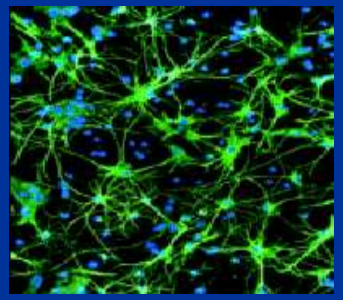
Cloning and Stem Cell research

&

Therapeutic Possibilities



Hans Bluyssen, 18-11-2020

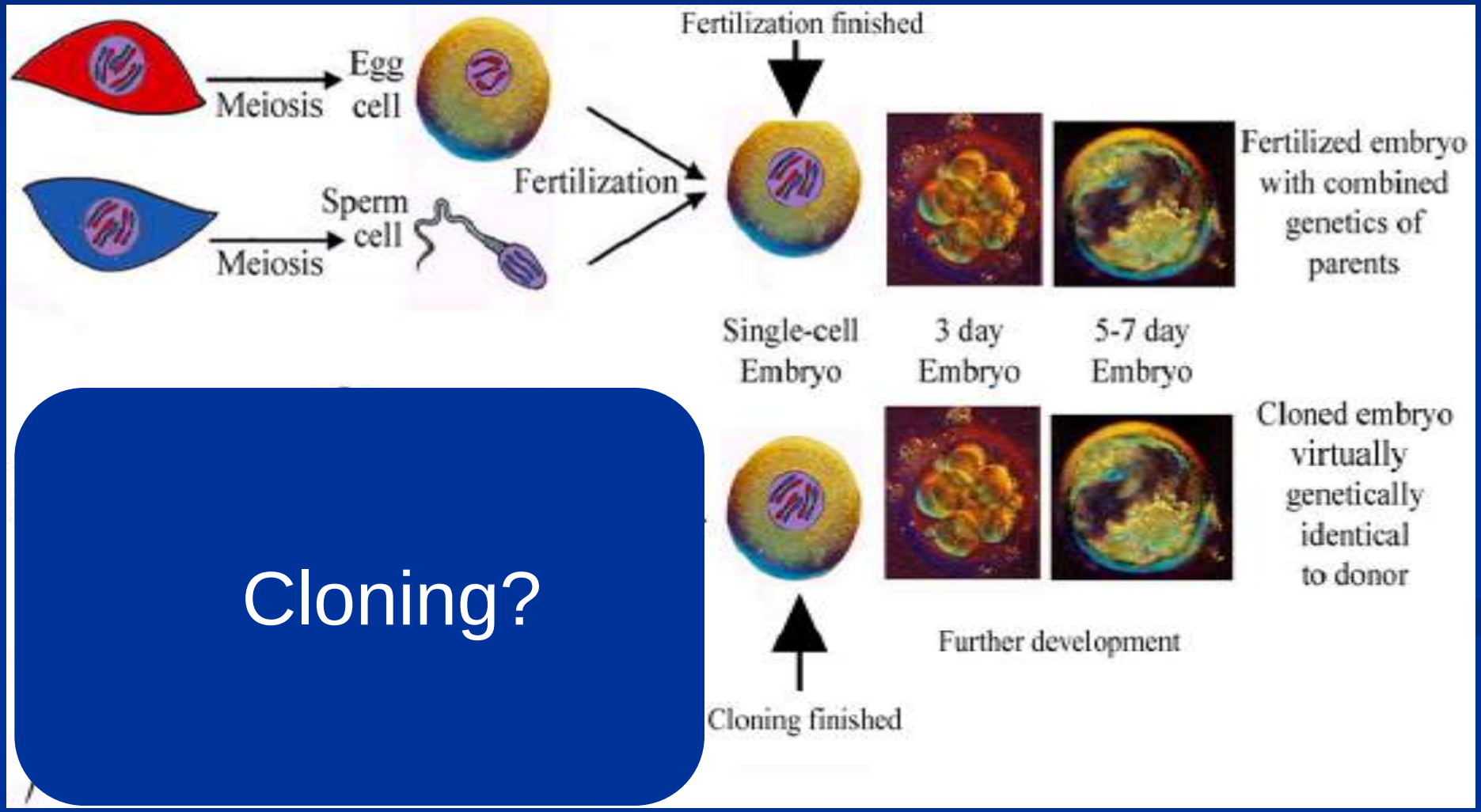
Cloning

- The process of making an identical copy of something

- In biology, it collectively refers to processes used to create copies of:
DNA fragments (molecular cloning),
cells (cell cloning),
or organisms

Fertilization *vs*. Cloning

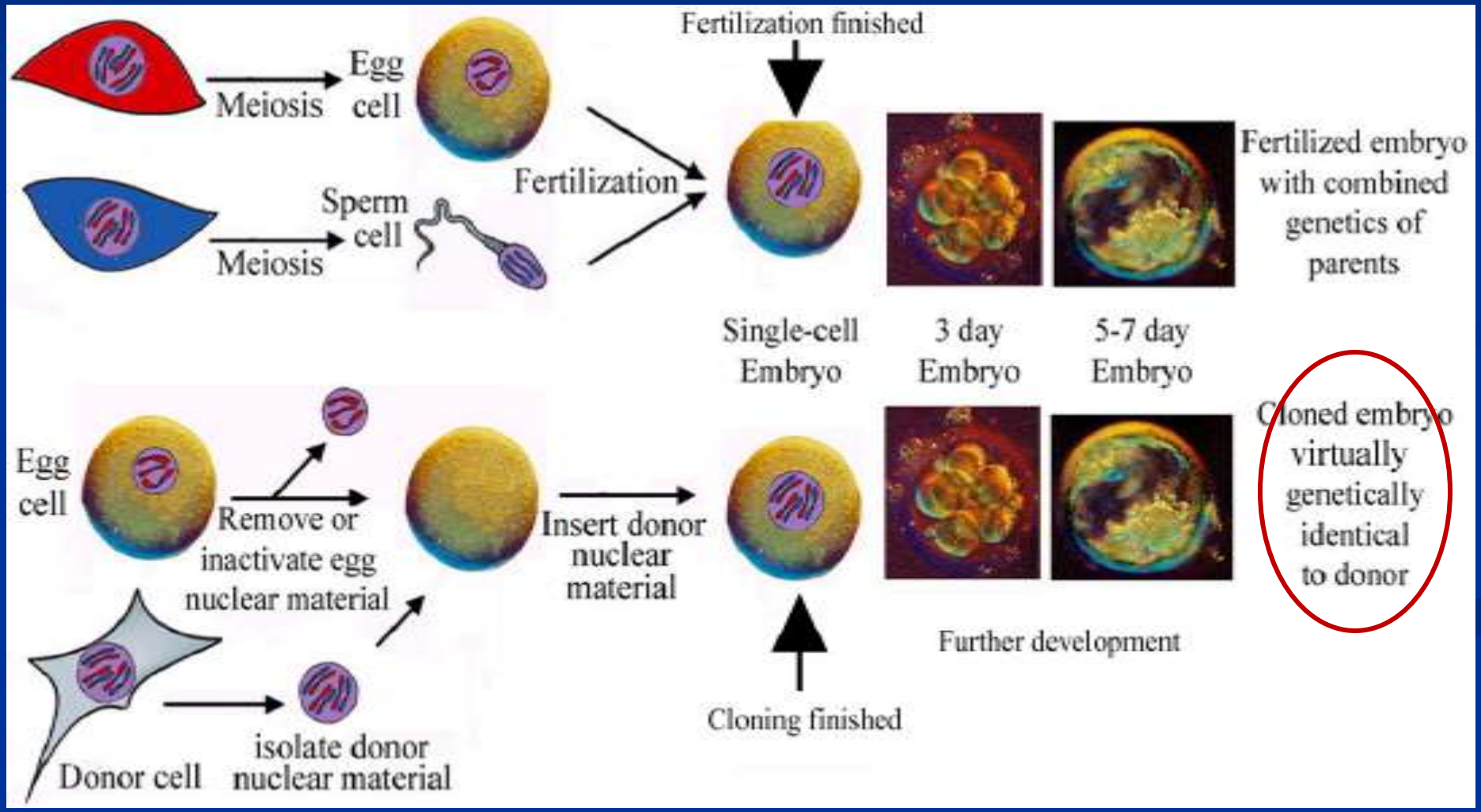
(somatic cell nuclear transfer, SCNT)



Cloning?

Fertilization *vs*. Cloning

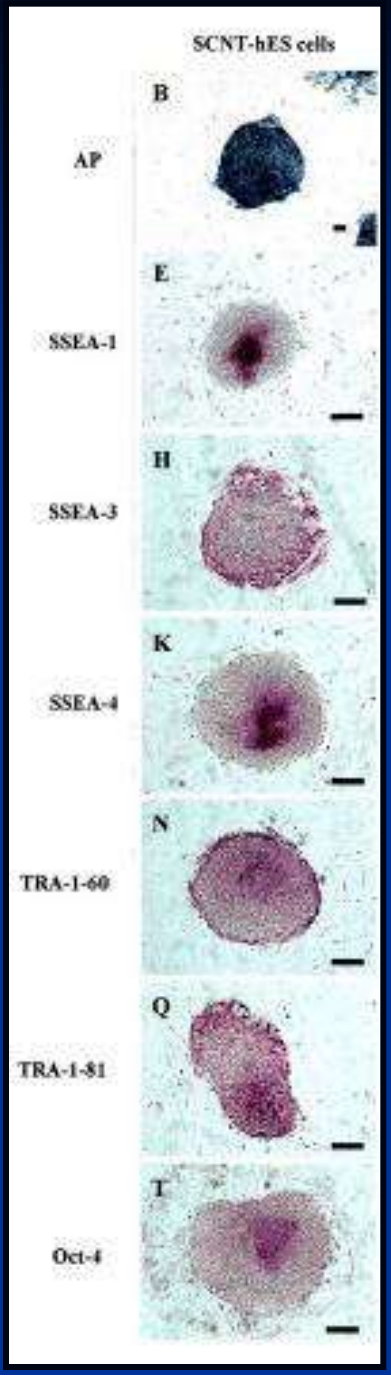
(somatic cell nuclear transfer, SCNT)



History of Somatic Cell Nuclear Transfer (Cloning)

- 1952 – Briggs and King cloned tadpoles
- 1996 – The first mammal cloned from adult cells was Dolly, the sheep.

- 1998 – Mice cloned
- 1998 – Cows cloned
- 2000 – Pigs cloned



Early Successes – Human Cloning

- 2001 – First cloned human embryos (only to six cell stage) created by Advanced Cell Technology (USA)
- 2004* – Claim of first human cloned blastocyst created and a cell line established (Korea) – later proved to be fraudulent

***Hwang, W.S., et al. 2004. Evidence of a Pluripotent Human Embryonic Stem Cell Line Derived from a Cloned Blastocyst. *Science* 303: 1669-1674.**

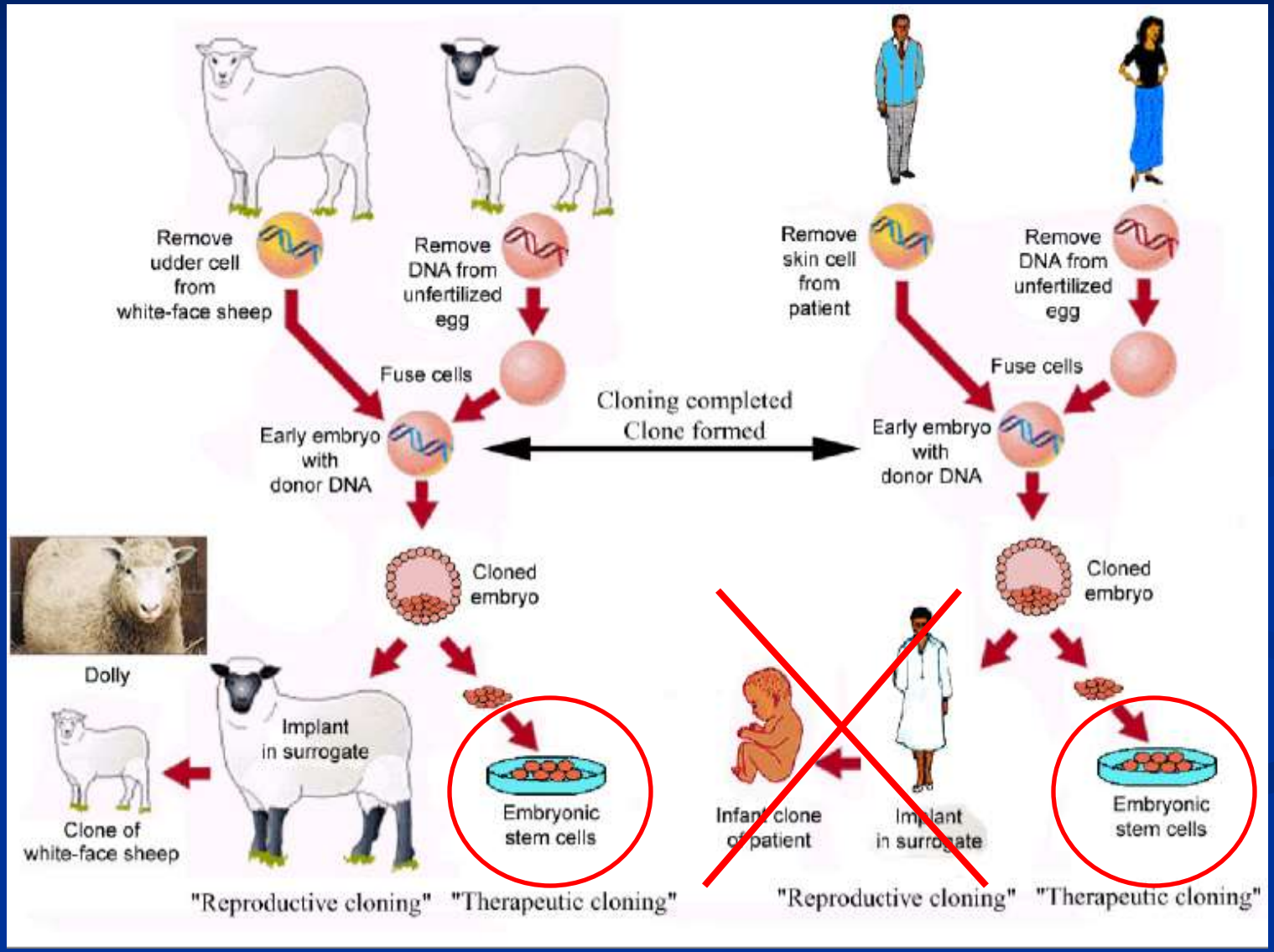
Cloning

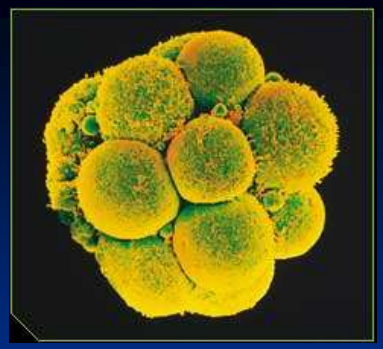
Reproductive cloning is a technology used to generate an animal that has the same nuclear DNA as another currently or previously existing animal.

Therapeutic cloning, also called "embryo cloning," is the production of human embryos for use in research,
not to create cloned human beings.

“Stem Cells”

Reproductive vs. Therapeutic Cloning





Embryo three days after fertilization

Stem cells

- cells found in most, if not all, multi-cellular organisms
- Self-renew in an undifferentiated state for prolonged times while retaining the ability to differentiate
- Potency - the capacity to differentiate into specialized cell types. Unipotent, multipotent, pluripotent or totipotent
- Embryonic (blastocysts) and Adult (adult tissues)
- progenitor cell has limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell.

Stem cells

- In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues.

- In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin or intestinal tissues.

- Understanding how stem cells develop into healthy and diseased cells will assist the search for cures.

Potency

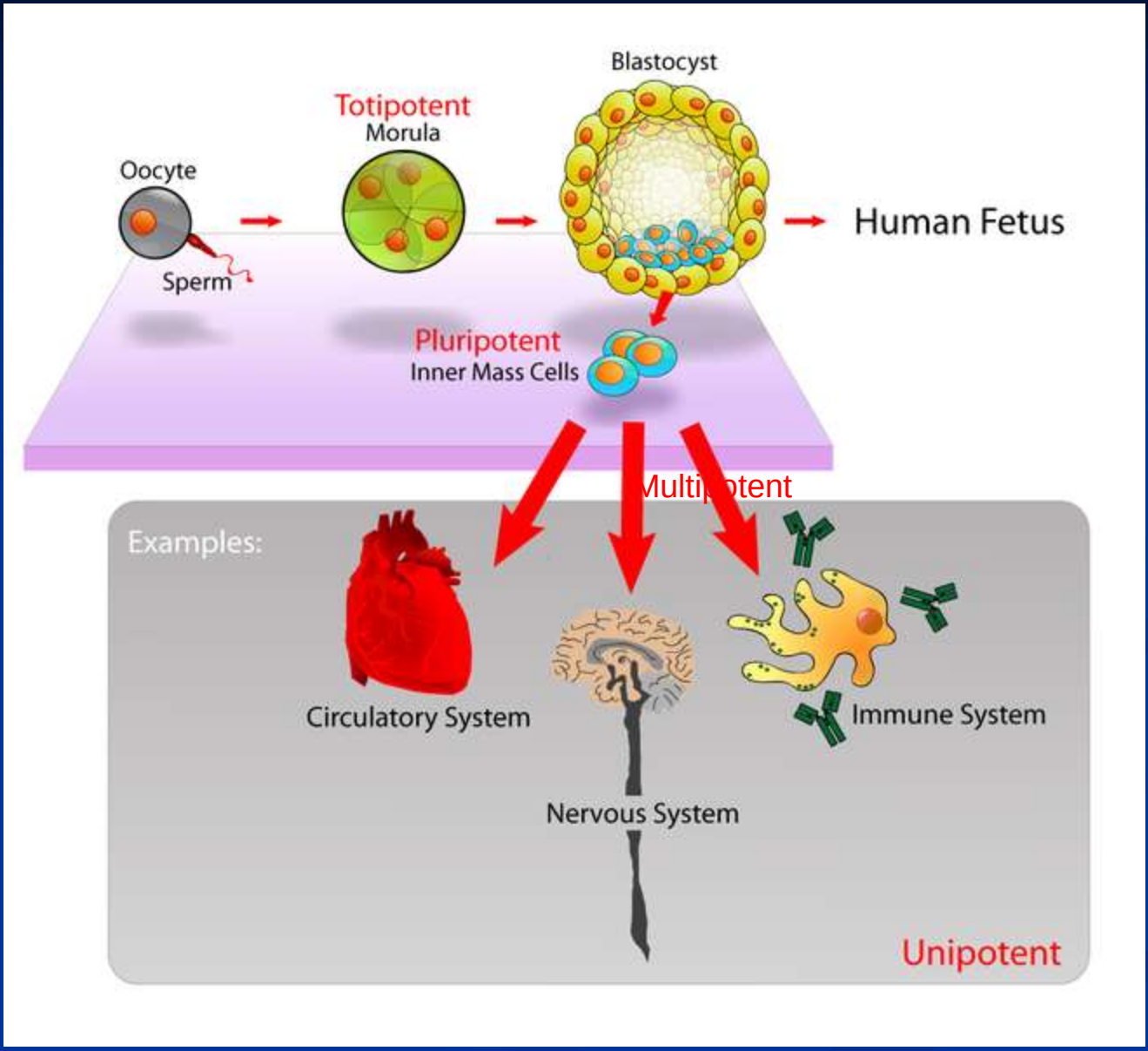
- **Totipotent** stem cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent. These cells can differentiate into embryonic and extraembryonic cell types

- **Pluripotent** stem cells are the descendants of totipotent cells and can differentiate into cells derived from any of the three germ layers

Potency

- **Multipotent** stem cells can produce only cells of a closely related family of cells (e.g. hematopoietic stem cells differentiate into red blood cells, white blood cells, platelets, etc.)

- **Unipotent** cells can produce only one cell type, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells)


Blastocyst
Totipotent
Morula
Oocyte
Sperm
Human Fetus
Pluripotent
Inner Mass Cells
Multipotent
Examples:
Circulatory System
Nervous System
Immune System
Unipotent

Embryonic Stem Cell Culture

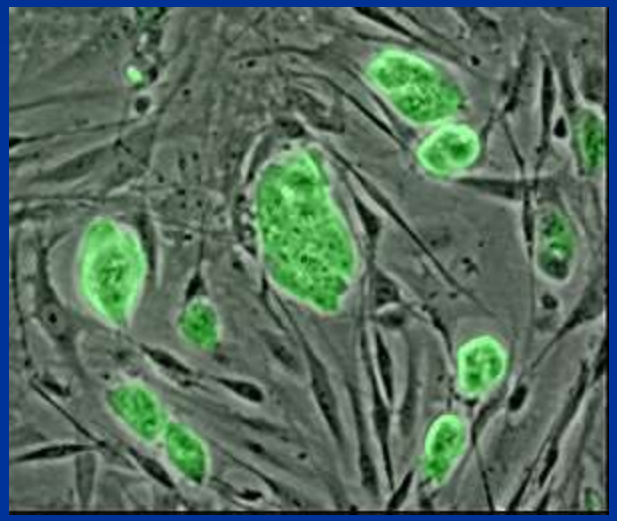
Researchers extract stem cells from 5-7 days old blastocyst.

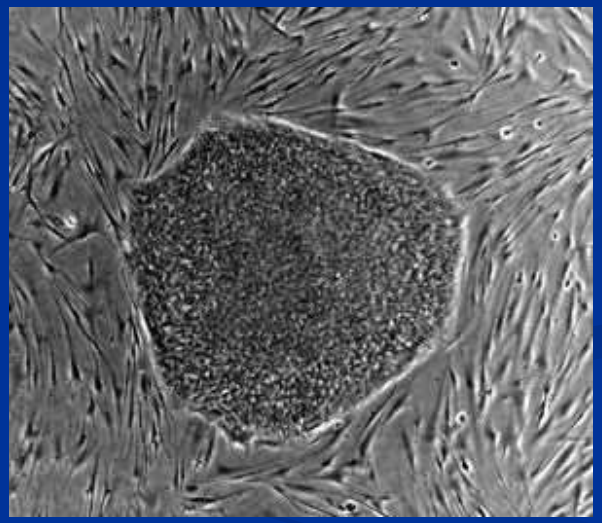
Stem cells can divide in culture to form more of their own kind, thereby creating a stem cell line.

The research aims to induce these cells to generate healthy tissue needed by patients.

Embryonic stem cells in culture

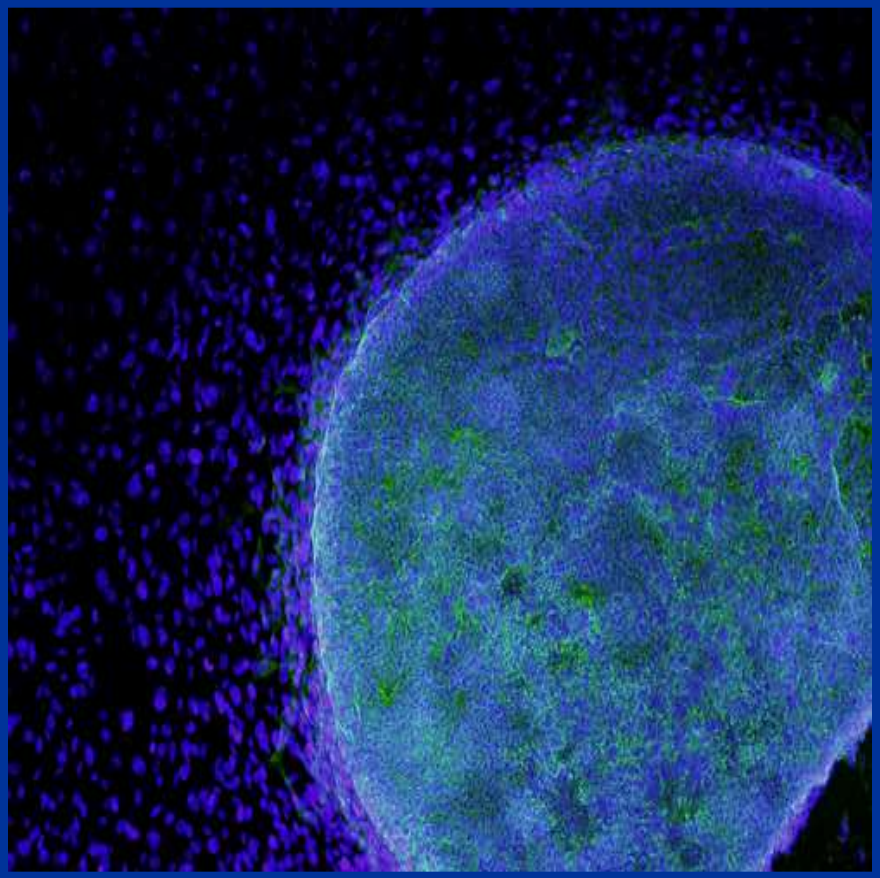
Mouse ES cells are grown on a layer of gelatin and require the presence of Leukemia Inhibitory Factor (LIF)

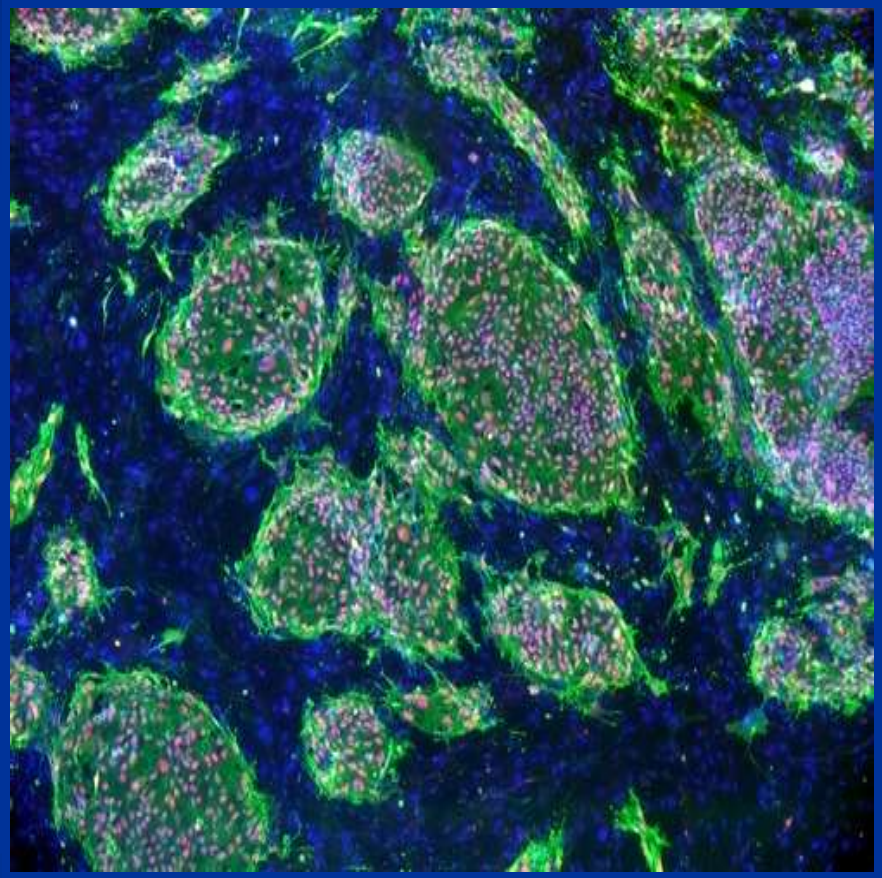




Human ES cells are grown on a feeder layer of mouse embryonic fibroblasts and require the presence of basic Fibroblast Growth Factor (bFGF or FGF-2)

Fluorescent imaging of embryonic stem cell colonies.

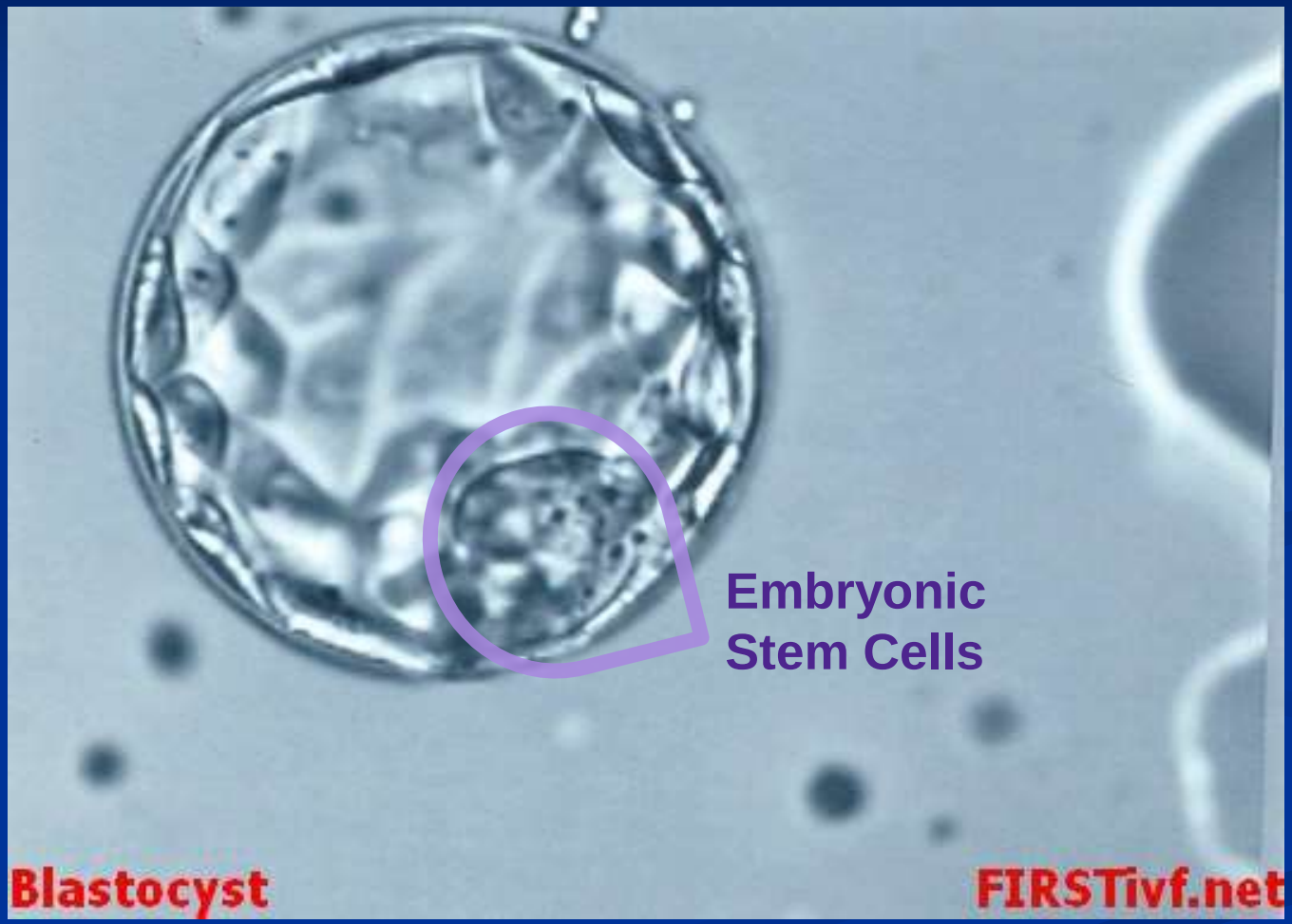




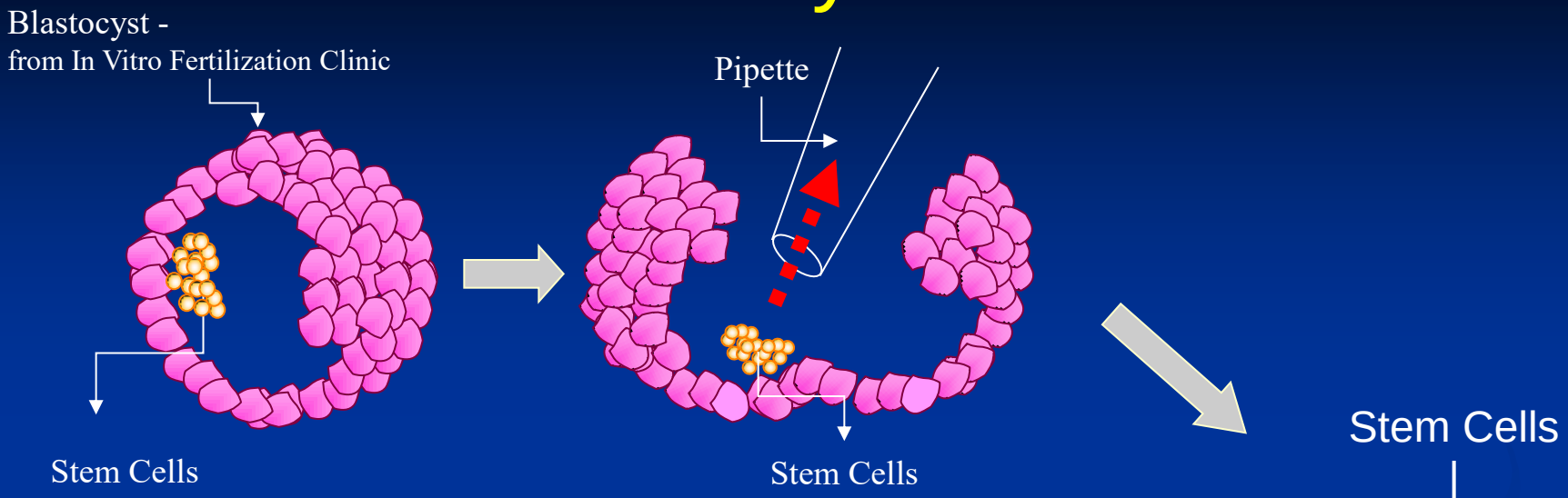
Sources of Embryonic Stem Cells

- Embryonic stem cell lines
- Excess embryos from IVF clinics

Intra-Cytoplasmic Sperm Injection


Embryonic
Stem Cells
Blastocyst
FIRSTivf.net

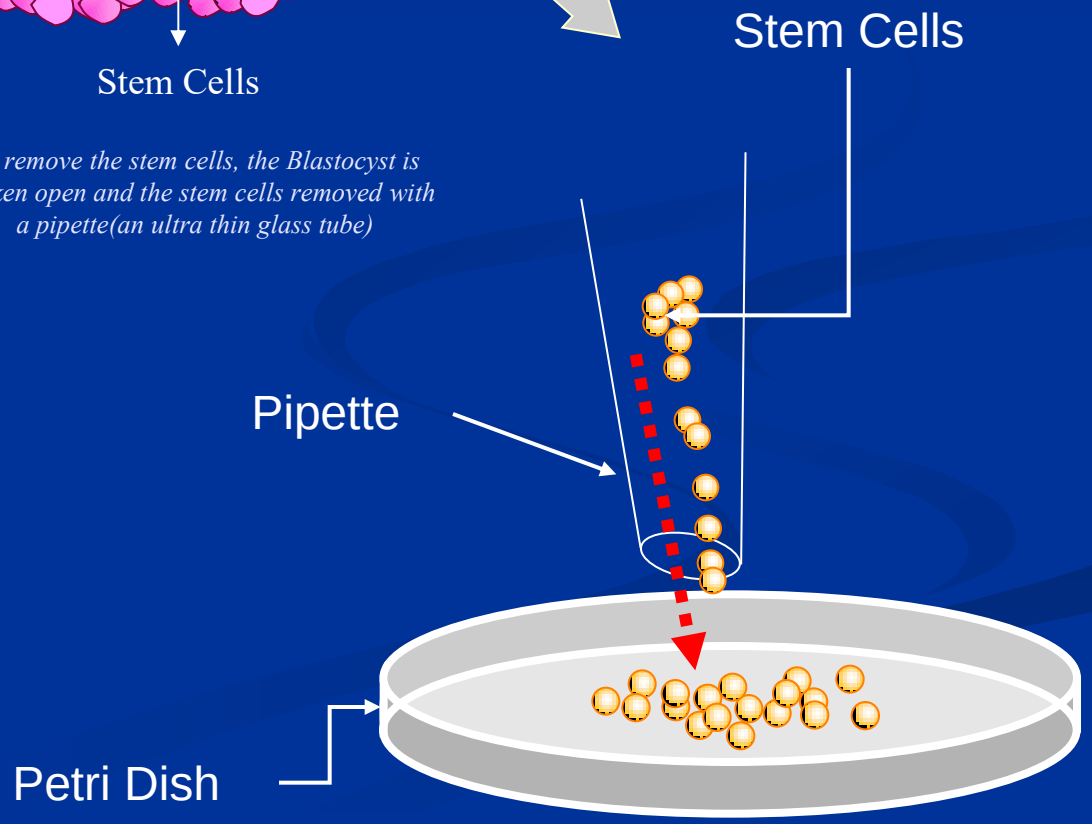
Human Embryonic Stem Cells

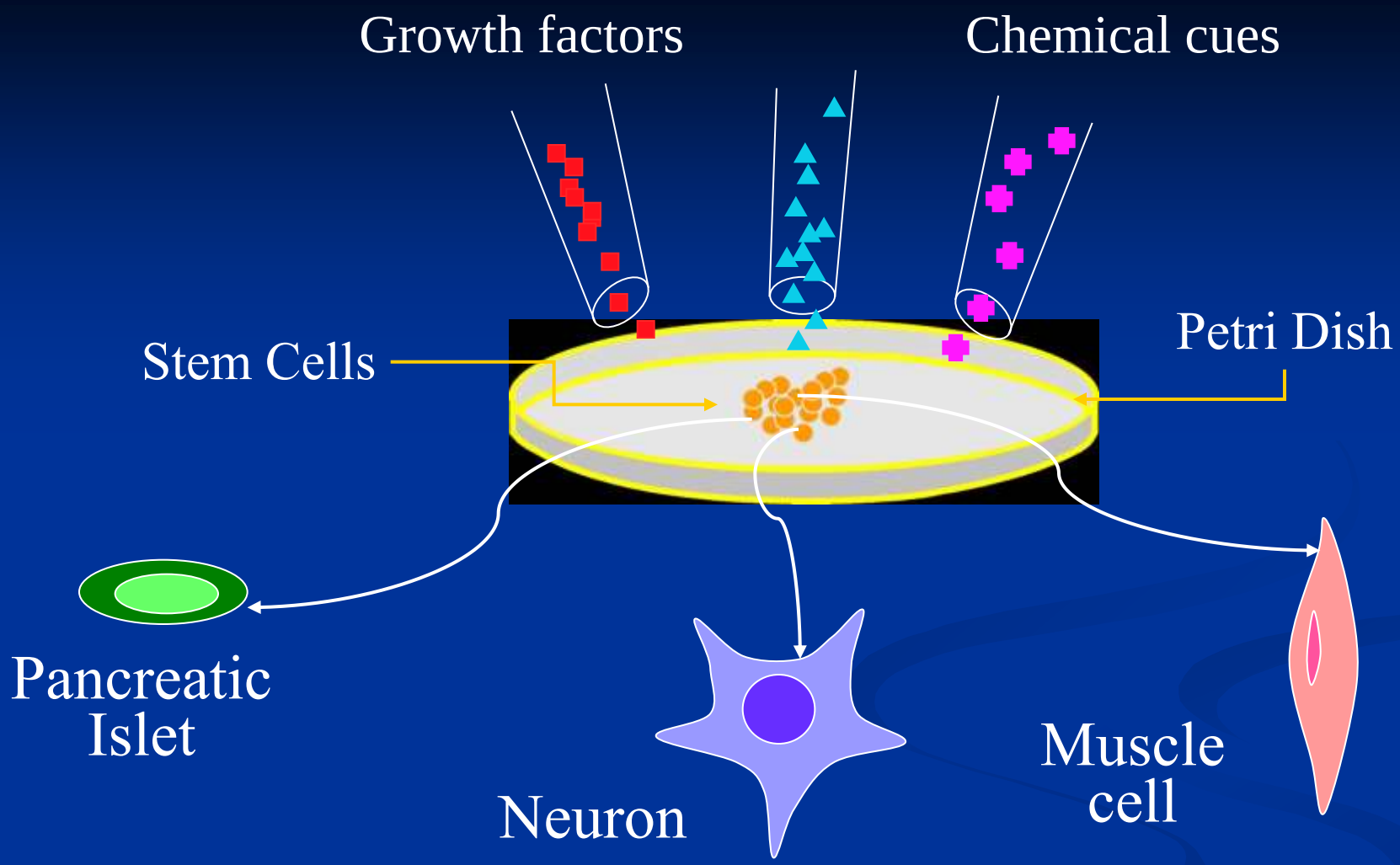


A Blastocyst is a hollow ball of cells with a small clump of stem cells inside

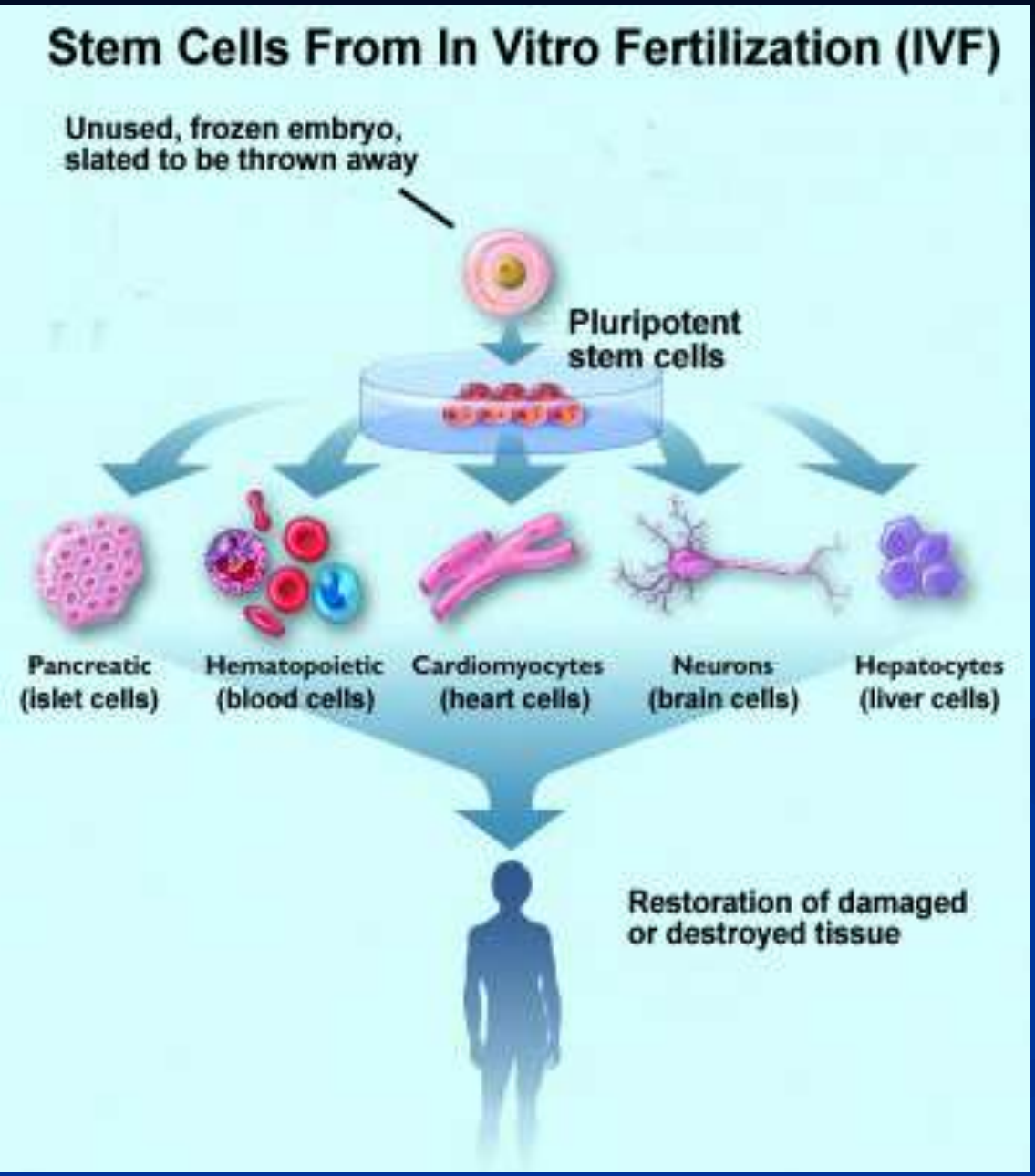
To remove the stem cells, the Blastocyst is broken open and the stem cells removed with a pipette(an ultra thin glass tube)

The stem cells are placed in a dish and are fed and cared for (each blastocyst = 1 stem cell line)





Different chemicals / molecules are added to the stem cells to make them become specific types of cells.



- Tens of thousands of frozen embryos are routinely destroyed when couples finish their treatment.
- These surplus embryos can be used to produce stem cells.
- Regenerative medical research aims to develop these cells into new, healthy tissue to heal severe illnesses.

Adult stem cells

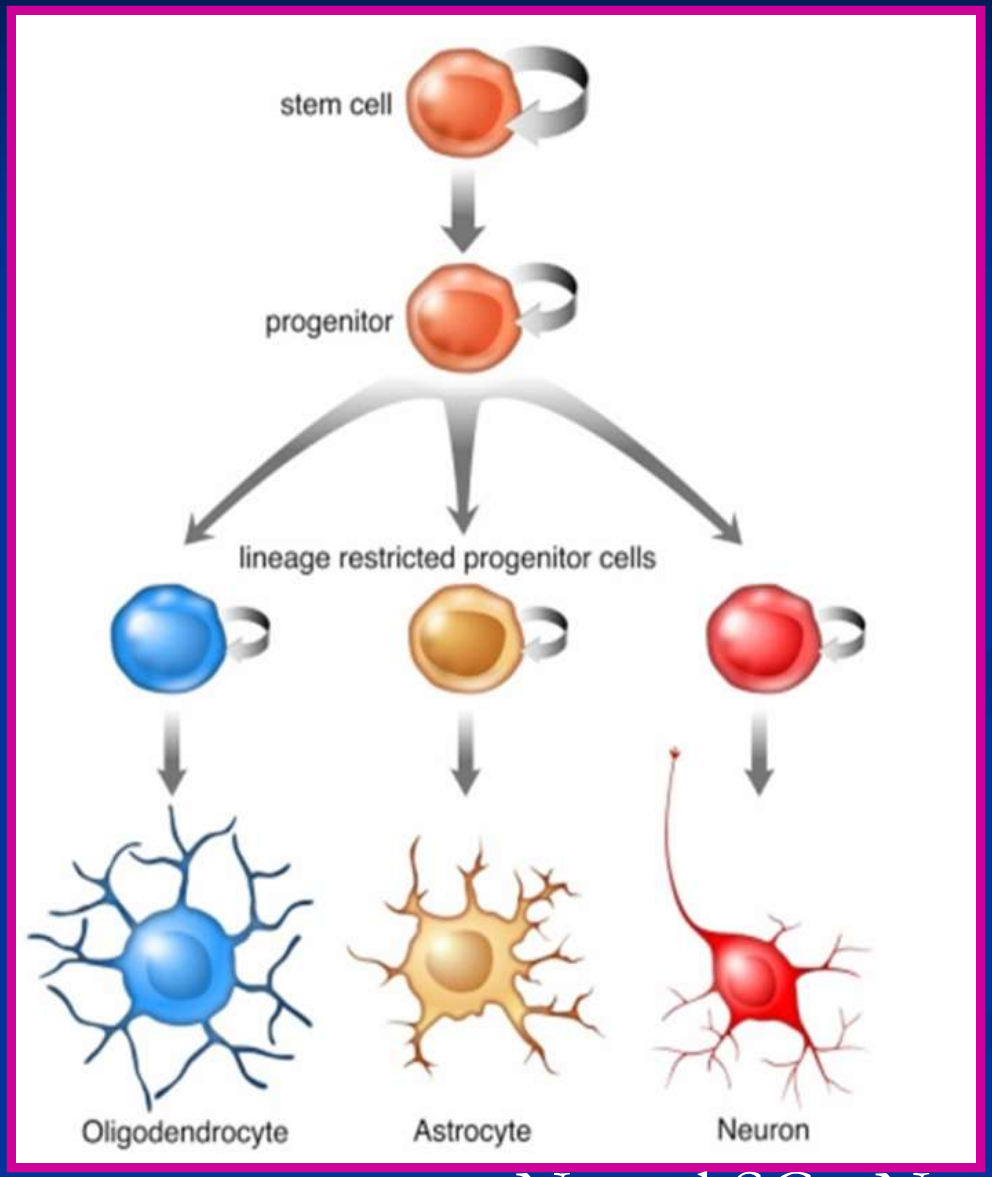
- Adult stem cells are rare: 1 in 10,000 to 15,000 cells in the bone marrow is a hematopoietic stem cell (**HSC**)

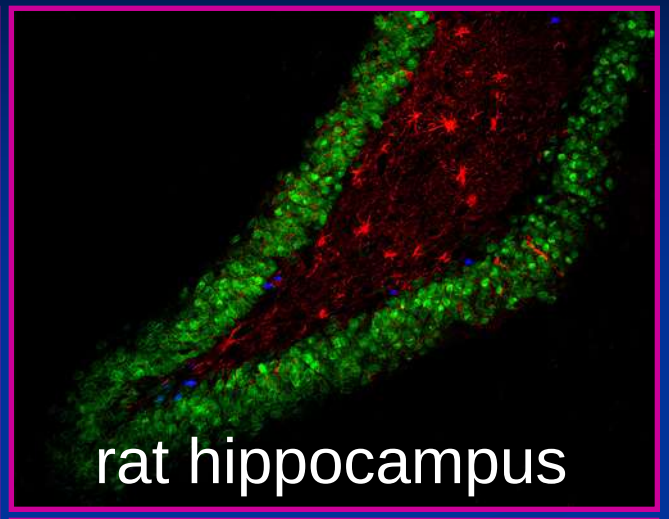
- Primary functions:
  - to maintain homeostasis
  - with limitations, to replace cells that die because of injury or disease

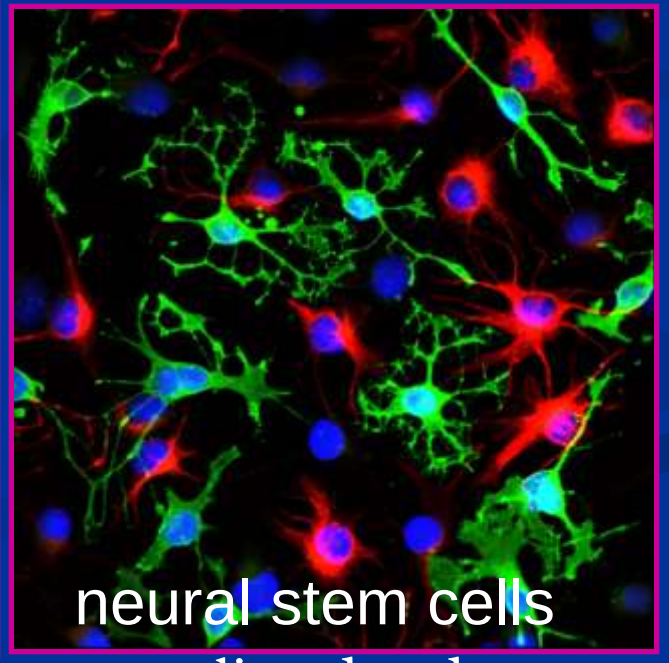
- Dispersed in tissues throughout the mature organism and behave very differently, depending on their local environment.
  - HSCs are constantly being generated in the bone marrow where they differentiate into mature types of blood cells (replace blood cells)
  - Stem cells in the small intestine are stationary, and physically separated from the mature cell types they generate. Occur at the bases of crypts— that line the lumen of the intestine.

Stem cells in the adult brain:

Neural SCs, Neurons, astrocytes,



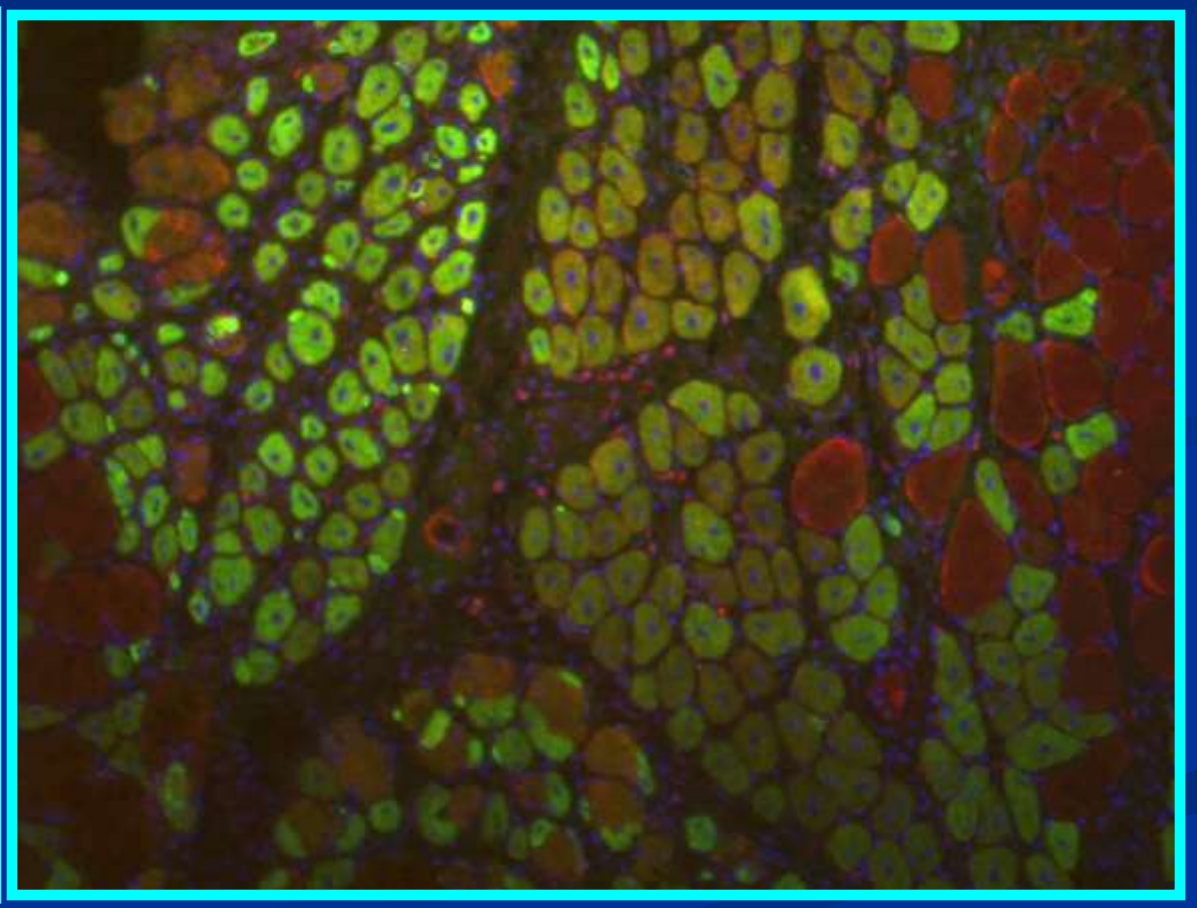




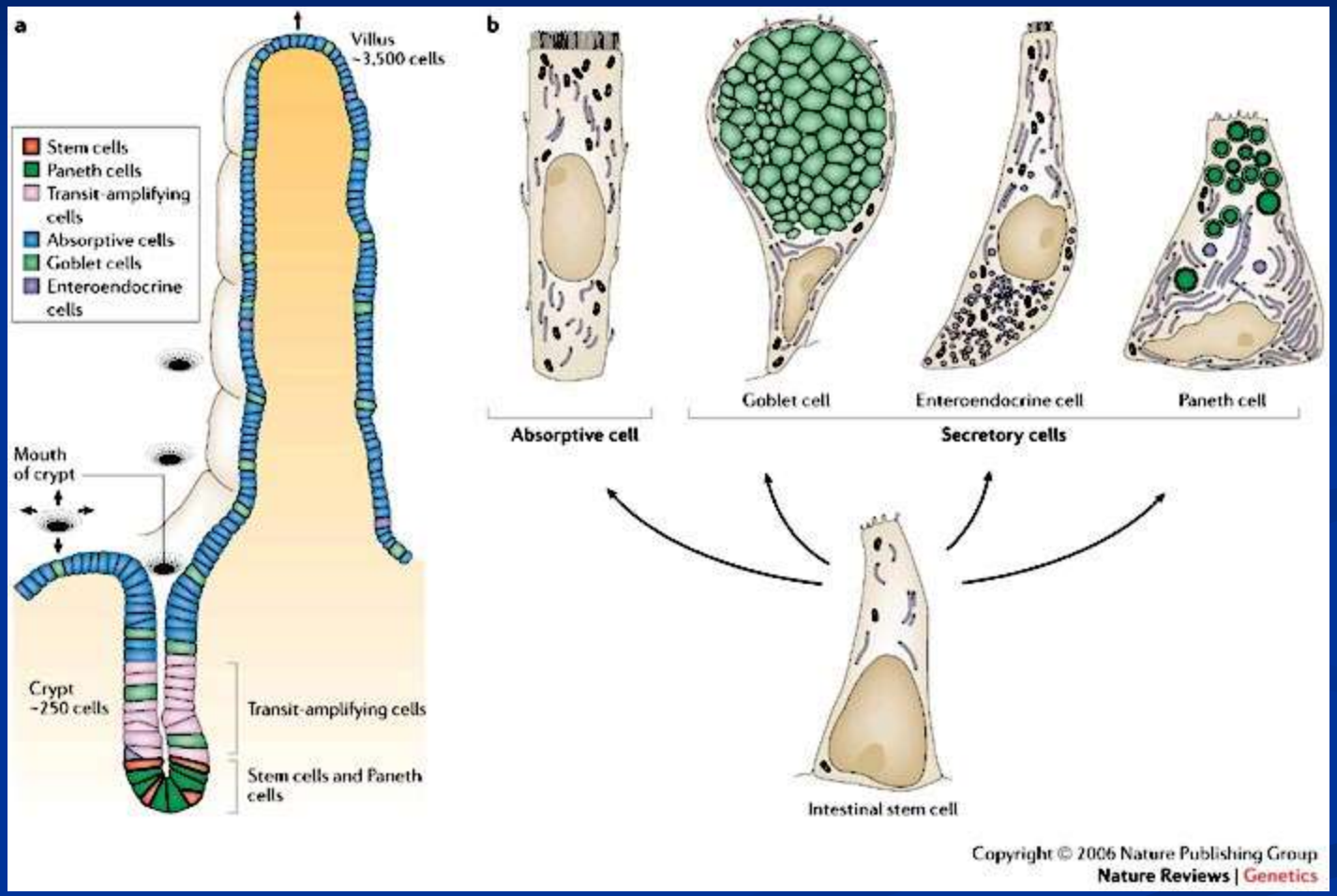
Neural SCs, Neurons, oligodendrocytes,

Stem cells in mature skeletal muscle:

Healthy + new muscle fibers, Muscle SCs



Stem Cells in the Intestine



Nature **459**, 262-265 (14 May 2009)

Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche

Toshiro Sato[1], Robert G. Vries[1], Hugo J. Snippert[1], Marc van de Wetering[1], Nick Barker[1], Daniel E. Stange[1], Johan H. van Es[1], Arie Abo[2], Pekka Kujala[3], Peter J. Peters[3] & Hans Clevers[1]

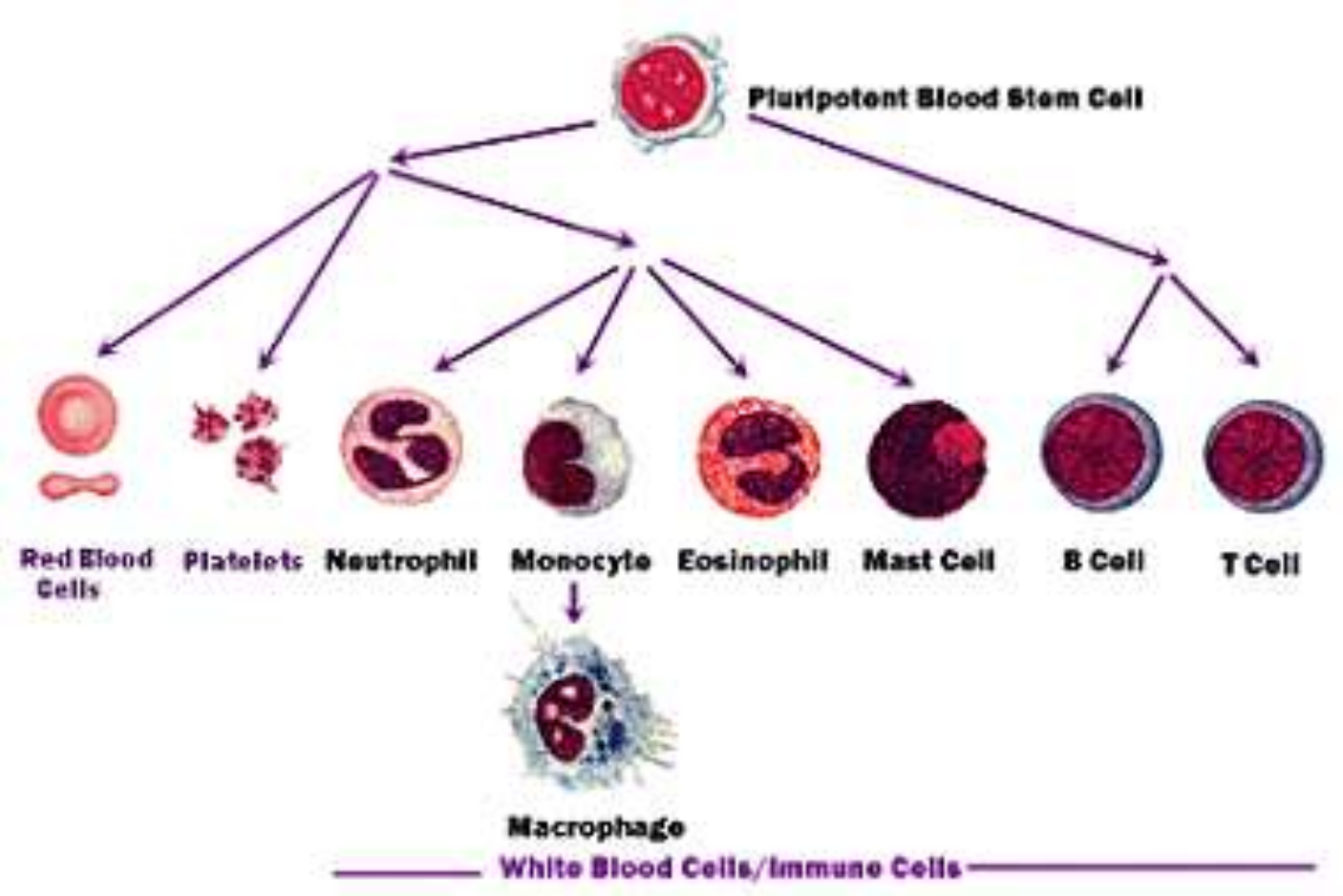
"Organoid"

Adult stem cells

- Evidence that Some Adult Stem Cells show Pluripotent Capacity

Umbilical Cord

Pluripotent Blood Stem Cells
Umbilical Cord



Umbilical Cord Blood Storage for Pluripotent Stem Cells

COMPANY WEBSITE

- Alpha Cordwww.alphacord.com
- California Cryobank, Inc.www.cryobank.com
- Cord Blood Registrywww.cordblood.com
- CorCellwww.corcell.com
- CORD,Inc.www.cordbloodforlife.com
- CordPartnerswww.cordpartners.com
- Cryo-Cell Int.www.cryo-cell.com
- Future Health Technologies(UnitedKingdom)www.futurehealthtechnologies.com
- GeneAngelwww.geneangel.com
- Lifebank, Inc.www.lifebankusa.com
- Lifebank Cryogenics Corp.(Canada) www.lifebank.com
- New England Cord Blood Bank, inc.www.cordbloodbank.com
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  Full Report
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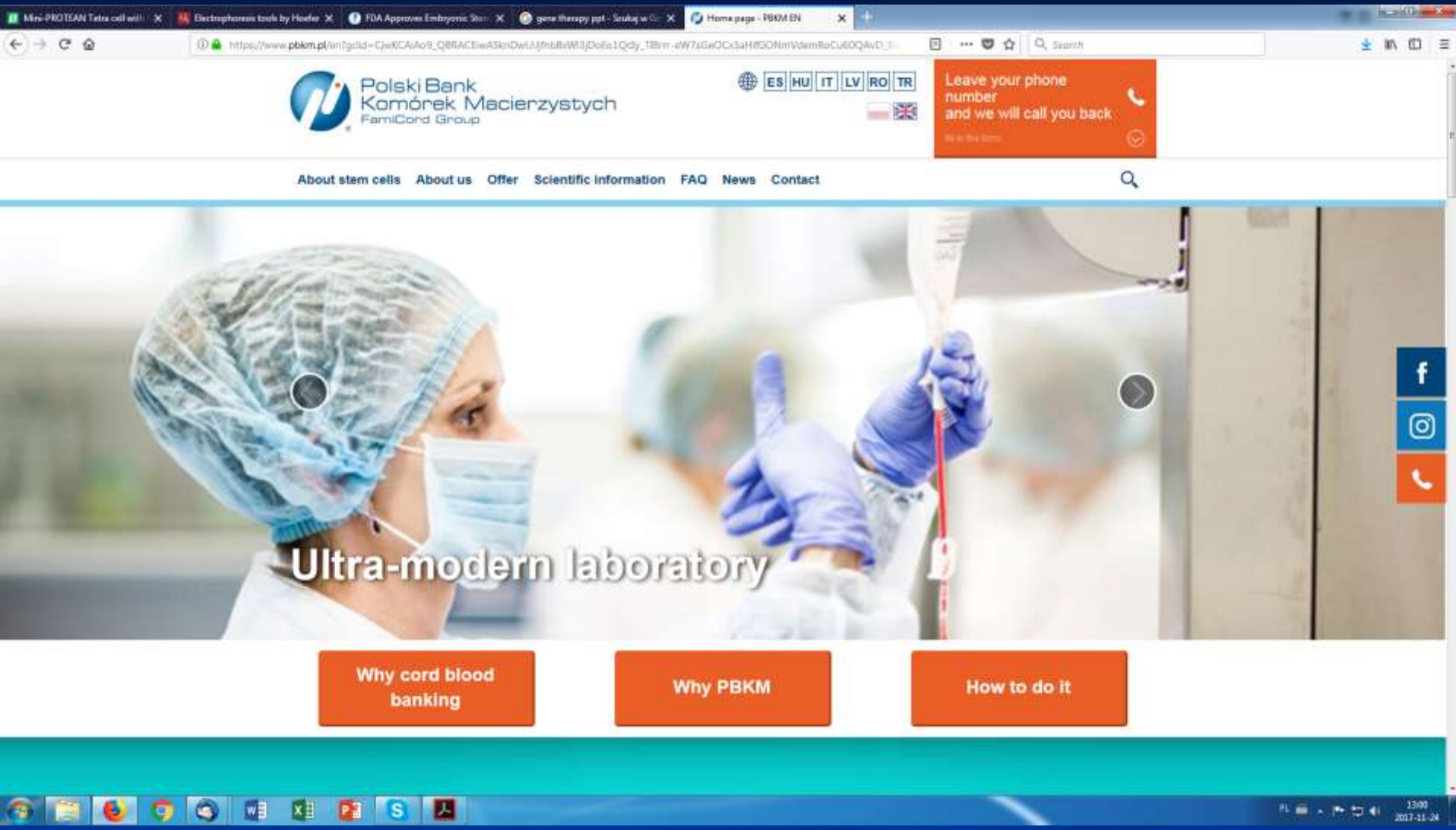

Polski Bank
Komórek Macierzystych
FamiCord Group
ES HU IT LV RO TR
Leave your phone
number
and we will call you back
About stem cells
About us
Offer
Scientific information
FAQ
News
Contact
Ultra-modern laboratory
Why cord blood
banking
Why PBKM
How to do it

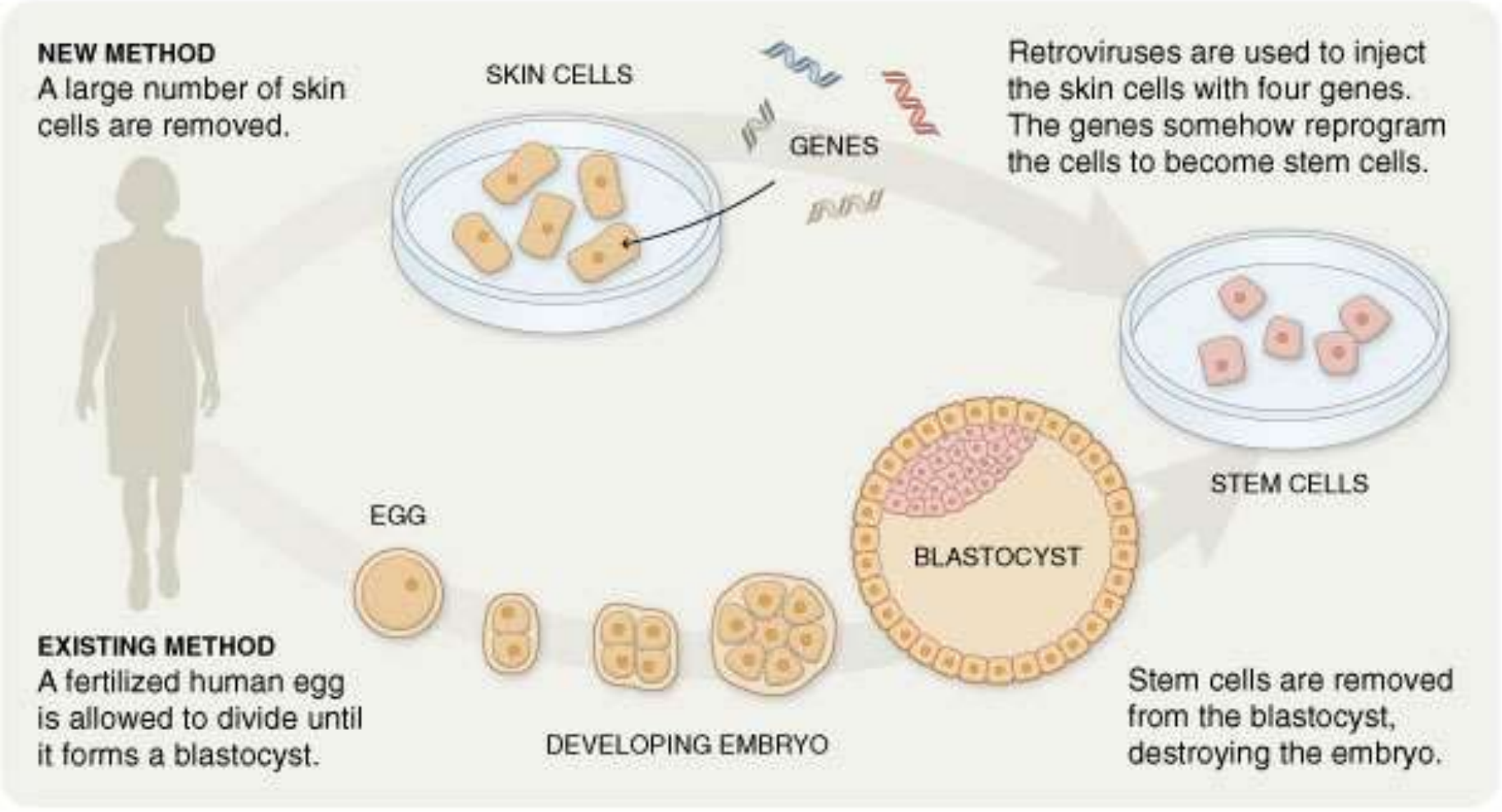
Induced Pluripotent Stem (iPS) Cells

Genetically engineering new stem cells

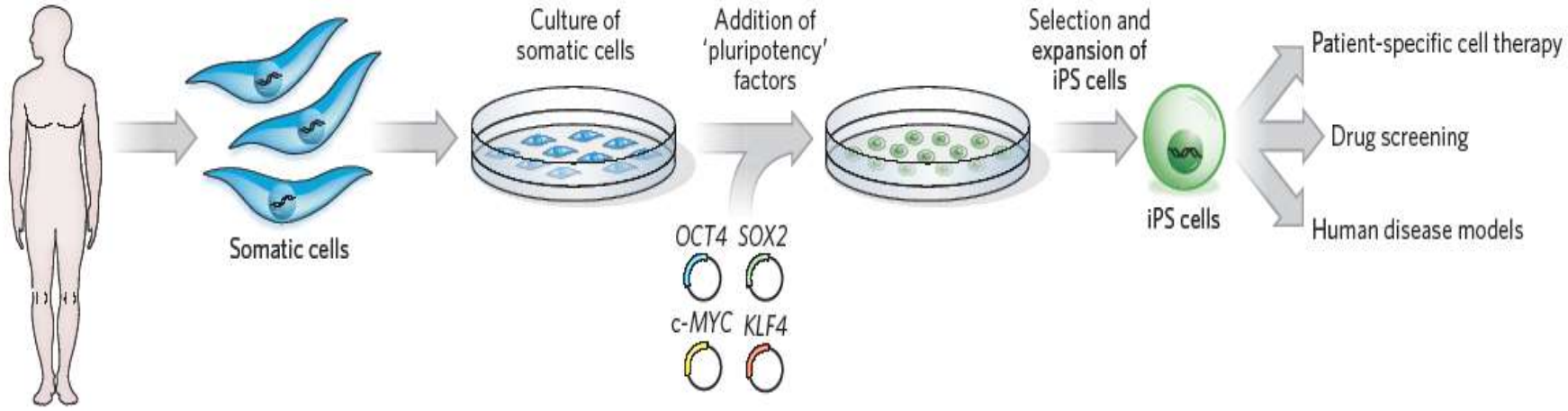
- Creating induced Pluripotent Stem cells is like turning back the clock in a subset of cells.
- This method creates pluripotency, rather than directly harvesting or cloning embryonic stem cells.

Reprogramming Human Skin Cells

Researchers have developed a technique for creating stem cells without the controversial use of human eggs or embryos. If the method can be perfected, it could quell the ethical debate troubling the field.

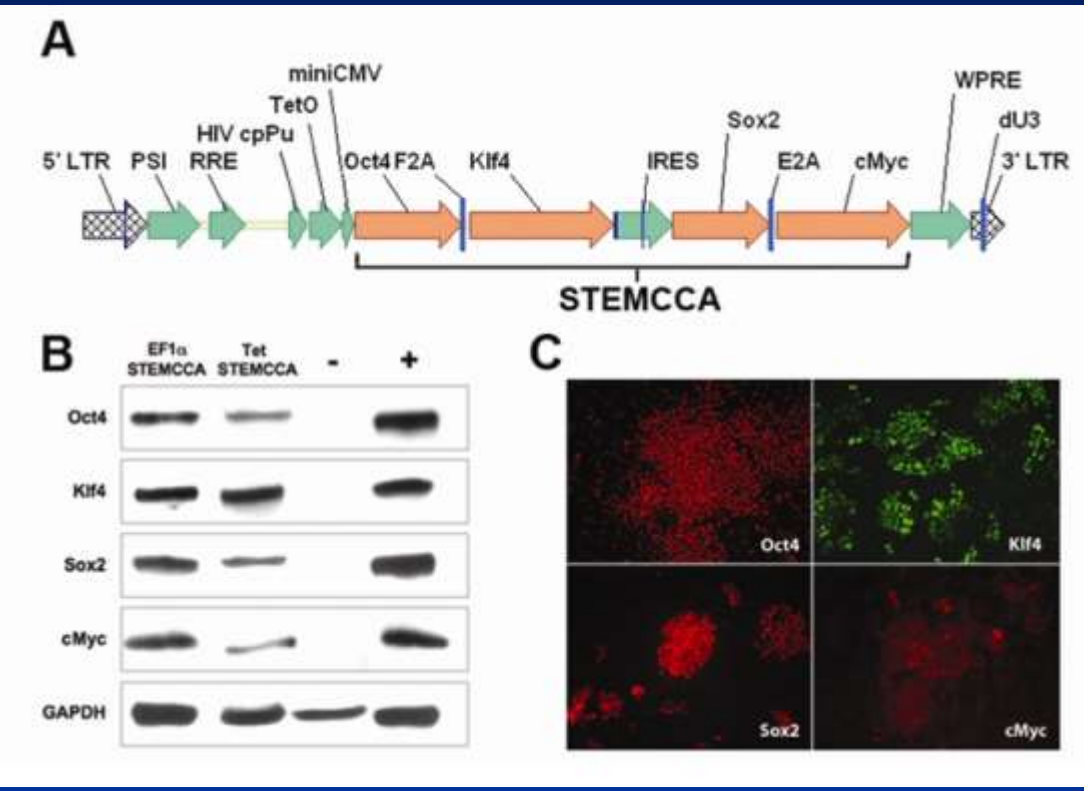


Nuclear reprogramming

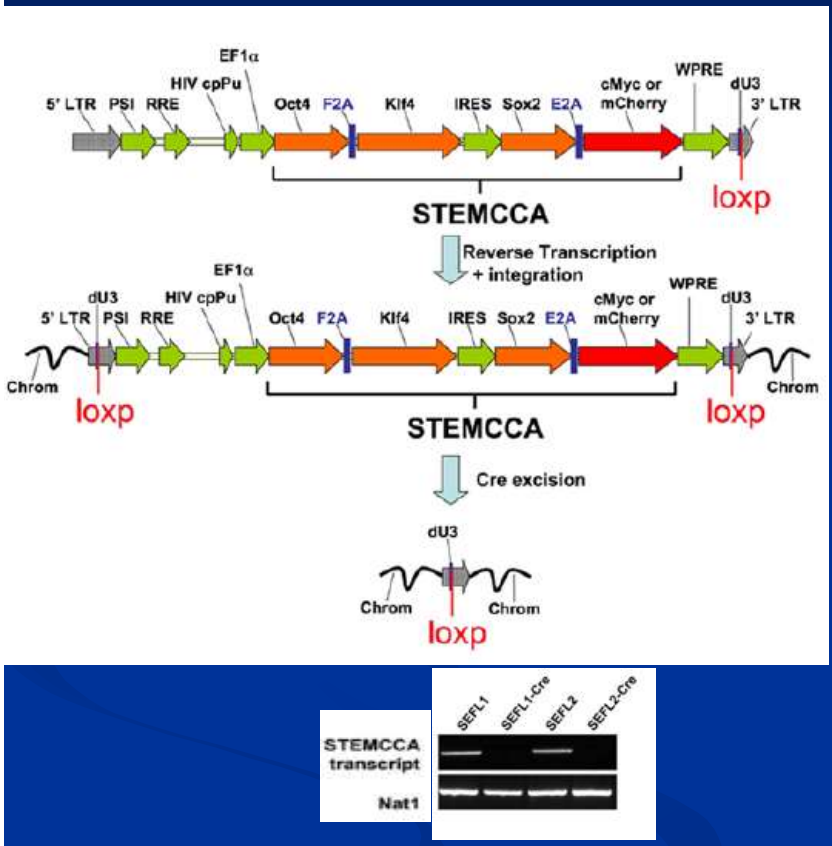


Nuclear reprogramming to a pluripotent state by three approaches
Shinya Yamanaka & Helen M. Blau
NATURE|Vol 465|10 June 2010|doi:10.1038/nature09229

STEMCCA: Single Vector Delivery of 4 Transcription Factors



TetOn/TetOff Inducible Promoter



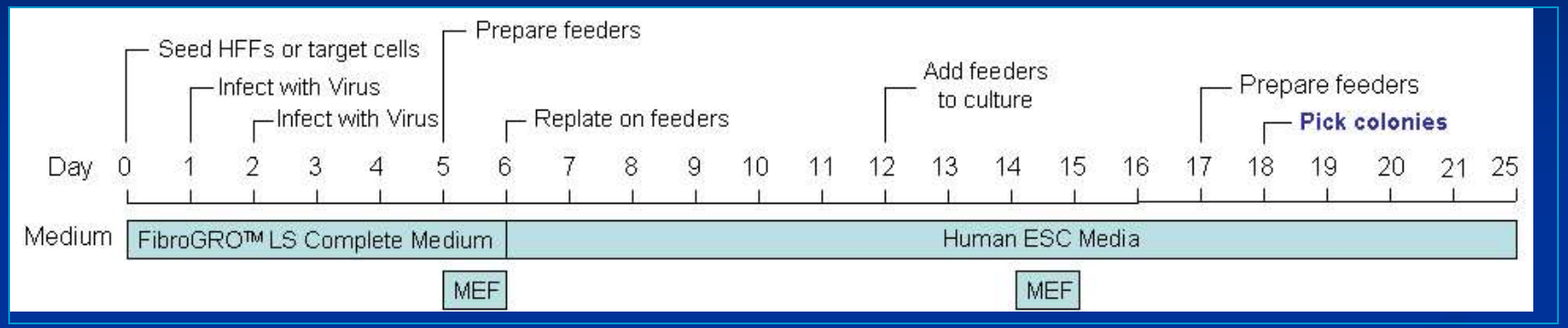
Constitutive Promoter/loxP-Flanked

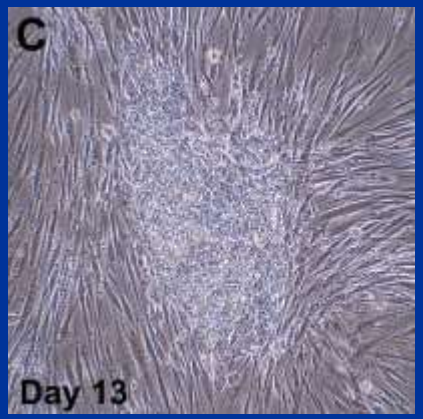
Sommer, C.A.; et al. 2009. Stem Cells 27(3): 543-549.

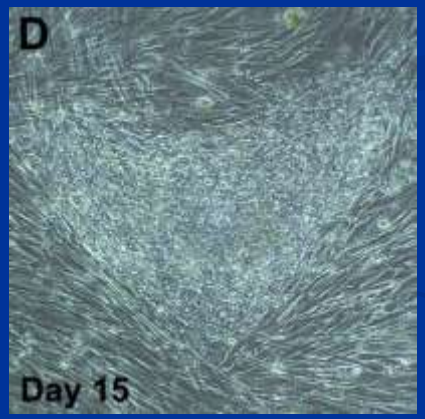
Sommer, C.A.; et al. 2010. Stem Cells 28(1): 64-74.

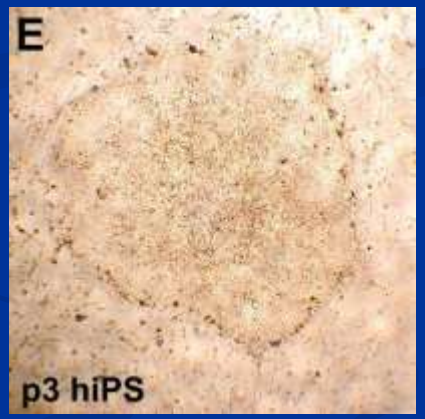
Time Course of Human iPS Colony Formation

using mouse STEMCCA lentivirus







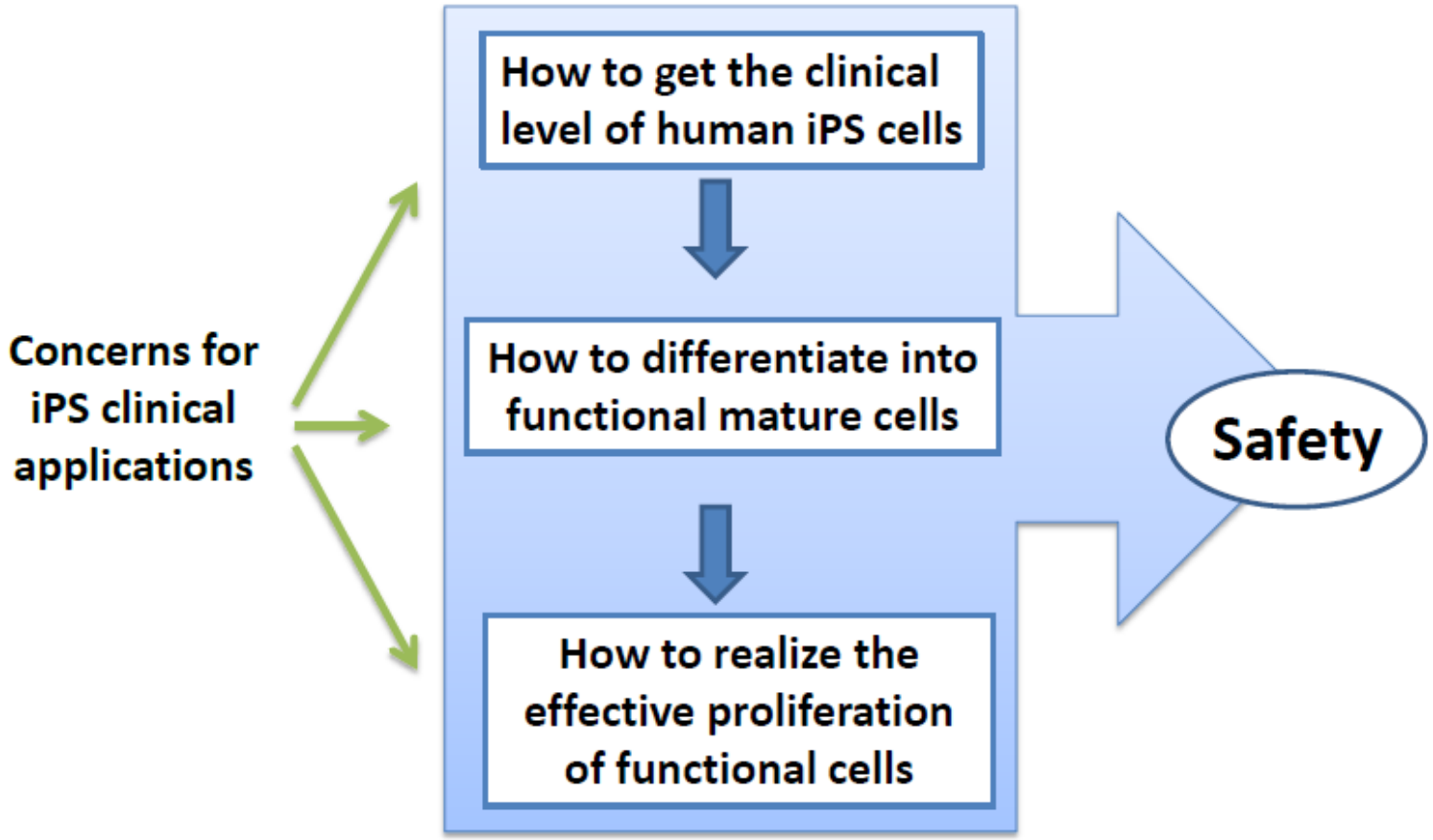


Timing: Infection to colony formation (p0): 18-25 days
p0 to p3: 10-12 days for each passage; 50-60 days total
p3 to p4: 7 days

Pros and Cons to iPS cell technology

- Pros:
  - Cells would be genetically identical to patient or donor of skin cells (no immune rejection!)
  - Do not need to use an embryo
- Cons:
  - Cells would still have genetic defects
  - One of the pluripotency genes is a cancer gene
  - Viruses might insert genes in places we don't want them (causing mutations)

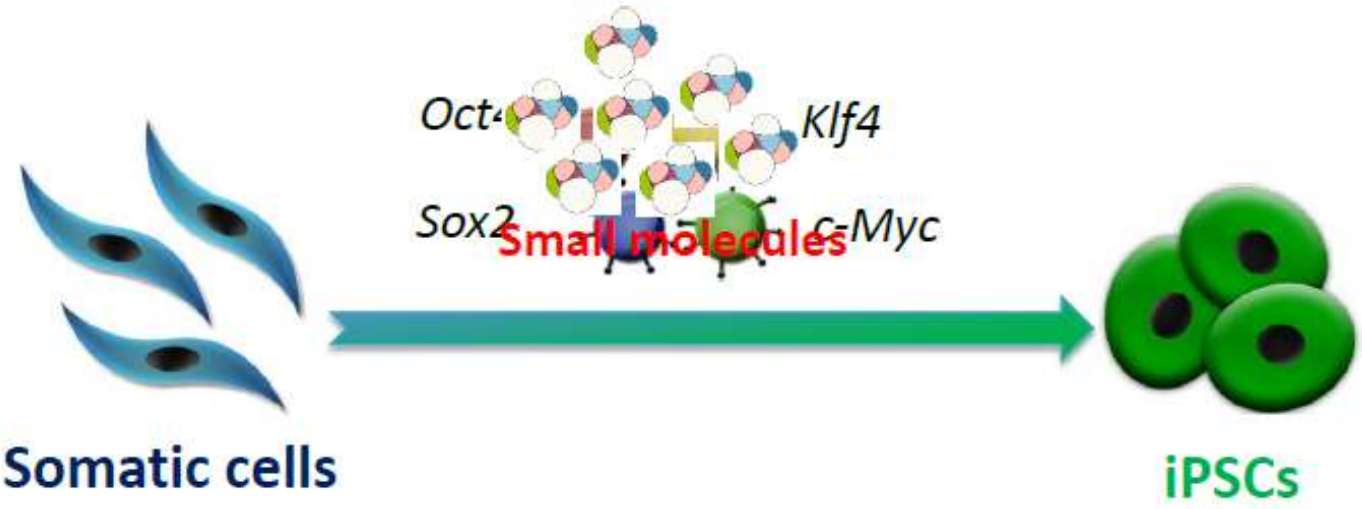
The Concerns Regarding the Clinical Applications of iPSCs



Transgenes increase the risk of dangerous mutations or cancer, gene genetic maniplation limited the clinical application.

Grand Challenge:

Can somatic reprogramming be achieved by using only small molecules?

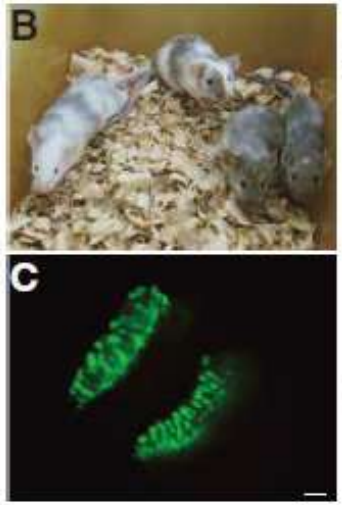


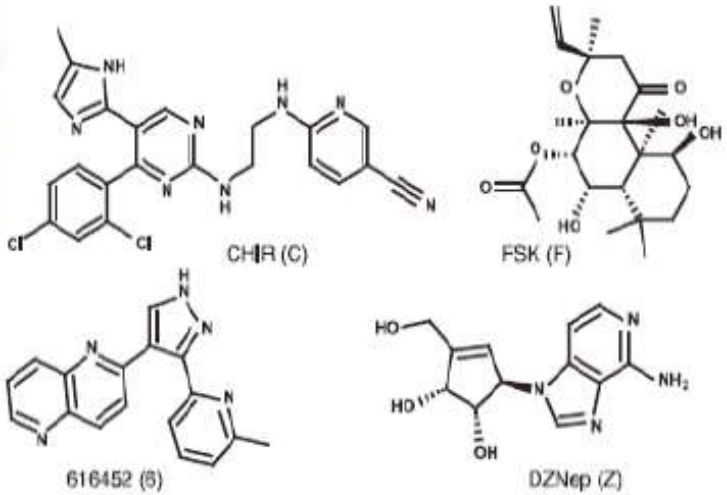
Researchers have been striving to achieve a new way to reprogram somatic cells to iPS cells without the addition of extra genes.

A New Way to Reprogram Adult Tissue to iPS Cells—without Extra Genes

Science
AAAS

Pluripotent Stem Cells Induced from Mouse Somatic Cells by Small-Molecule Compounds
Pingping Hou *et al.*
Science **341**, 651 (2013);
DOI: 10.1126/science.1239278





Deng, H. et. al., *Science* (2013)

nature
International weekly journal of science

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NATURE | NEWS

Stem cells reprogrammed using chemicals alone

Patient-specific cells could be made without genetic manipulation.

David Cyranoski

18 July 2013

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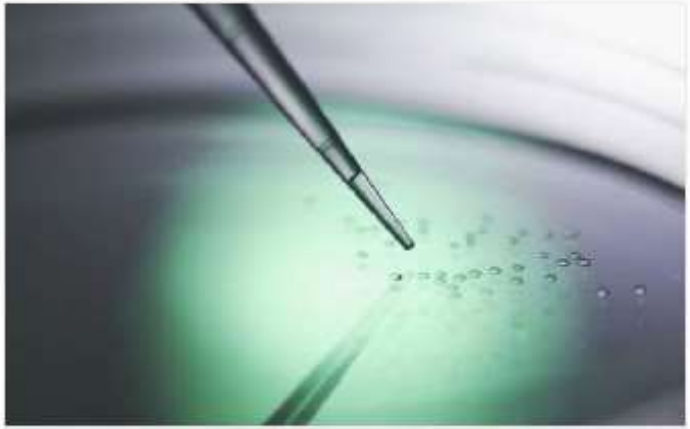


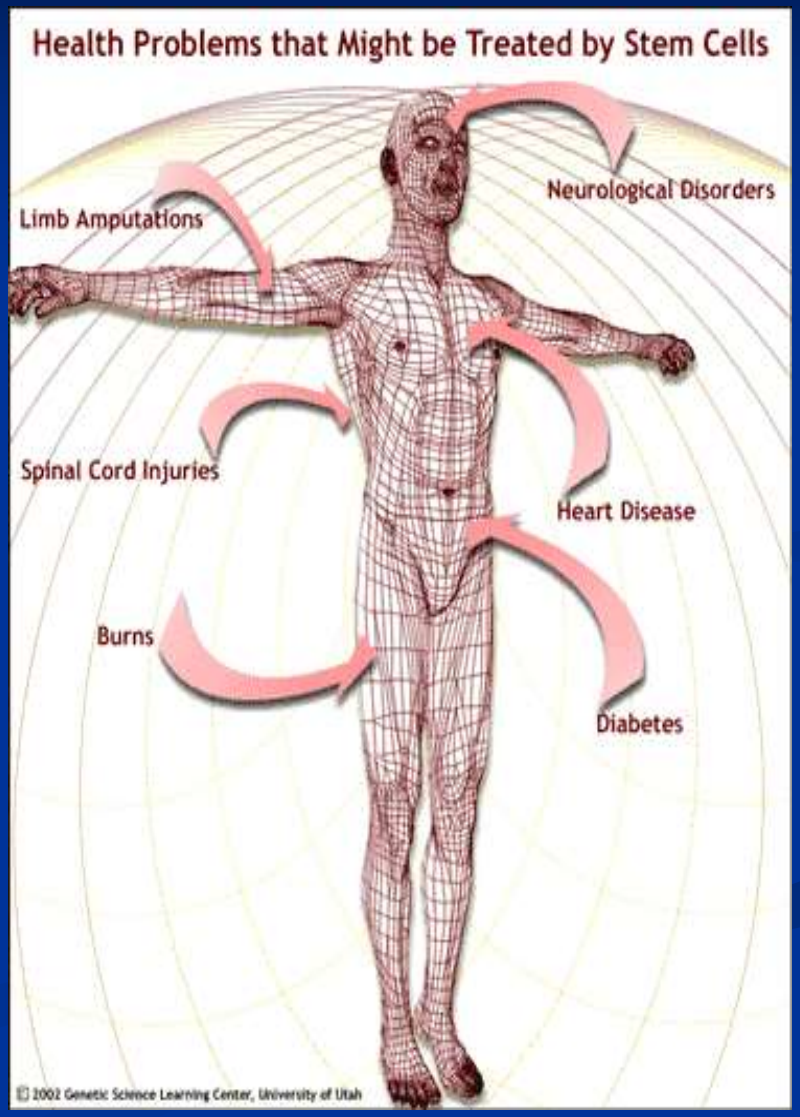
Applications of Stem Cells

- Disease

  Blood cancer, Diabetes, Spinal cord injury, Parkinson's disease, heart disease

- Genetic based Disease

  Cystic fibrosis, Huntington's

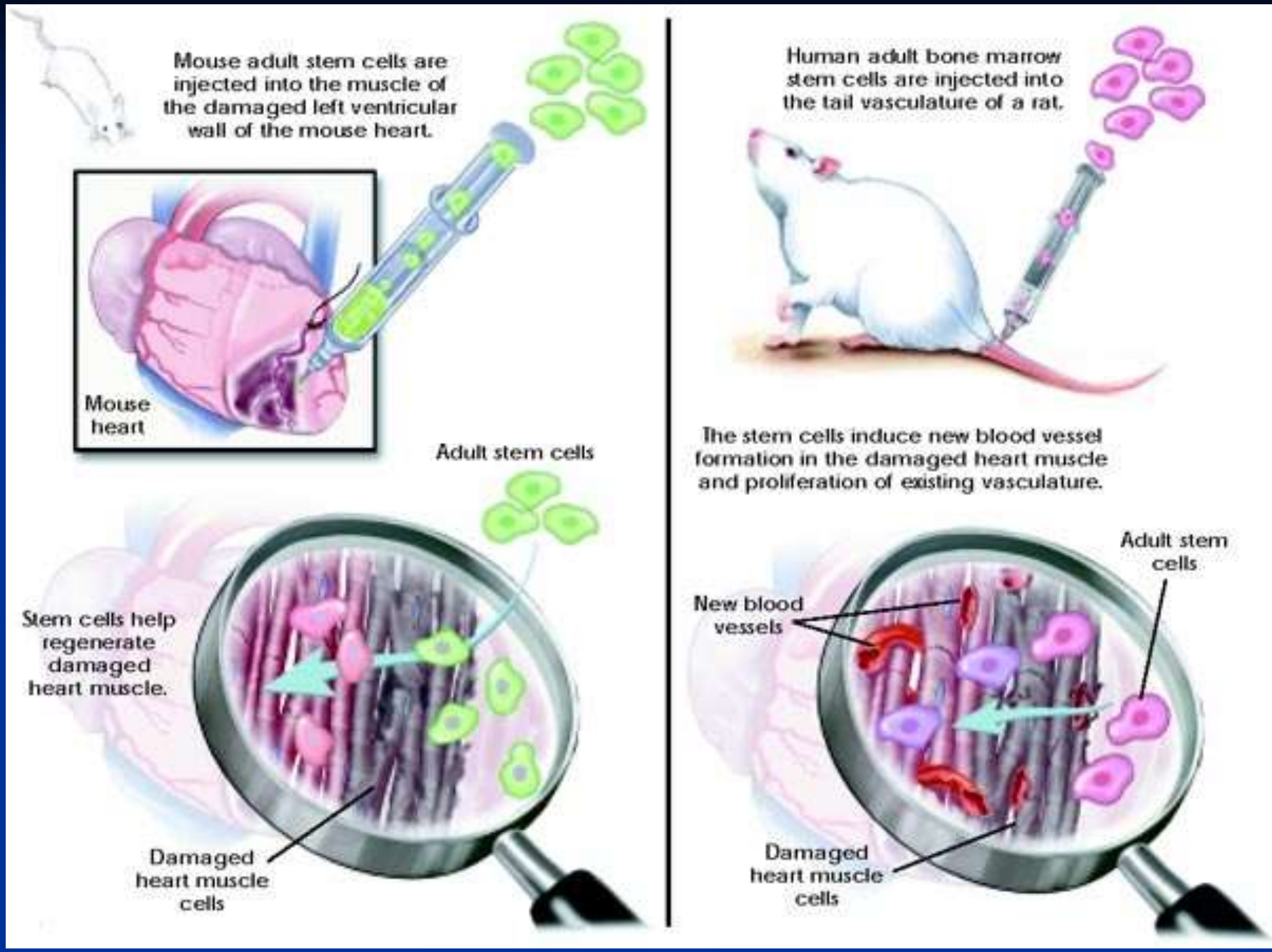


Bone marrow transplant:

Adult stem cell treatments have been used for many years to treat successfully leukemia and related bone/blood cancers through bone marrow transplants

Heart Disease

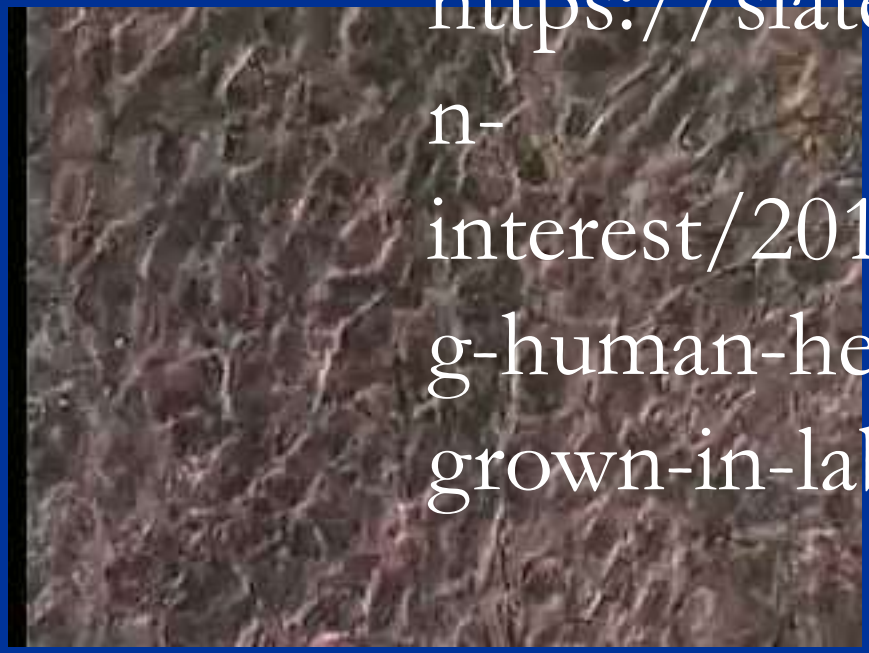
- Adult bone marrow stem cells injected into the hearts arteries are believed to improve cardiac function in victims of heart failure or heart attack

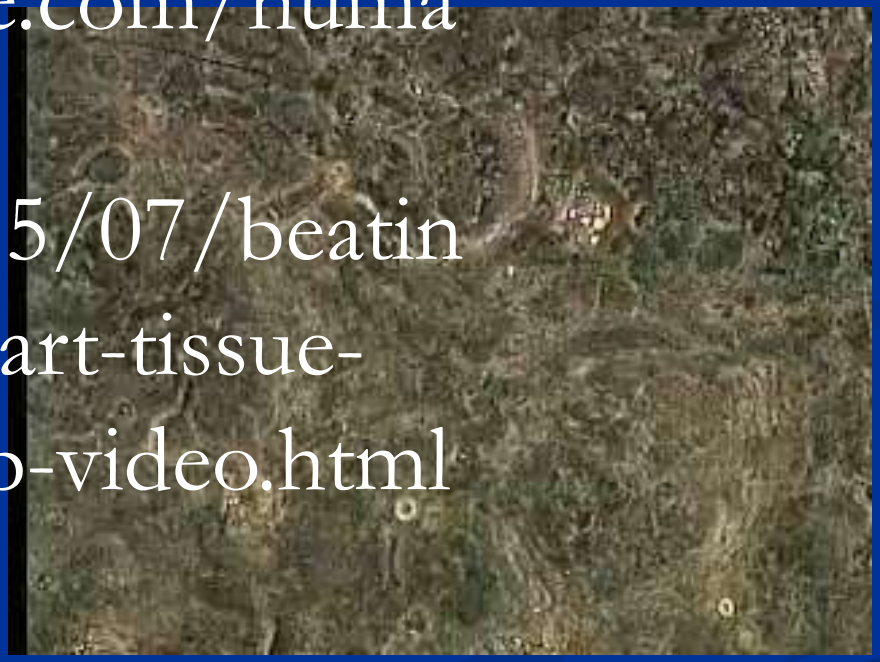

Mouse adult stem cells are injected into the muscle of the damaged left ventricular wall of the mouse heart.
Mouse heart
Adult stem cells
Stem cells help regenerate damaged heart muscle.
Damaged heart muscle cells
Human adult bone marrow stem cells are injected into the tail vasculature of a rat.
The stem cells induce new blood vessel formation in the damaged heart muscle and proliferation of existing vasculature.
Adult stem cells
New blood vessels
Damaged heart muscle cells

Experimental model system

Heart muscle cells beating in a petri dish!

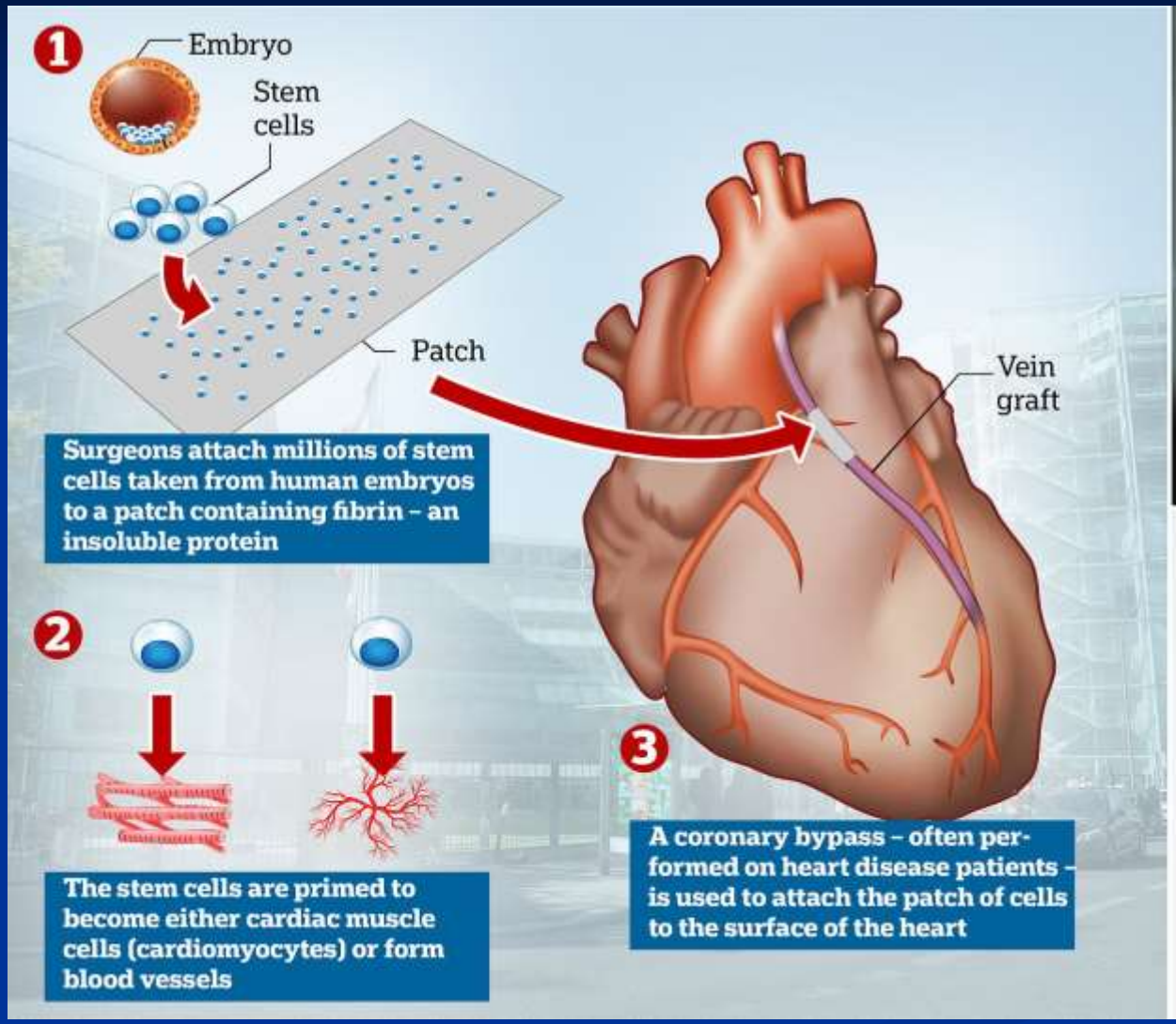
https://slate.com/human-interest/2015/07/beating-human-heart-tissue-grown-in-lab-video.html



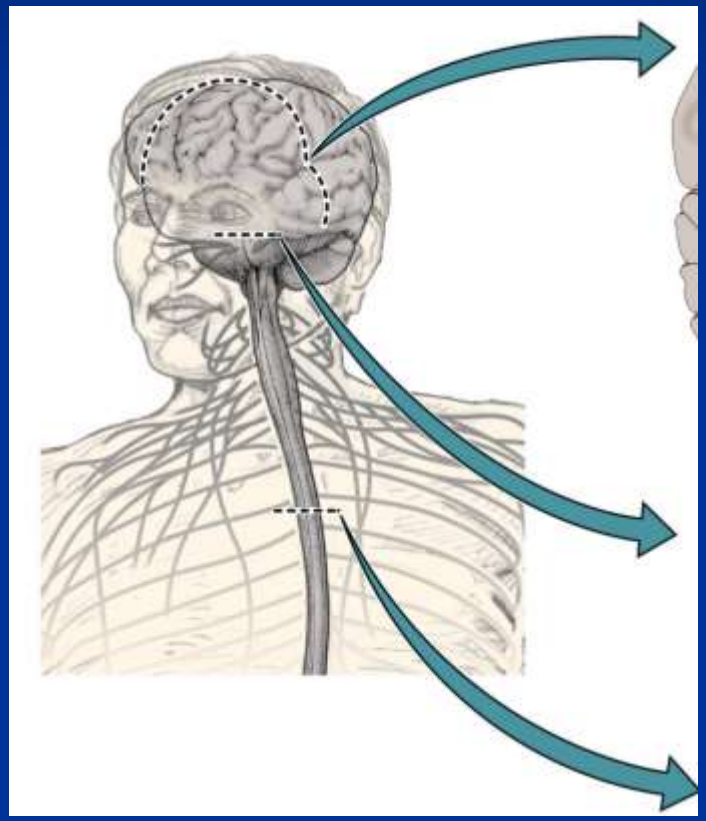


from mouse embryonic stem cells

September 2019



Adult stem cells effective in tissue repair



- Brain and spinal cord injury.
- Stroke.
- Neurodegenerative diseases
  - Parkinson's Disease
  - Huntington's Disease
  - Alzheimer's Disease
  - Multiple Sclerosis
  - Lou Gerhig's Disease (ALS)

Neurological disorders involve the loss of particular cell types in the nervous system

- Brain and spinal cord injury and stroke (loss of nerve cells and myelin-forming oligodendrocytes).

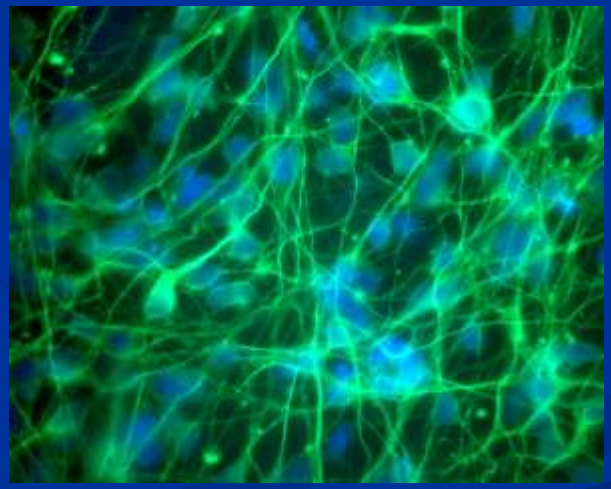
- Neurodegenerative diseases
  - Parkinson's Disease (loss of dopamine-containing nerve cells in the brainstem).
  - Huntington's Disease (loss of nerve cells in the striatum).
  - Alzheimer's Disease (loss of nerve cells in the cerebral cortex).
  - Multiple Sclerosis (loss of myelin-forming oligodendrocytes).
  - Lou Gerhig's Disease-ALS (loss of motor neurons from the spinal cord).

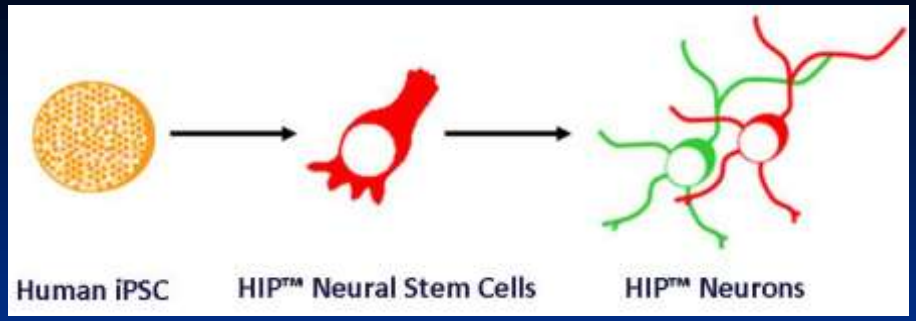
- The vision: To use stem cells to restore the cells that are lost as a result of injury or neurodegenerative diseases.

Make stem cells into nerve cells

The stem cells are treated with factors to cause them to differentiate into particular cell types

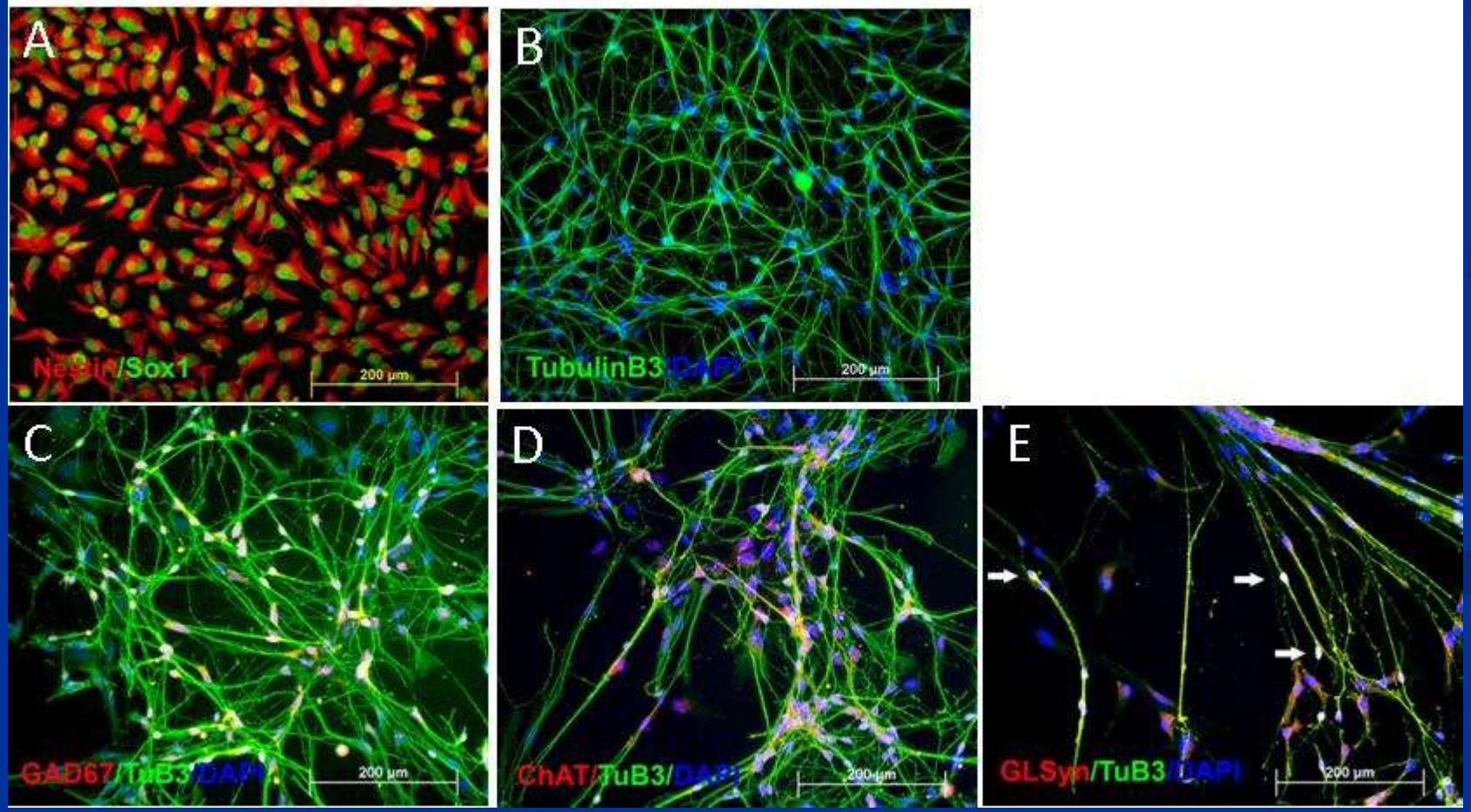
Stem cells differentiated into neurons





MTI-GlobalStem

Human iPSC-Derived Neural Cells



Christopher Reeve

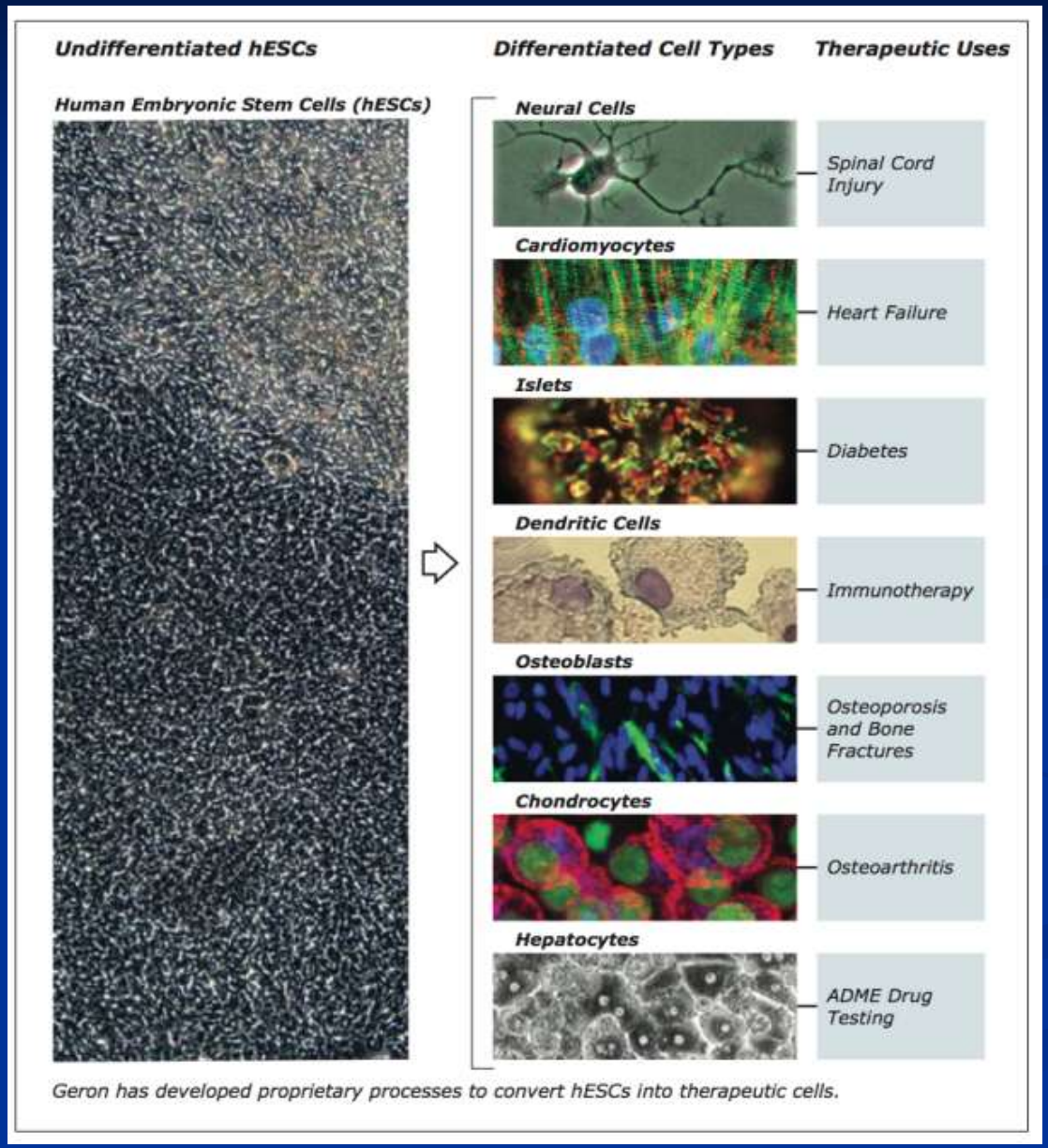
1952-2004

<u>Spinal Cord Injury</u>—Adult stem cells capable of re-growth and reconnection in spinal cord. Clinical trials in progress.

Geron Stem Cell Therapy

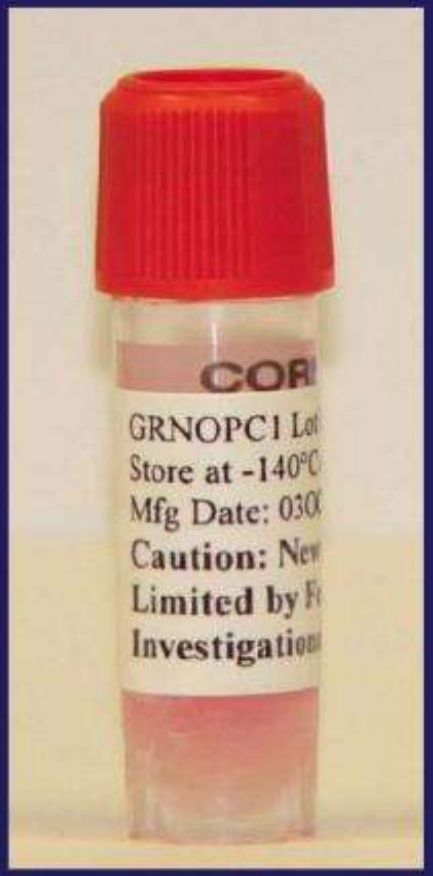
•Tom Okarma - Geron

Human Embryonic Stem Cells



Human Embryonic Stem Cell (hESC) Based Therapy

Geron Oligodendrocyte Progenitor Cells GRNOPC1



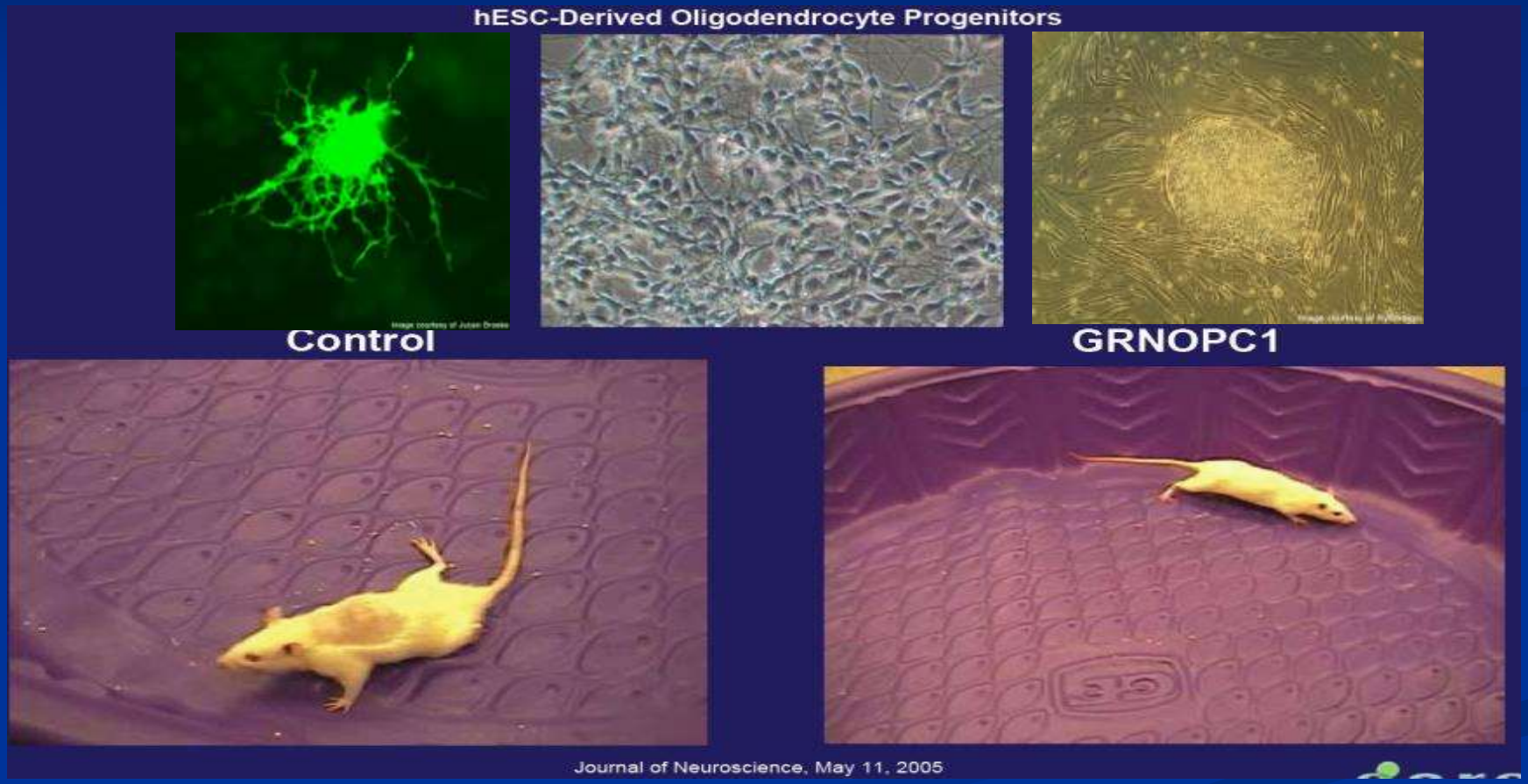
GRNOPC1

- Cryopreserved Allogeneic Cell Population
- Derived from Human Embryonic Stem Cells
- Characterized Composition of Cells
- Contain Oligodendrocyte Progenitor Cells
- Produces Neurotrophic Factors
- Induces Myelination of Denuded Axons

Intended Application

- "Off-the-Shelf" Product
- Spinal Cord Injury
- Other CNS Disorders

GRNOPC1 Improves Locomotor Behavior after Spinal Cord Injury



The oligodendrocyte precursor cells in GRONPC1 turn into oligodendrocytes to form myelin fatty sheath.

•Tom Okarma - Geron

https://globalgenes.org/raredaily/fda-approval-of-gerons-embryonic-stem-cell-trial-has-widespread-implications-for-rare-disease/

Properties of GRNOP1

24 Studies

1977 Rodents

858 Injected with GRNOPC1

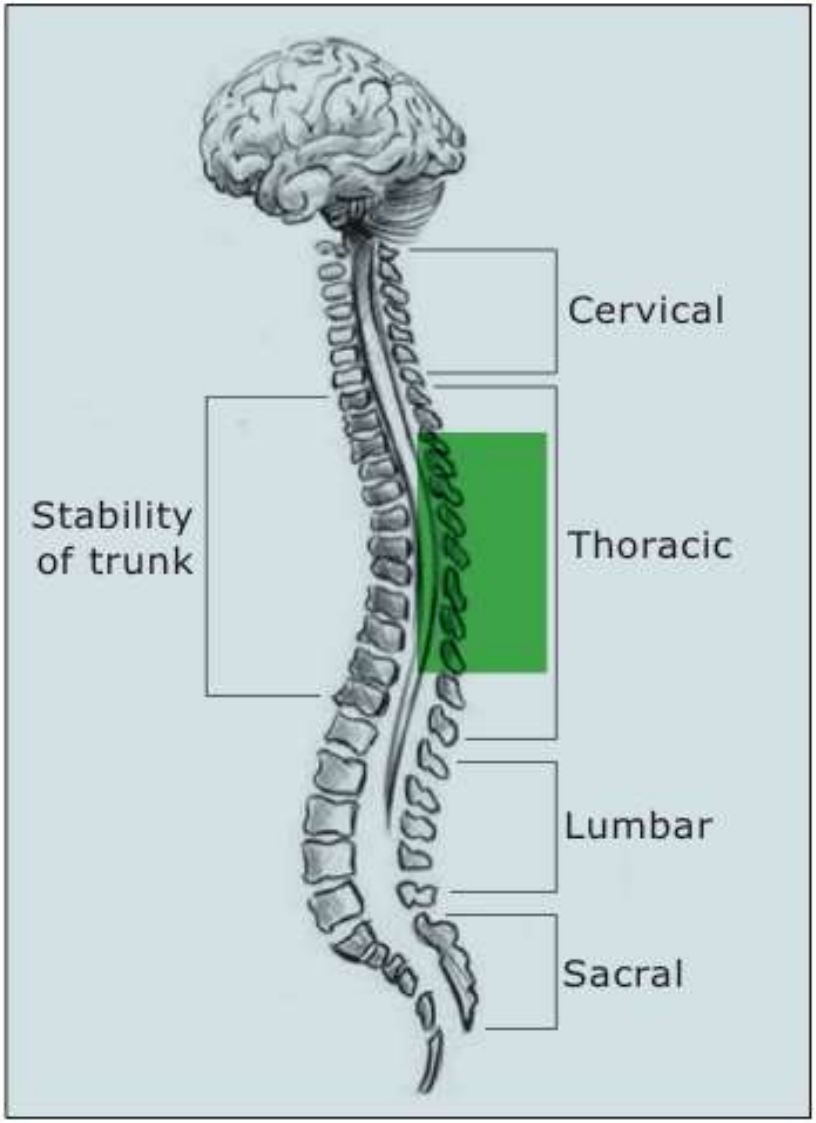
5 x 10^9 OPC1 Tested in Studies

GRNOPC1

- Survives in the Spinal Cord
- Produces Neurotrophic Factors
- Can Induce Myelination
- Improves Locomotor Activity
- Reduces Parenchymal Cavitation
- Migrates Through the Spinal Cord
- Does Not Increase Mortality
- Does Not Induce Allodynia
- Does Not Induce Systemic Toxicity
- Predominantly Neural Cells Types
- Some Non-Neural Cell Types Observed
- Does Not Produce Teratomas
- Not Highly Susceptible to Direct Immune Responses

GRNOP1 Phase 1 Multi-Center Spinal Cord Injury Trial

- **Open Label Trial**
- **Subacute, Functionally Complete Spinal Cord Injury with a Neurological Level of T3 to T10**
- **2x10⁶ Cells**
- **Transplant 7-14 Days Post Injury**
- **Temporary Immunosuppression with Low Dose Tacrolimus**
- **Primary Endpoint: Safety**
  - *Neurological*
  - *Overall*
- **Secondary Endpoint: Efficacy**
  - *ASIA Sensory Score*
  - *Lower Extremity Motor Score*



Embryonic Stem Cells

Table 1. ESC Trials

Trial Sponsor (Location)	Disease Target	Cell Therapy	No. Patients	Phase
Chabiotech Co. Ltd. (S. Korea)	macular degeneration	human-ESC-derived RPE	12	phase I/II
Ocata Therapeutics (MA, USA)	Stargardt's macular dystrophy	human-ESC-derived RPE	16	phase I/II
	macular degeneration	human-ESC-derived RPE	16	phase I/II
	myopic macular degeneration	human-ESC-derived RPE	unknown	phase I/II
Pfizer (UK)	macular degeneration	human-ESC-derived RPE	10	phase I
Cell Cure Neurosciences Ltd. (Israel)	macular degeneration	human-ESC-derived RPE	15	phase I/II
ViaCyte (CA, USA)	type I diabetes mellitus	human-ESC-derived pancreatic endoderm cell	40	phase I/II
Assistance Publique-Hopitaux de Paris (France)	heart failure	human-ESC-derived CD15+ Isl-1+ progenitors	6	phase I
International Stem Cell Corp. (Australia)	Parkinson's disease	human parthenogenetic-derived neural stem cells	unknown	phase I/II
Asterias Biotherapeutics (CA, USA)	spinal cord injury	human-ESC-derived oligodendrocyte precursor cells	13	phase I/II

Neural Stem Cells

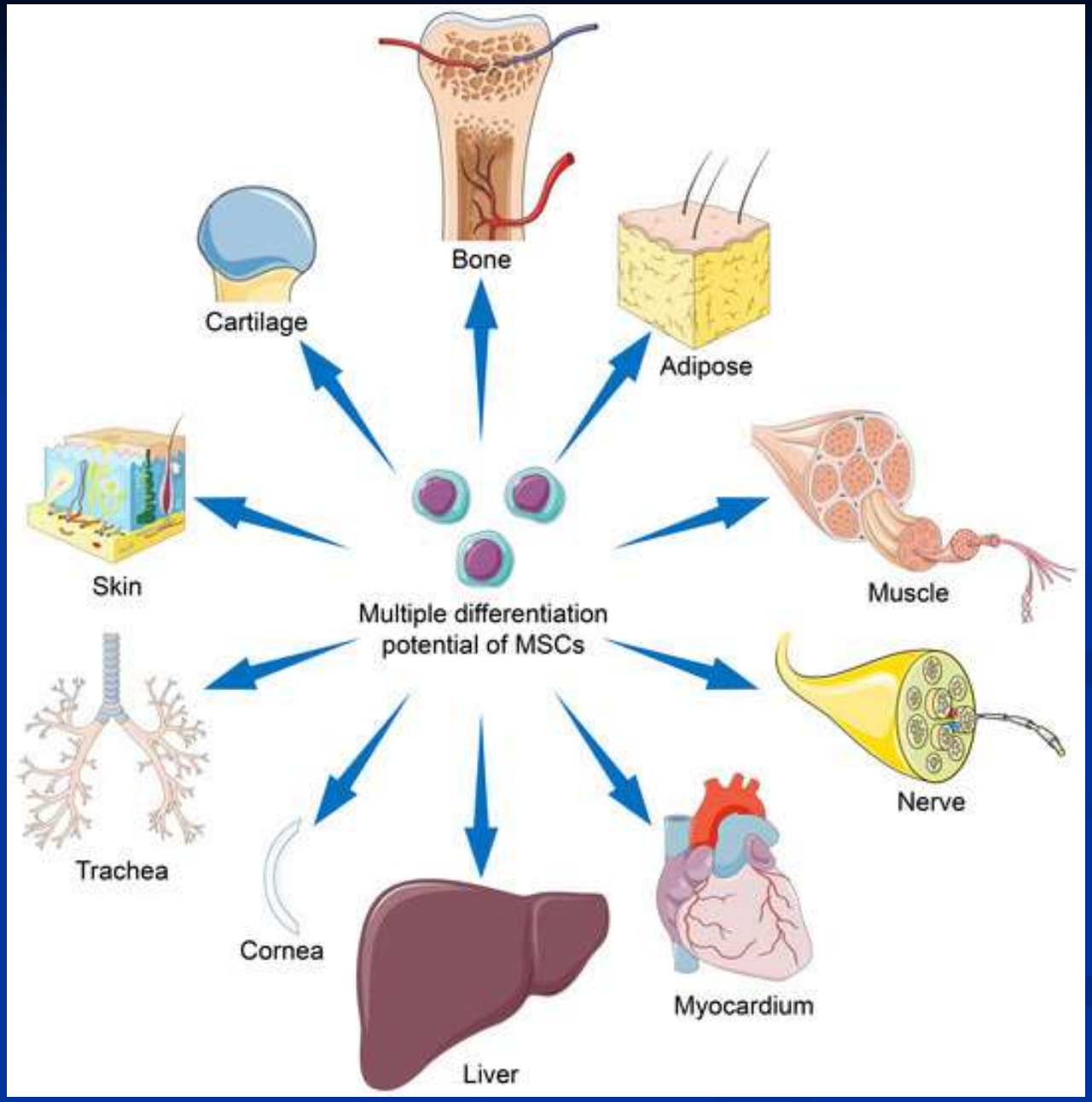
Table 2. Neural Stem Cell trials

Trial Sponsor (Location)	Disease Target	Cell Therapy	No. Patients	Phase
City of Hope (CA, USA)	recurrent high grade gliomas	*E. Coli* CD-expressing neural stem cells	24	phase 1
	recurrent high grade gliomas	carboxylesterase-expressing neural stem cells	53	phase I
Neuralstem Inc. (MD, USA)	ALS	fetal-derived neural stem cells	18	phase I
	ALS	fetal-derived neural stem cells	18	phase II
	chronic spinal cord injury	fetal-derived neural stem cells	4	phase I
ReNeuron Ltd. (UK)	stroke	human neural stem cells	12	phase I
	stroke	human neural stem cells	41	phase II
	lower limb ischemia	human neural stem cells	9	phase I
Stem Cells Inc. (CA, USA)	neuronal ceroid lipofuscinosis	human CNS stem cells	6	phase I
	cervical spinal cord injury	human CNS stem cells	50	phase II
	macular degeneration	human CNS stem cells	15	phase I/II
	thoracic spinal cord injury	human CNS stem cells	12	phase I/II
	Pelizaeus-Merzbacher disease	human CNS stem cells	4	phase I
TRANSEURO (UK)	Parkinson's disease	fetal-derived dopaminergic cells	40	phase I
Wroclaw Medical University (Poland)	spinal cord injury	olfactory ensheathing cells, autologous	10	phase I

Placental Stem Cells

Table 3. Placental Stem Cell Trials

Trial Sponsor (Location)	Disease Target	Cell Therapy	No. Patients	Phase
Celgene Corporation (NJ, USA)	stroke (terminated)	human placenta-derived cells	44	phase II
	pulmonary sarcoidosis (terminated)	human placenta-derived cells	4	phase I
	CD	human placenta-derived cells	14	phase I
	MS	human placenta-derived cells		phase I
	peripheral artery disease	human placenta-derived cells	24	phase I
	rheumatoid arthritis	human placenta-derived cells	26	phase II
Karolinska Institute (Sweden)	GVHD	decidual stromal cells (MSC-like)	30	phase I/II
	hemorrhagic cystitis	decidual stromal cells (MSC-like)	12	phase I/II
Prince Charles Hospital/Mater Medical Research Institute (Australia)	idiopathic pulmonary fibrosis	placental mesenchymal stromal cell	8	phase I
New York Medical College (NY, USA)	immune disorders	human placental-derived stem cells	30	phase I


Bone
Cartilage
Adipose
Skin
Muscle
Multiple differentiation potential of MSCs
Nerve
Trachea
Cornea
Liver
Myocardium

NIH clinical trials database: 374 registered clinical trials (2015)

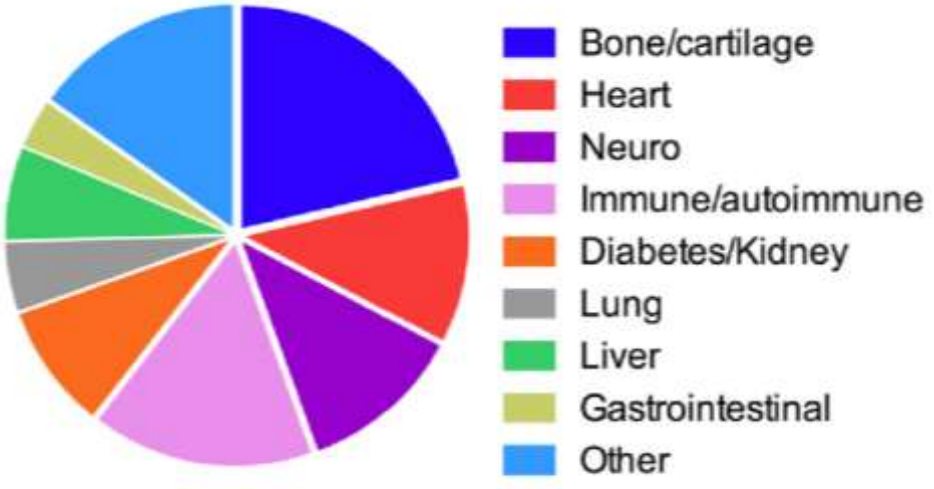


Figure 1. Indications Being Addressed using MSCs in Clinical Trials
Data for 352 registered clinical trials.

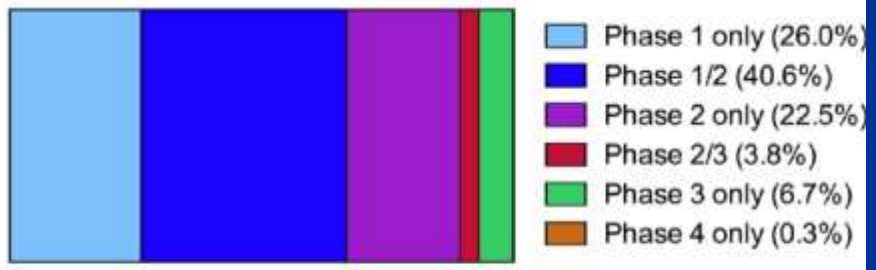
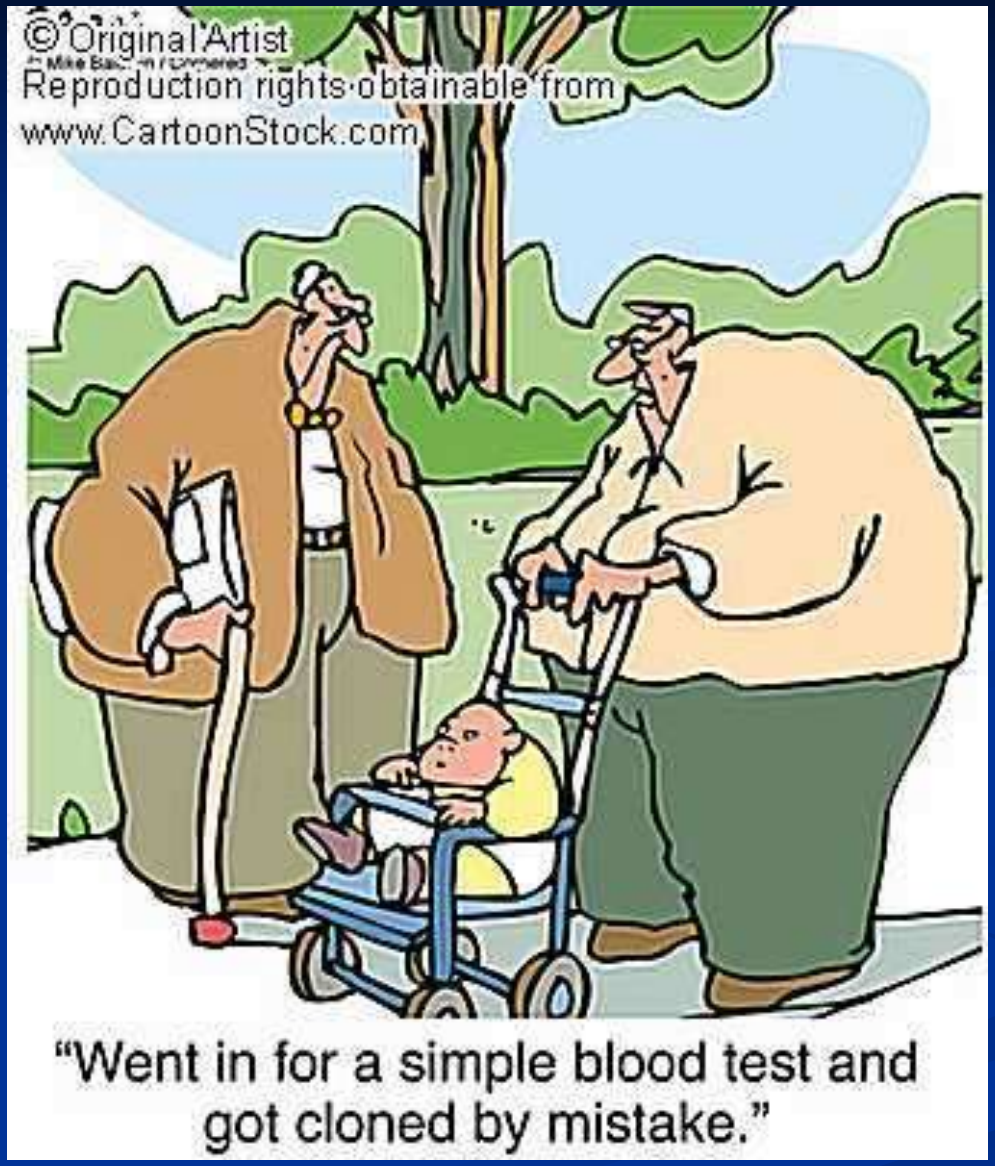


Figure 2. MSC Clinical Trials Classified by Clinical Phase
Data for 315 registered clinical trials.

https://www.youtube.com/watch?v=MwDZhkwpOtA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www.CartoonStock.com
"Went in for a simple blood test and got cloned by mistake."