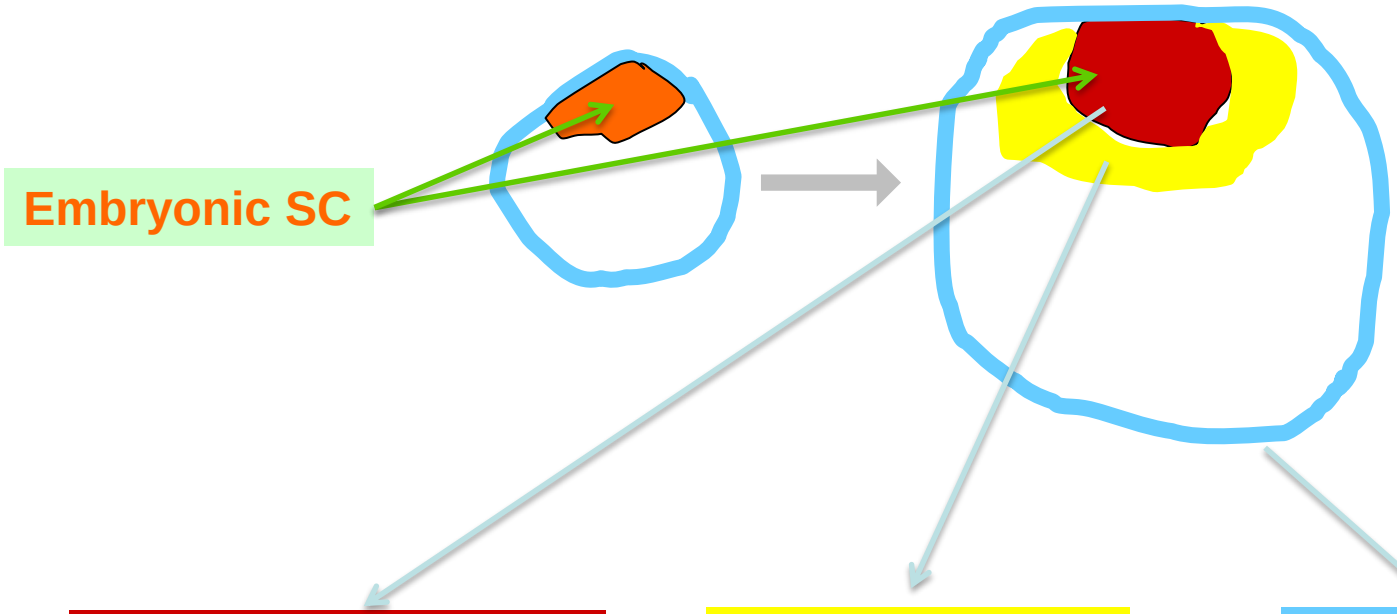


Kooreman, J R Soc Interface. 2010

(a) telomerase
(b) Absence of contact inhibition
(c) Strong antiapoptotic aparatus
(d) High proliferation

Current standard for clinical trials: Max 1 pluripotent cell per 10^6 differentiated cells in the off-the-shelf product!

Origin and developmental ontogenesis of stem cells (SC)



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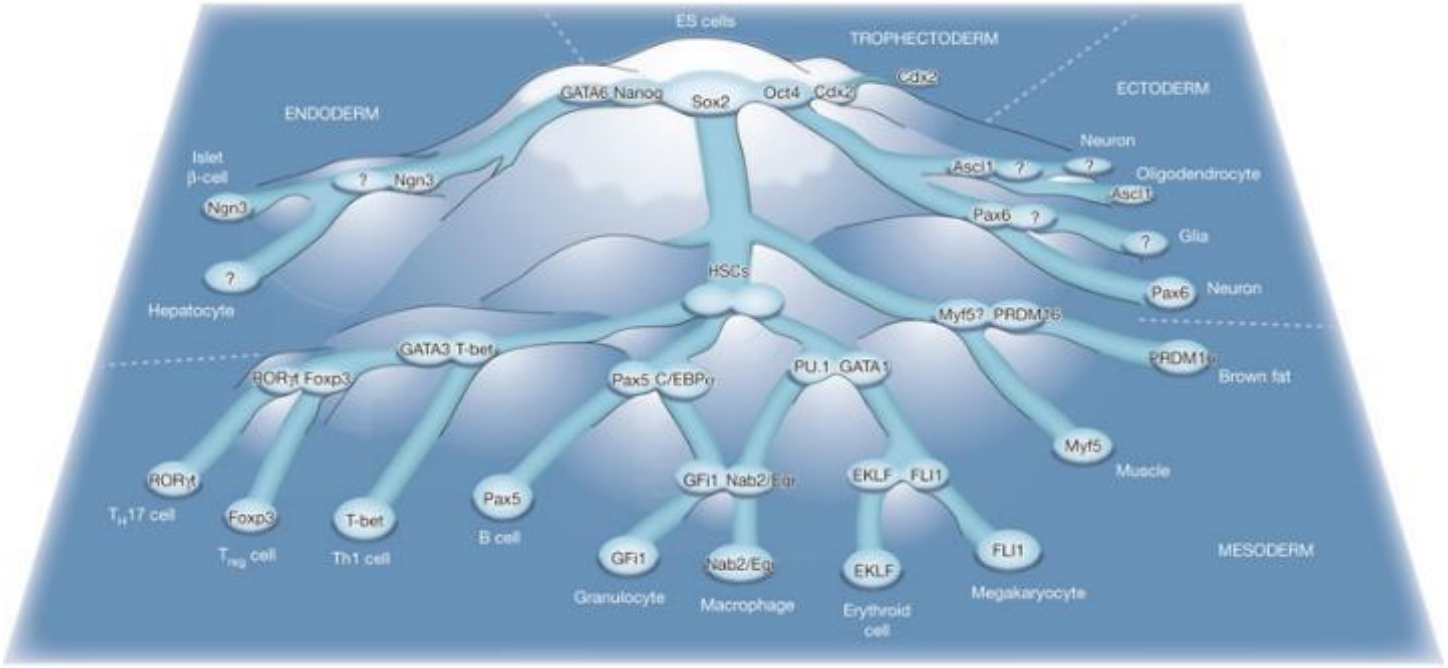
} placenta

Mural trofectoderm
Ectoplacentálnal cone
Giant cells of trophoblast
} Parietal yolk sac

What determines differentiation *in vivo* and *in vitro*:

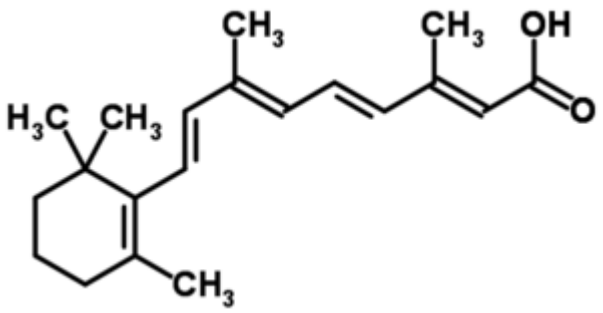
Cell line differentiation of embryonic stem cells in complex landscape of epigenetic bariers (=cells are unstable on the hills), mountain platos (=RELATIVELY STABLE BUT REVERSIBLE CELL STATUS) and deep walleys (=TERMINAL DIFFERENTIATION)



Thomas Graf & Tariq Enver *Nature* **462**, 587-594 (2009) doi:10.1038/nature08533

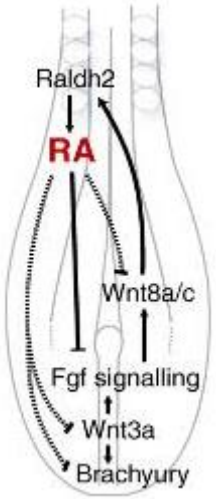
nature

DIFFERENTIATION OF PSC – embryo development analogy…



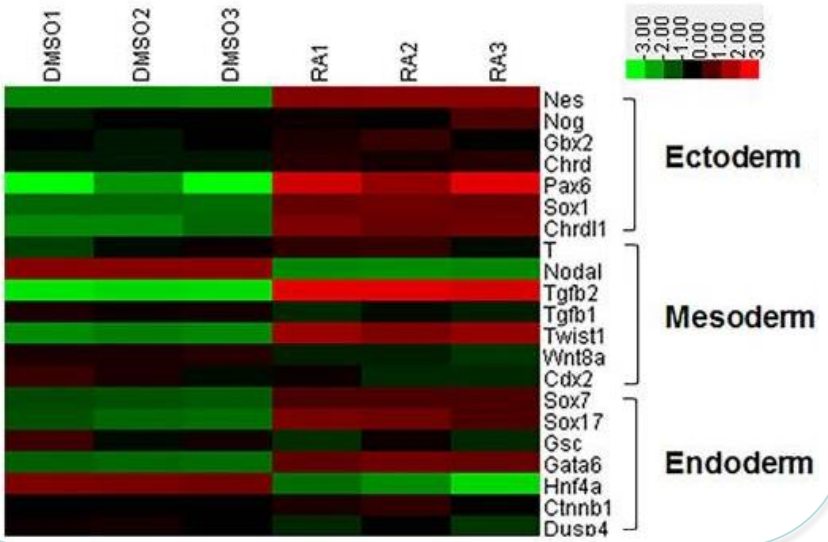
Retinoic acid

AP axis establishment



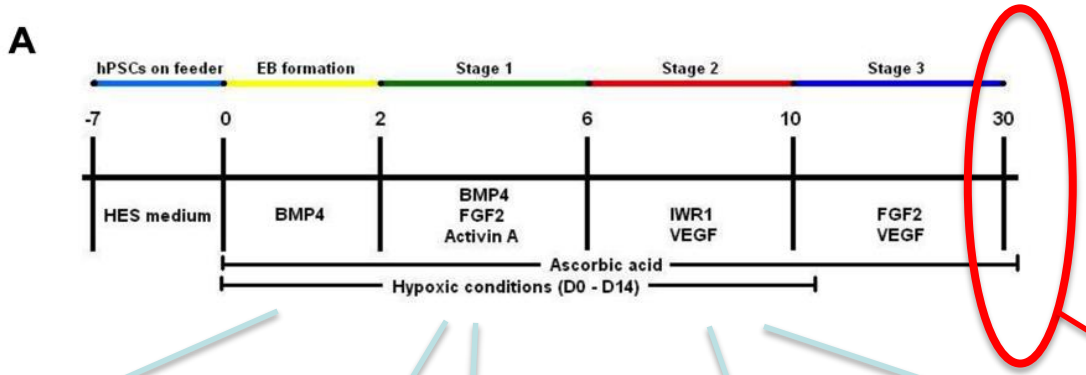
Ribes, Development 2009

RA ectoderm formation from PSC



Zhang, PLOS 2015

DIFFERENTIATION OF PSC INTO FUNCTIONAL CARDIOMYOCYTES…



Pešl, Heart and Vessels, 2014

BMP4 helps to polarize the embryo during gastrulation (primitive mesendoderm)

Activin A triggers mesoderm formation

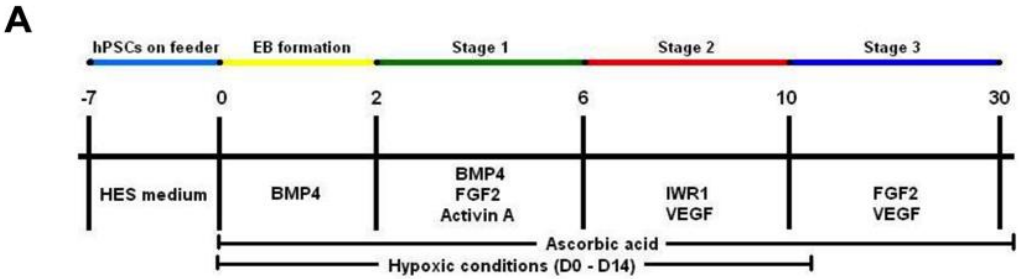
Hensen node with FGF2 triggers cardiogenesis

VEGF is needed for later embryo heart morphogenesis (ventricle formation)

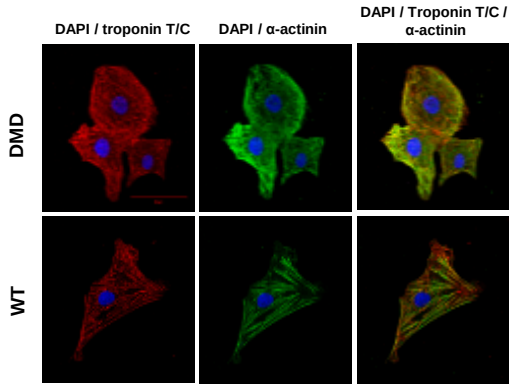
IWR inhibits Wnt signal – prevents neurodifferrenciation etc.

Cardiomyocytes starting spontaneous beating

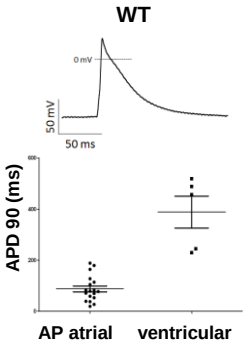
FUNCTIONAL CHARACTERISATION OF PSC DERIVED CARDIOMYOCYTES.



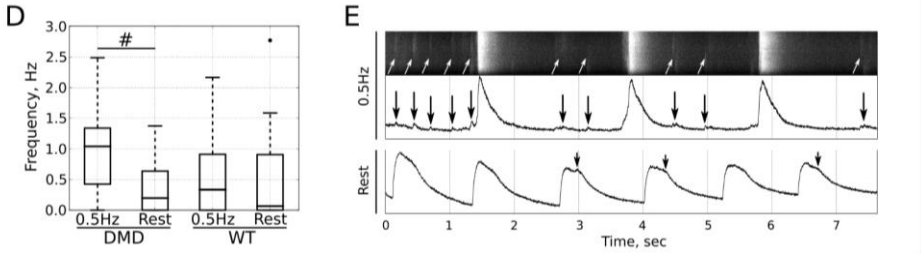
Cardiac Markers & Morphology



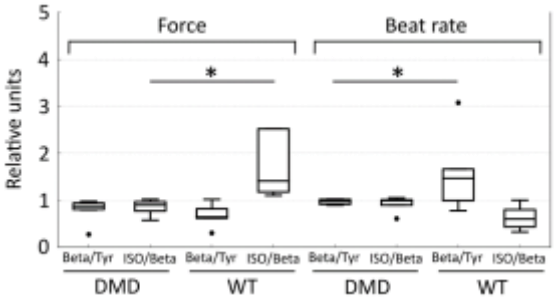
Electrophysiology (Patch Clamp)



Ca^{2+} Transients

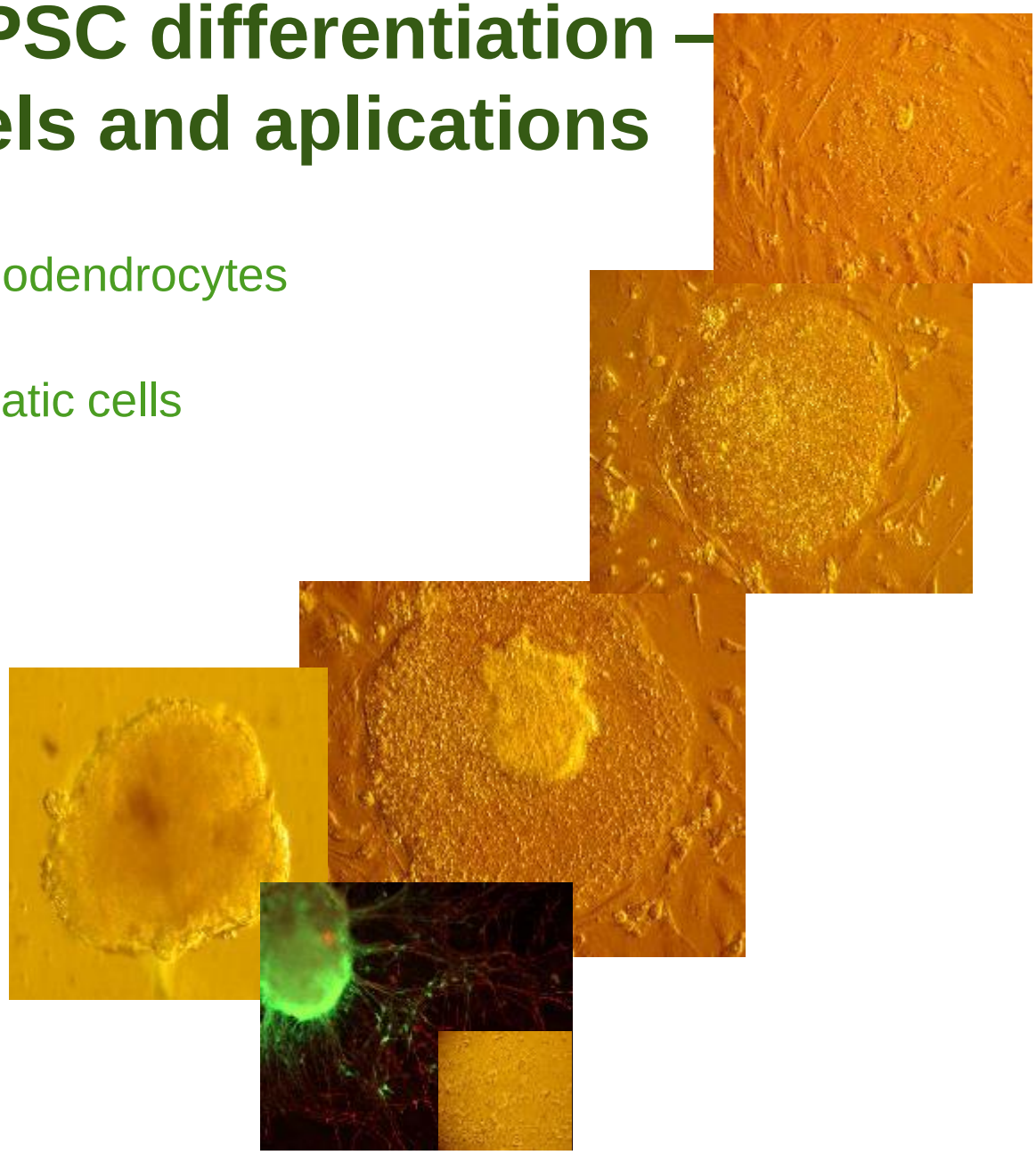


Atomic Force Micrscopy



Advances in hIPSC differentiation – biological models and aplications

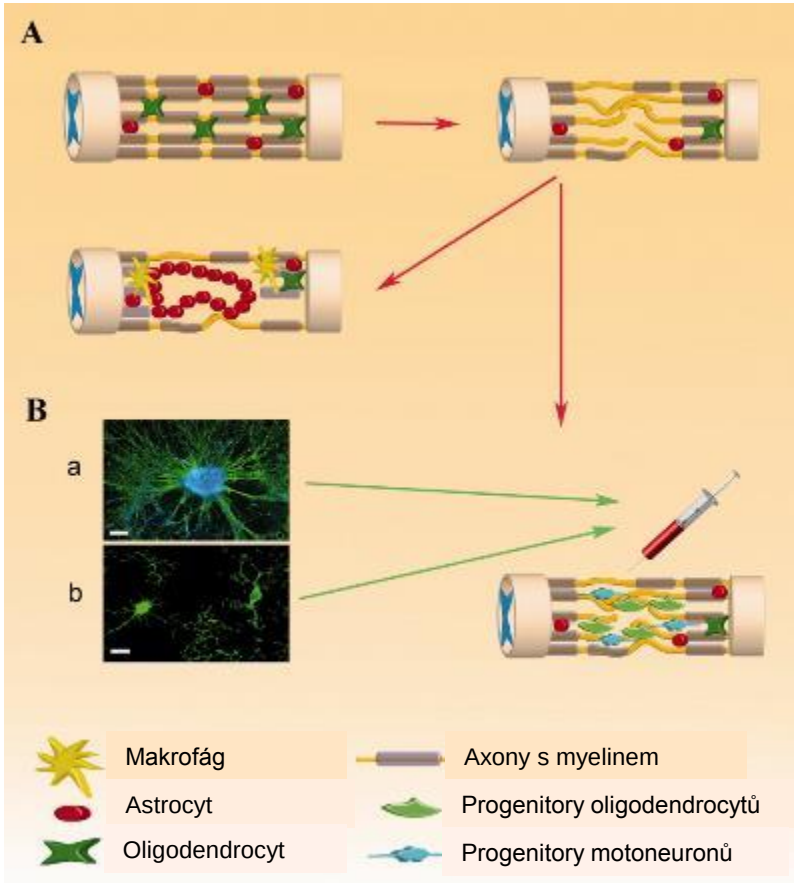
- Neurons, astrocytes, oligodendrocytes
- Cardiomyocytes
- Insulin-producing pankreatic cells
- Blood cells
- Immunocompetent cells
- Endotelial cells
- Trofoblast cells
- Respiratory cells
- Osteoblasts
- Hepatocytes
- Melanocytes
- Prostate cells
- Germ cells

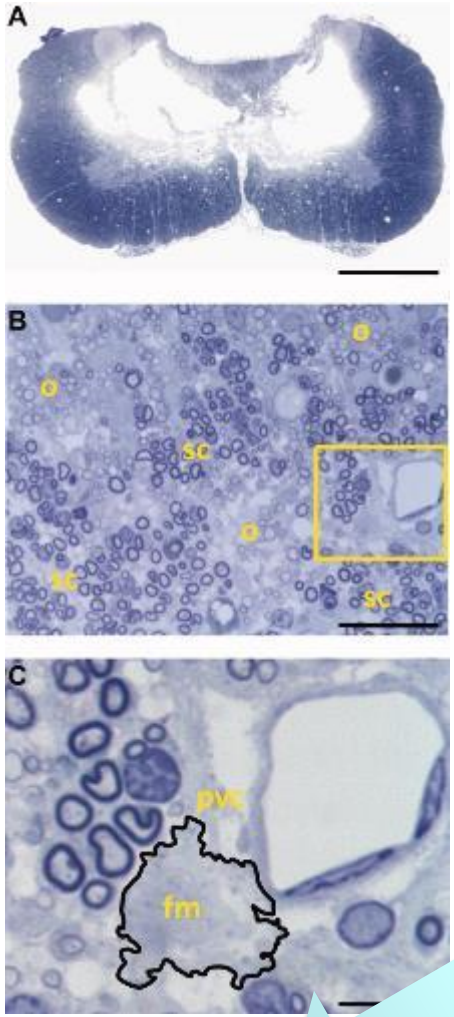


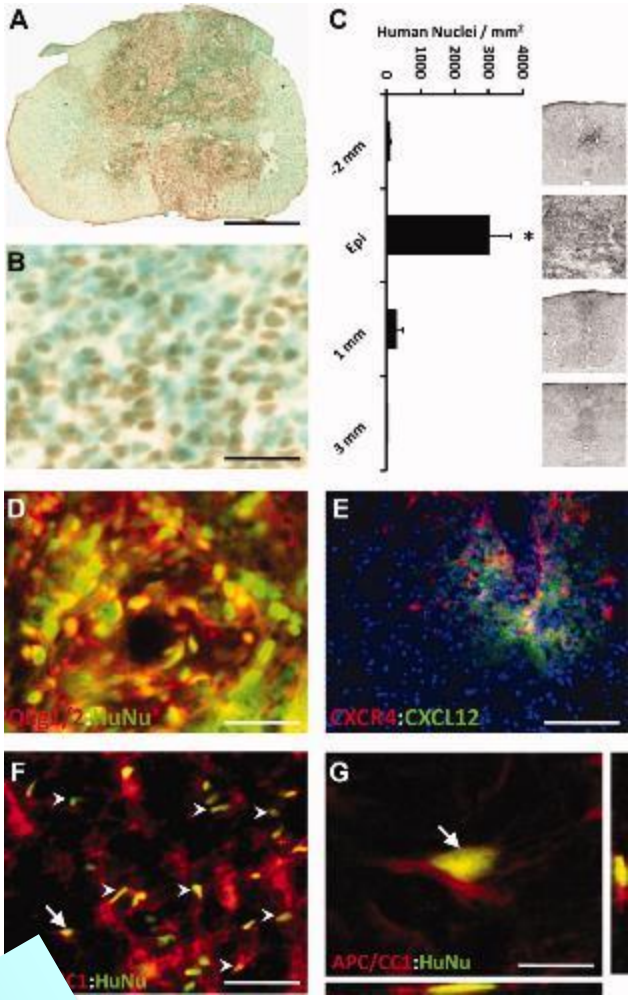
Example: thorasic spinal cord injury, myelopaty and SC treatment

Damage before the treatment

After the treatment







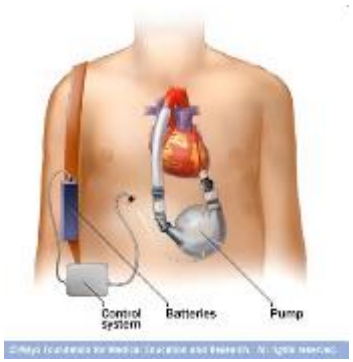
Result: reconstruction of gray and white matter
reconstruction of motoric neurons
return of the mobility

Model: rat

Adapted from Stem Cells, 2010

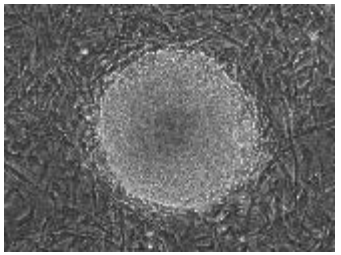
Successes: hiPSC

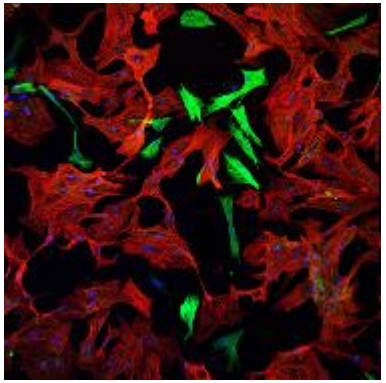
…Heart patches…



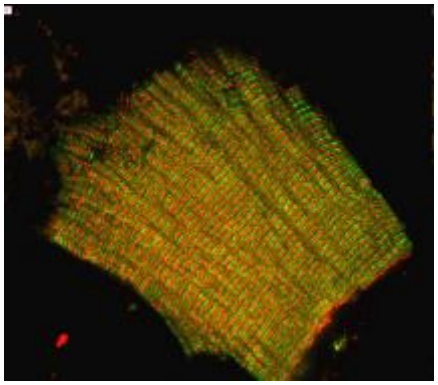
Patient suffering heart failure

5% ejection fraction





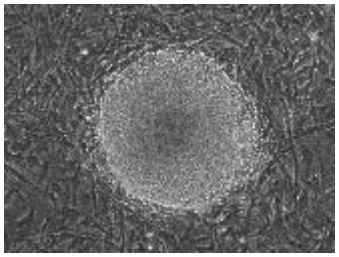
30% ejection fraction
… patient leaves the ER



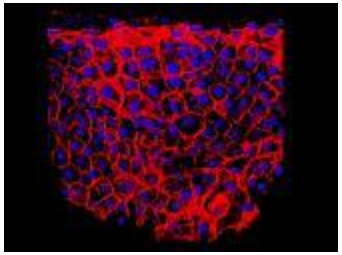
Teruo Okano: ISSCR Yokohama 2012

Succsesses: hESC

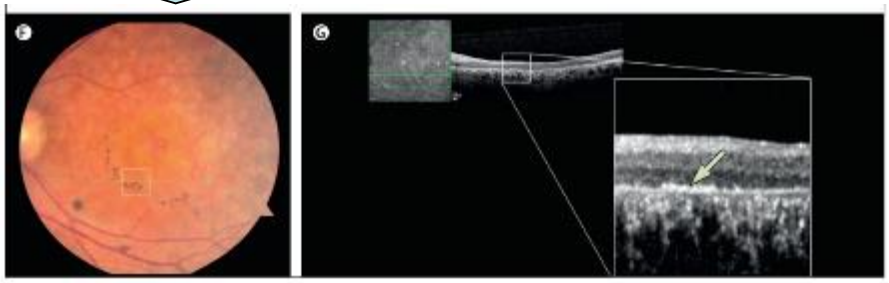
…RPE regeneration…



Human embryonic stem cells



Pigment epithelium differenciation



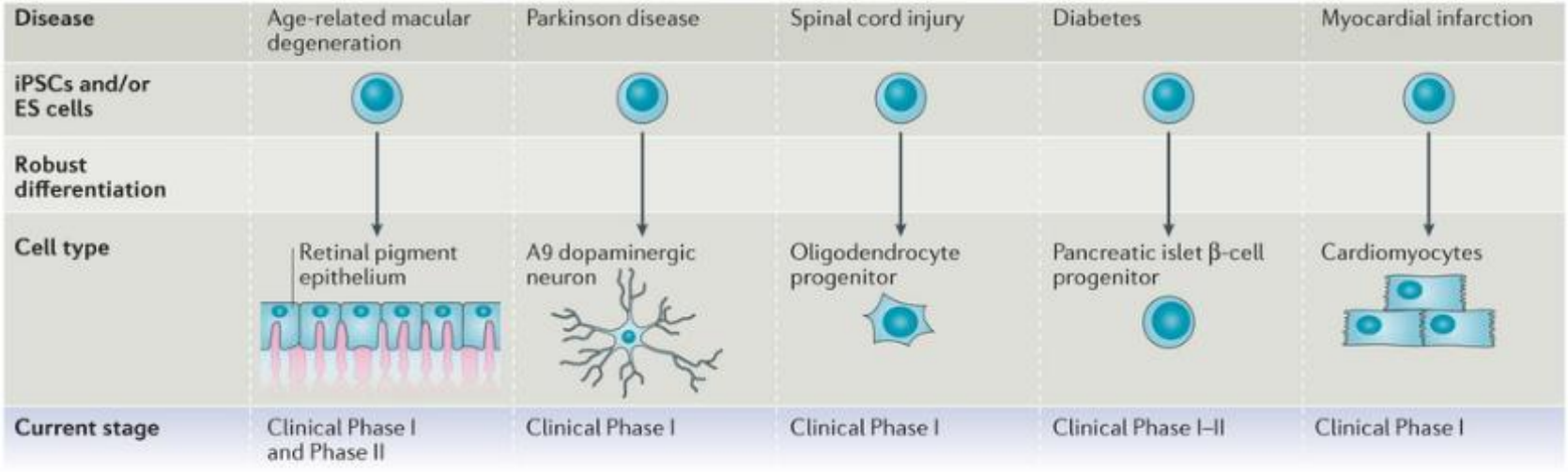
	BCVA	ETDRS (number of letters)
Fellow eye		
Baseline	Hand motion	0
1 week	Hand motion	0
2 weeks	Hand motion	0
3 weeks	Hand motion	0
4 weeks	Hand motion	0
6 weeks	Hand motion	0
8 weeks	Hand motion	0
12 weeks	Hand motion	0
Operated eye		
Baseline	Hand motion	0
1 week	Counting fingers	0
2 weeks	Counting fingers	1
3 weeks	Counting fingers	3
4 weeks	20/800	5
6 weeks	20/800	5
8 weeks	20/800	5
12 weeks	20/800	5

hESC=human embryonic stem cells. RPE=retinal pigment epithelium. BCVA=best corrected visual acuity. ETDRS=Early Treatment Diabetic Retinopathy Study visual acuity chart.

Table: Change in visual acuity after hESC-RPE transplantation in patient with Stargardt's macular dystrophy

Schwartz a kol. Lancet 2012; 379:713-20

Shortlist of recent applications of PSC in clinical trials
(more info in part II – with Dr. Martin Pesl)



Nature Reviews | Molecular Cell Biology

Trounson, 2016

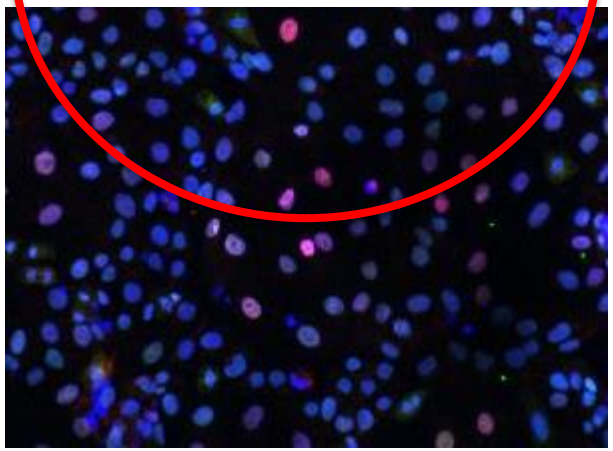
What is this for?

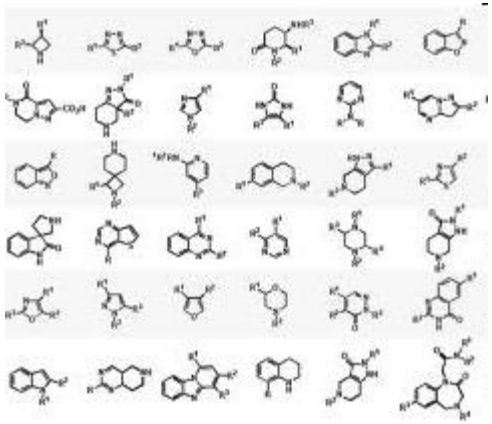
Disease modelling

Regenerative and reconstructive medicine

Understanding pathological processes

Drug development





Duchen Muscular Dystrophy

-Affected sceletal muscles

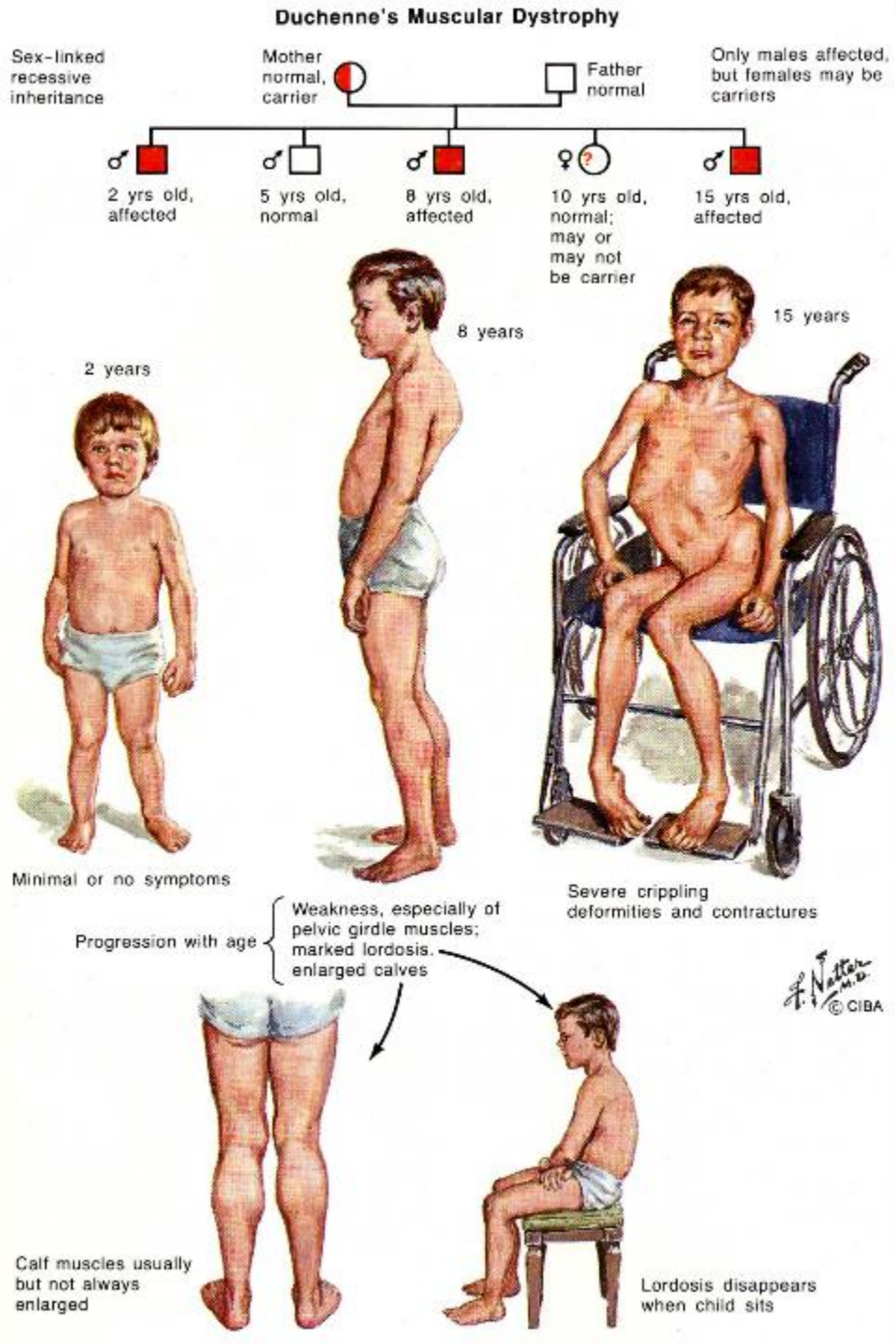
-Heart failure

Hypothesis:
-Missing dystropin

-Sarcoplasmic reticulum calcium leakage

-Would Ca2+ channel inhibitors help?

We want to test it on affected cells!!!



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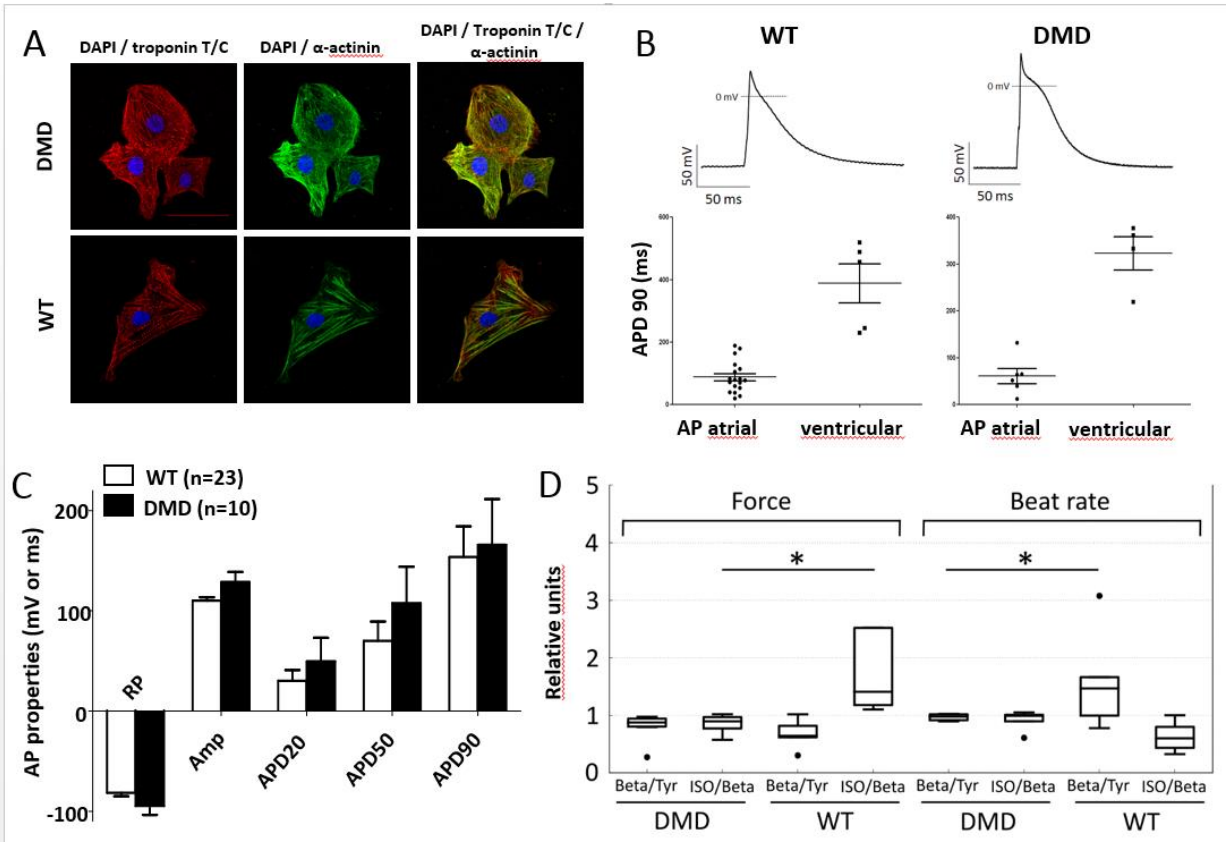
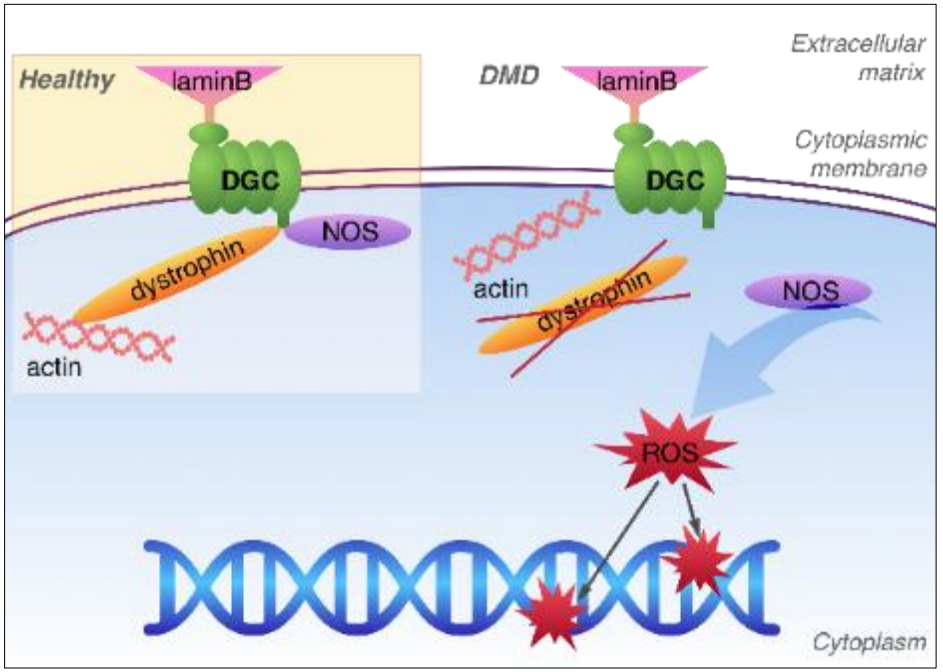


Found physiological differences but not severly preventing natural heart muscle regeneration and what would explain heart failure…

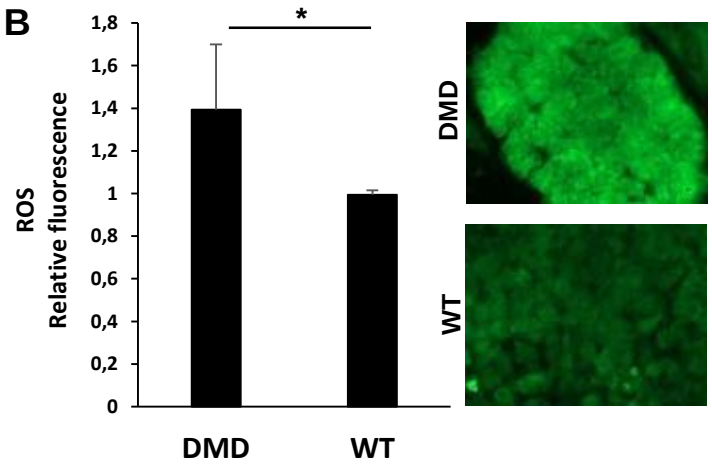
Duchen Muscular Dystrophy

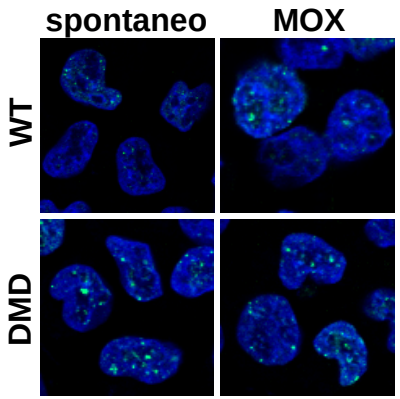
-Affected sceletal muscles

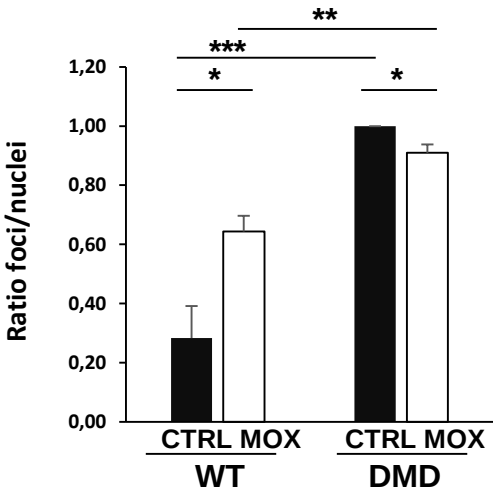
-Heart failure



D







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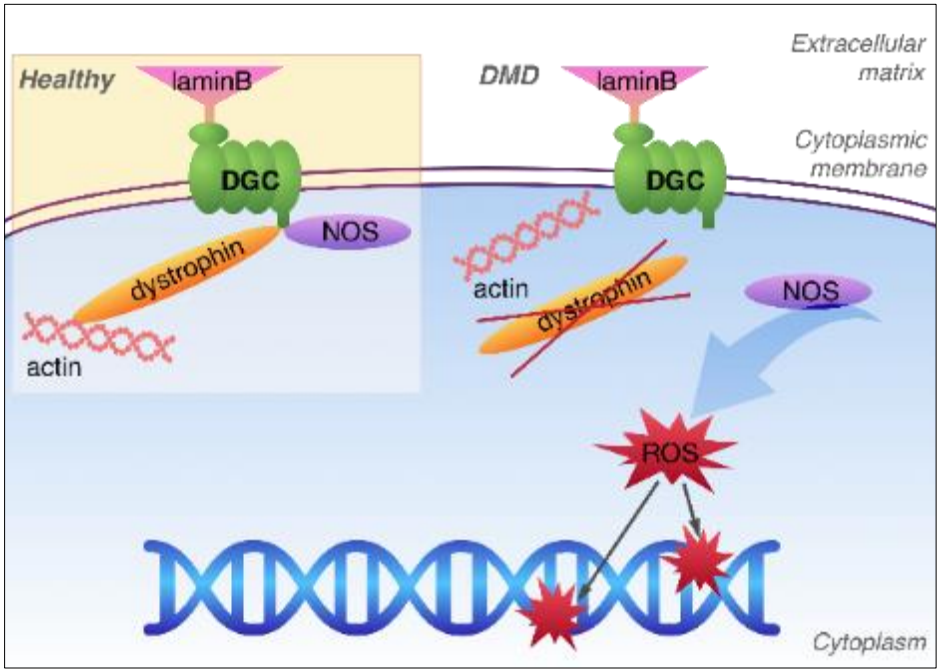

Conclusion: defective dystrophin causes elevated ROS production via NO synthase, causing SC mutagenesis and progenitor depletion

What is this for?

Disease modelling

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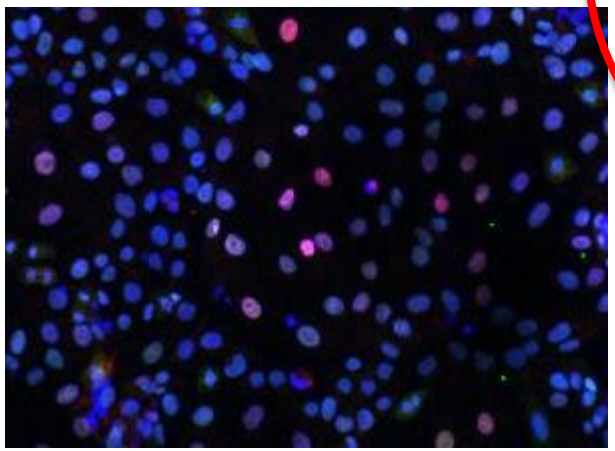
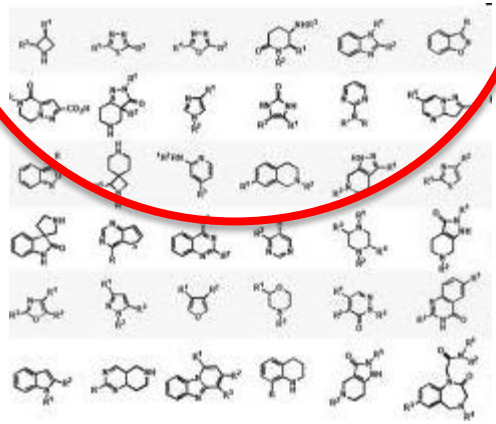
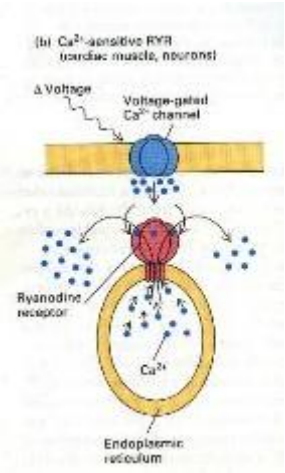




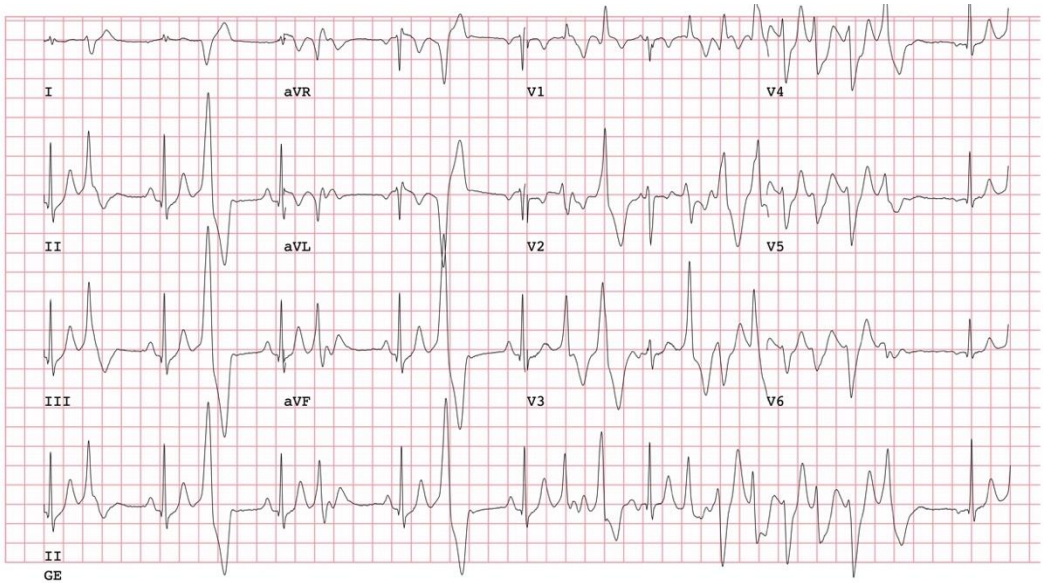
Catecholinergic Polymorphic Ventricular Tachycardia (CPVT)

Sportment‘s Sudden Cardiac Death Syndrome

Marc Vivian Foe



Mutace RyR:
Pomalý únik Ca2+

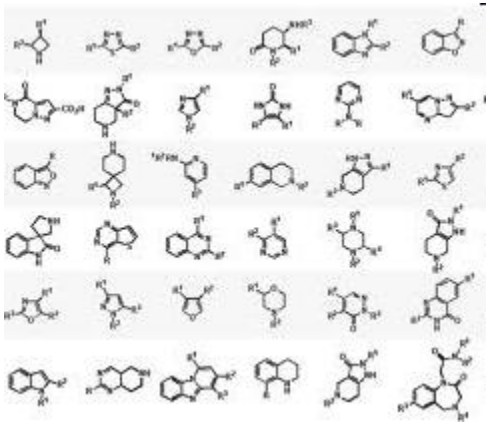


Ca^{2+} channel inhibitor screen needed – will hiPSC help?

Classical proces impossible

Target organ biopsy

We do not have access to the sufficiently sized heart biopsies

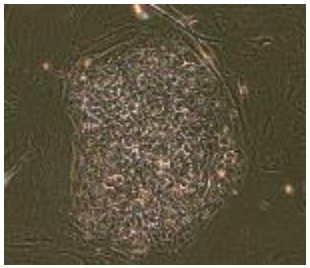


Putative drugs

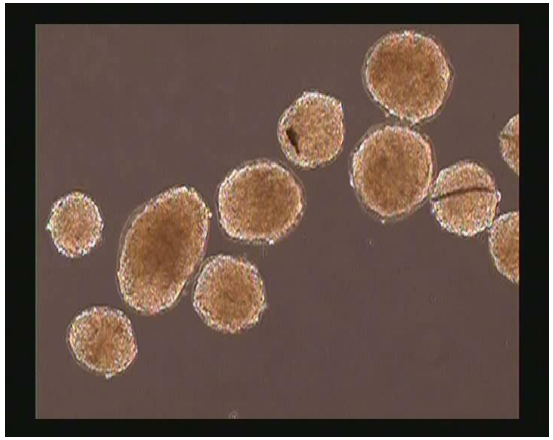
„ex vivo“/“in vitro“ cultivation

… how to get heart cells of the patient who cannot provide them..

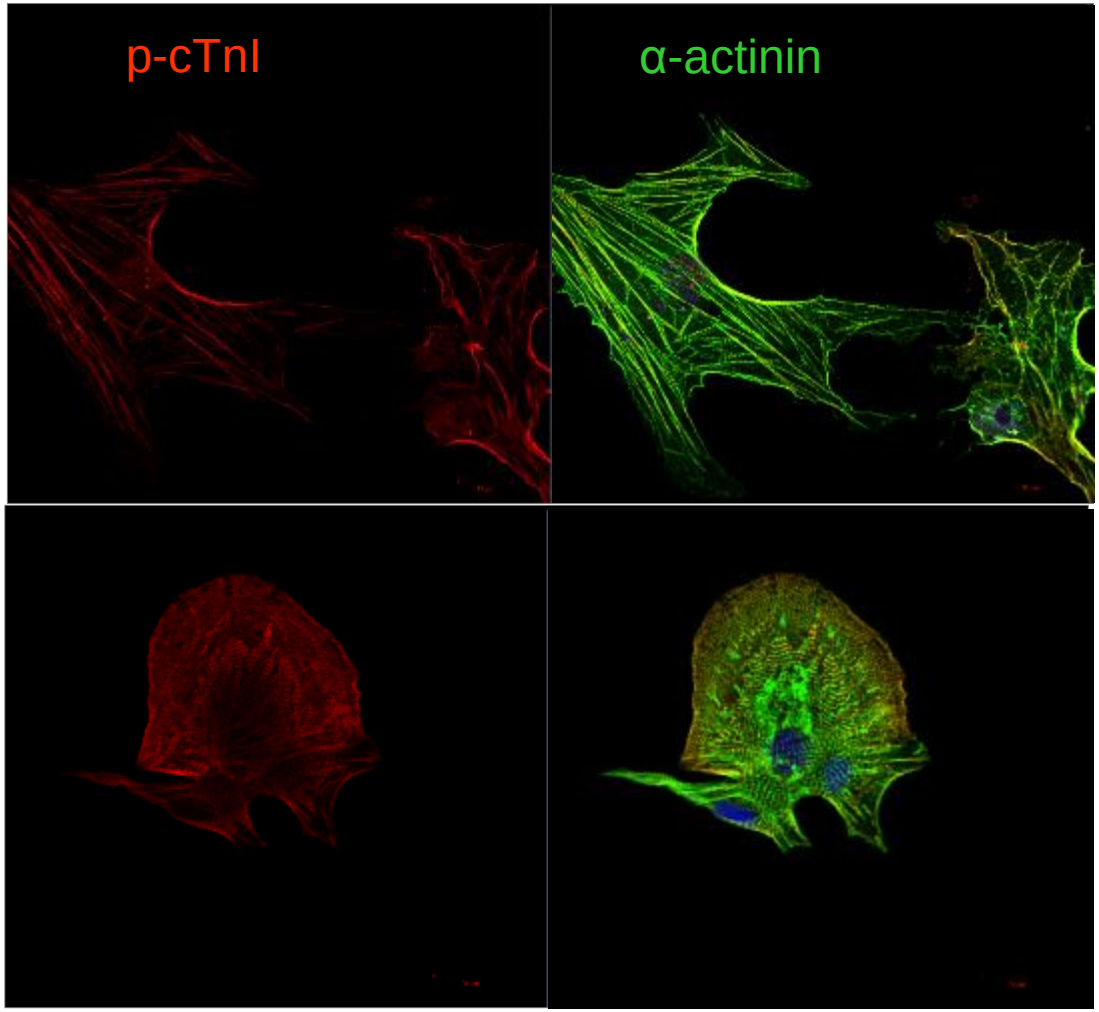
Skin biopsy



hiPSC



Embryonic bodies



Patient specific hiPSC derived cardiomyocytes

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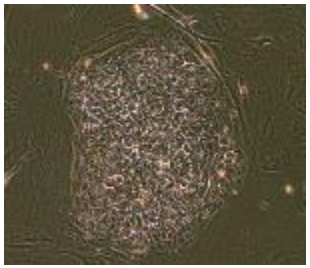

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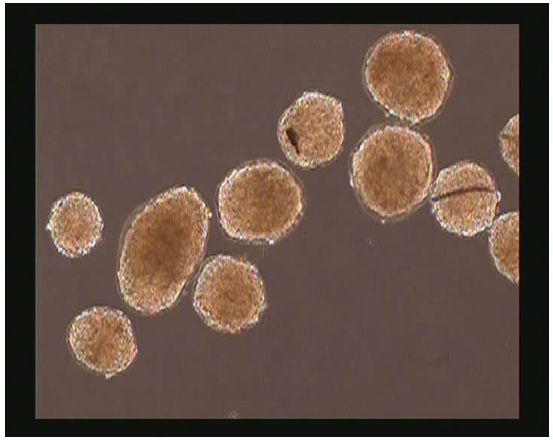
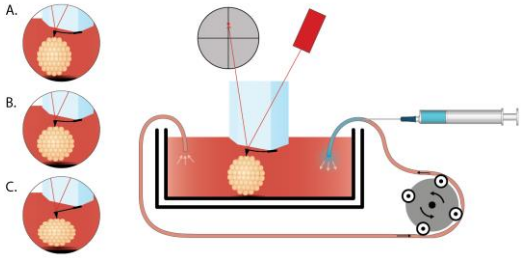
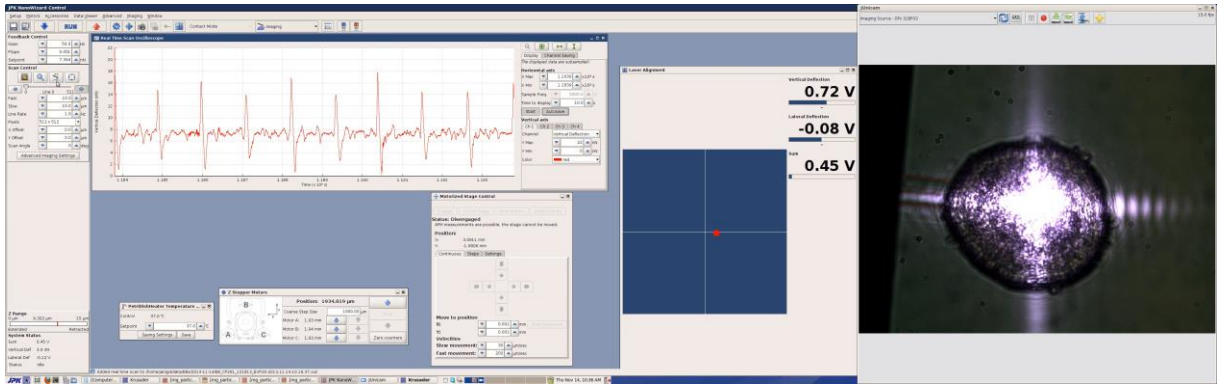


Embryonic bodies

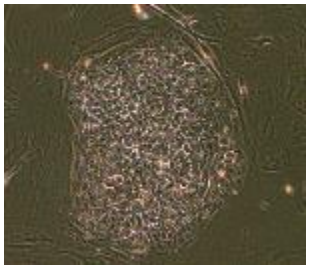
Biomechanical properties:



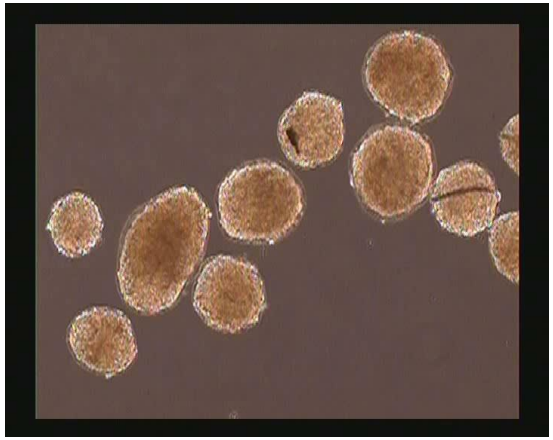


Analysis using atomic force microscope

Skin biopsy



hiPSC



Embryonic bodies

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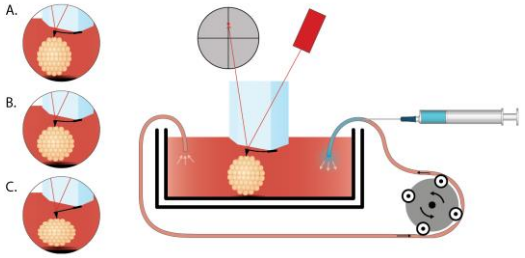

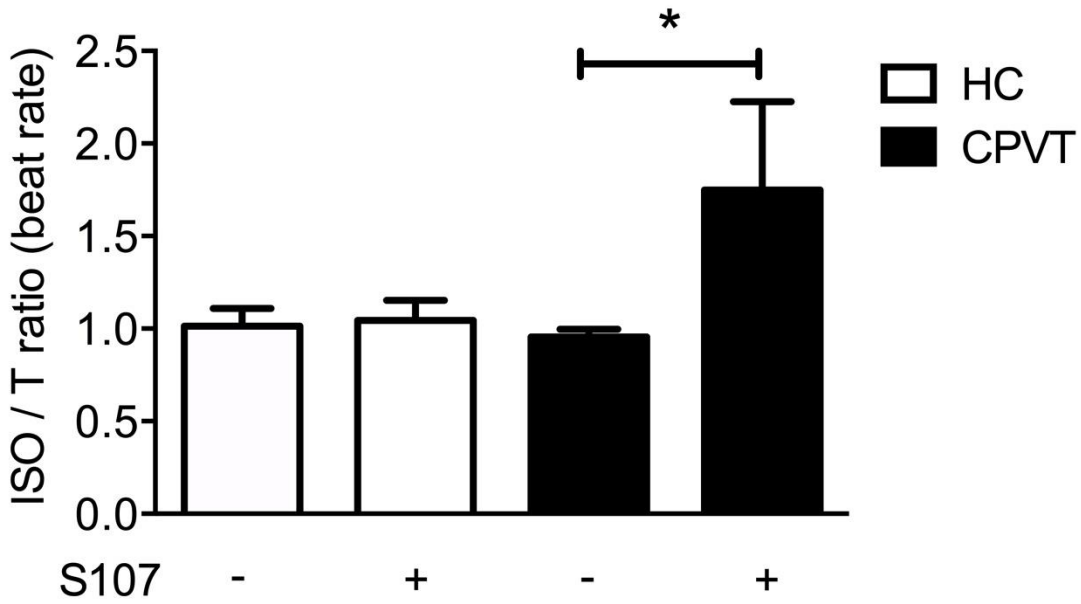

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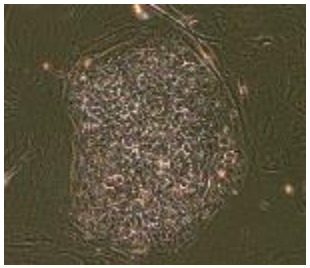
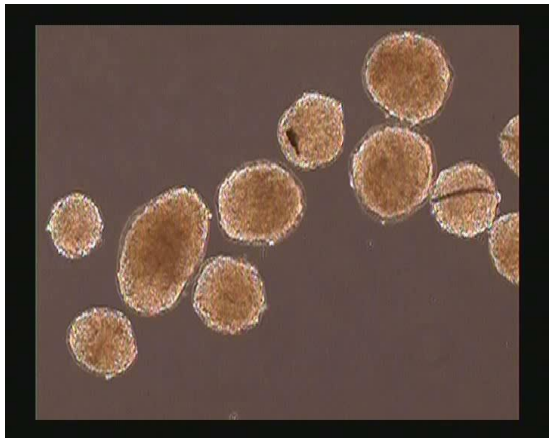


hiPSC



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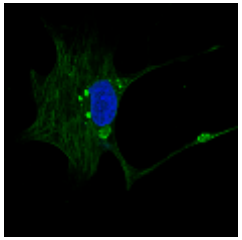
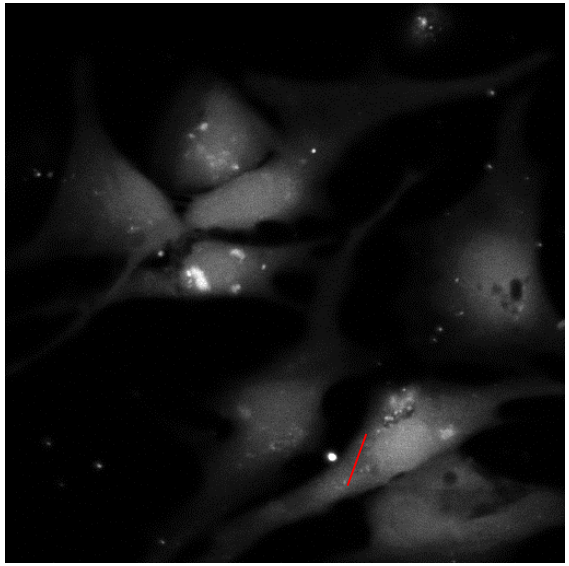
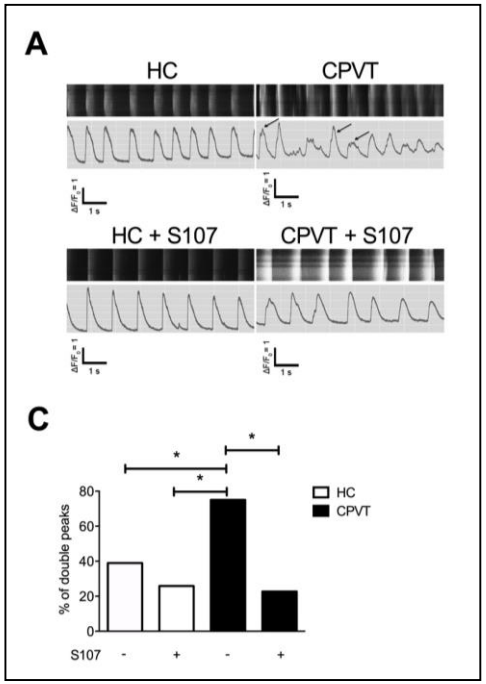


Fluorescent microscopy





Acimovic, Rotrekl a kol.

Conclusion: Succsessful stabilization of RyR2 and calstabin 2 binding by S107 compensated CPVT fenotype.

Final discussion – PART I (pluripotent stem cells)

- Ageing of the PSC in culture – dangerous genetic changes?
- Genetic reprogramming – is that a danger in iPS based therapy?
- Limitted diversity of PSC lines and associated GVH disease risk?
- Legislature sufficient/overcautios?