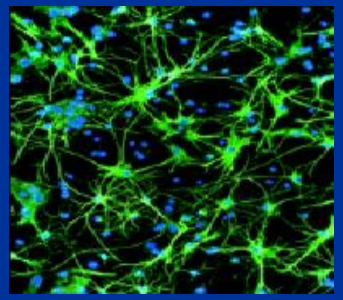
Cloning and Stem Cell research

&

Therapeutic Possibilities



Hans Bluyssen, 18-11-2020

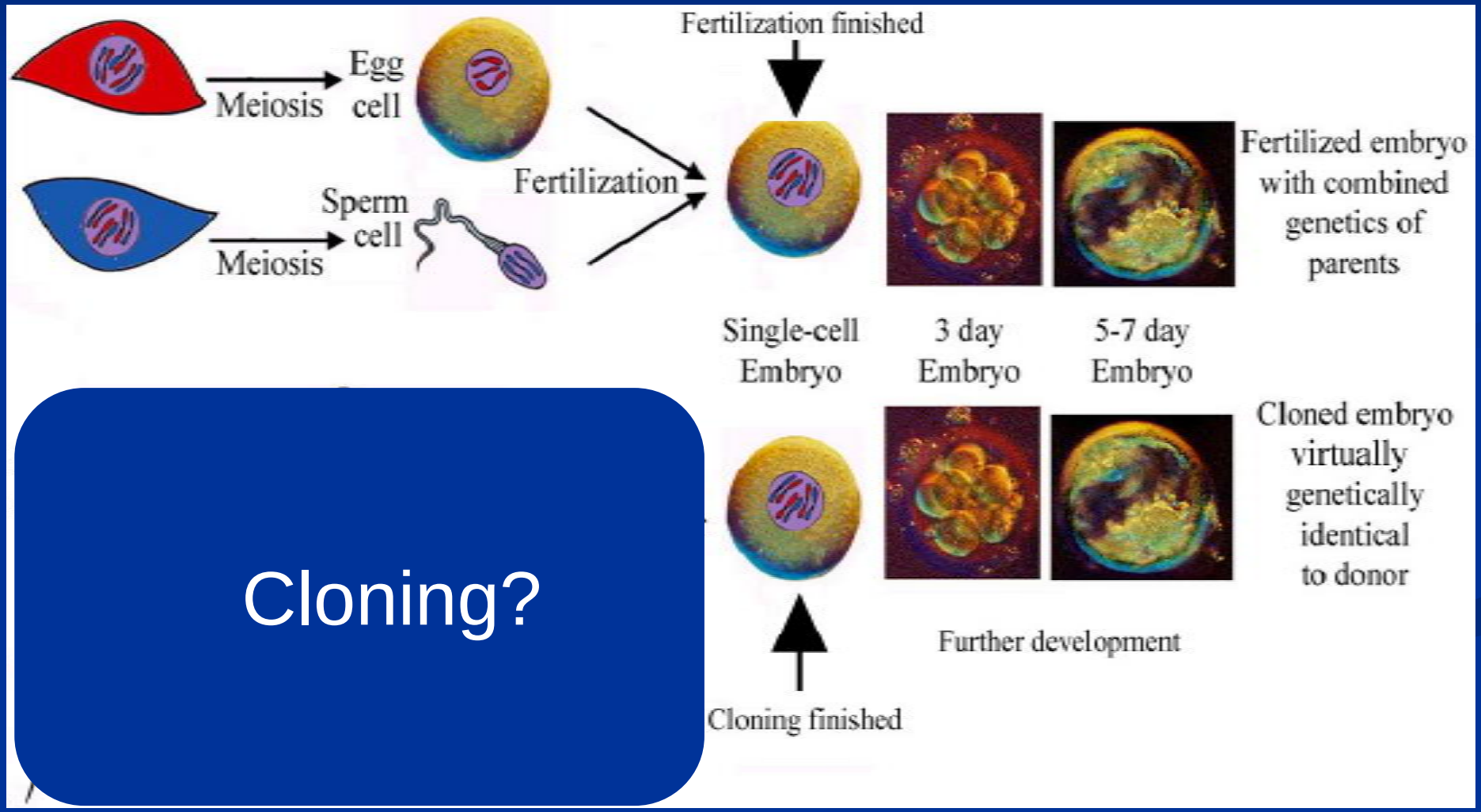
Cloning

- 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

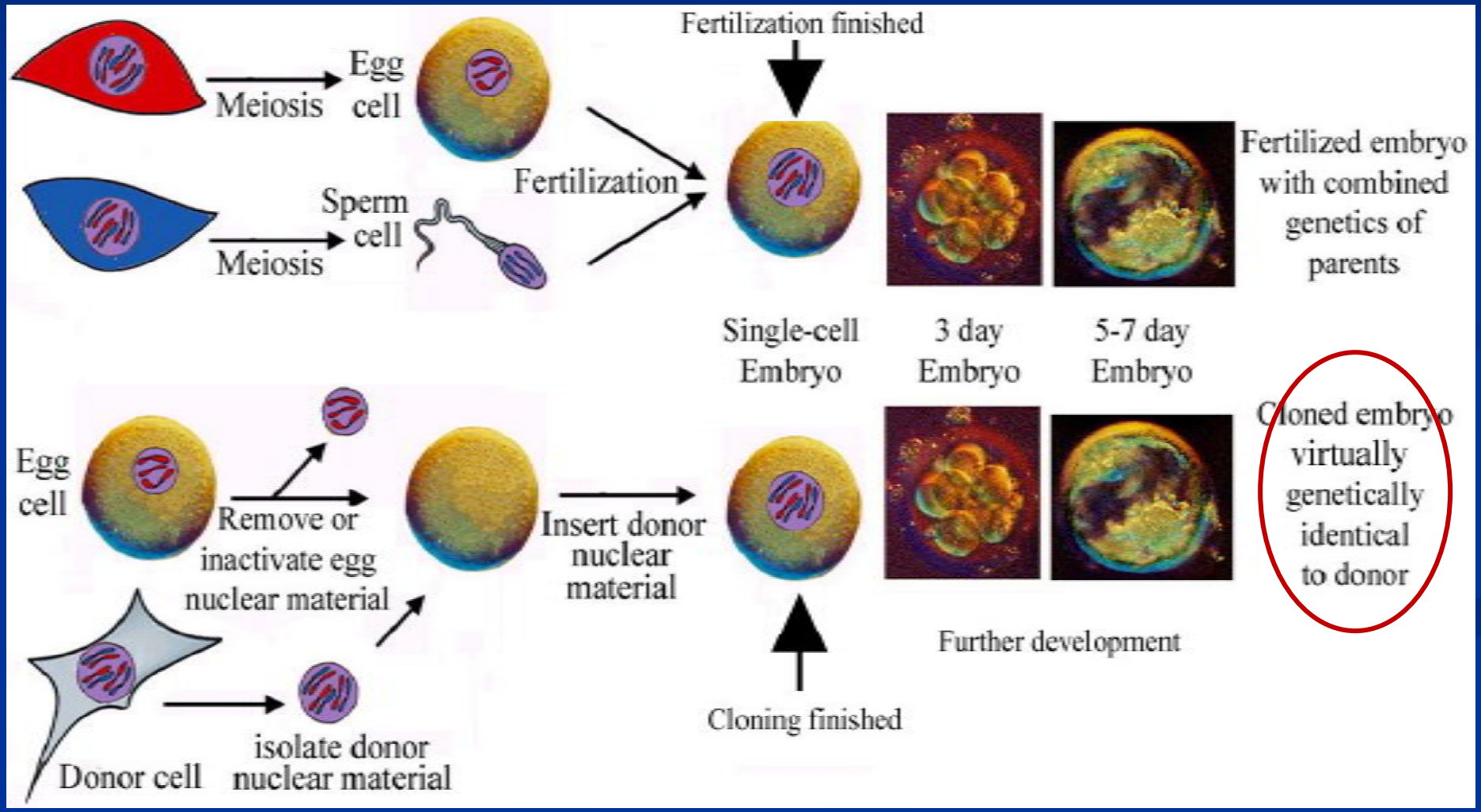
(somatic cell nuclear transfer, SCNT)



Cloning?

Fertilization *vs*. Cloning

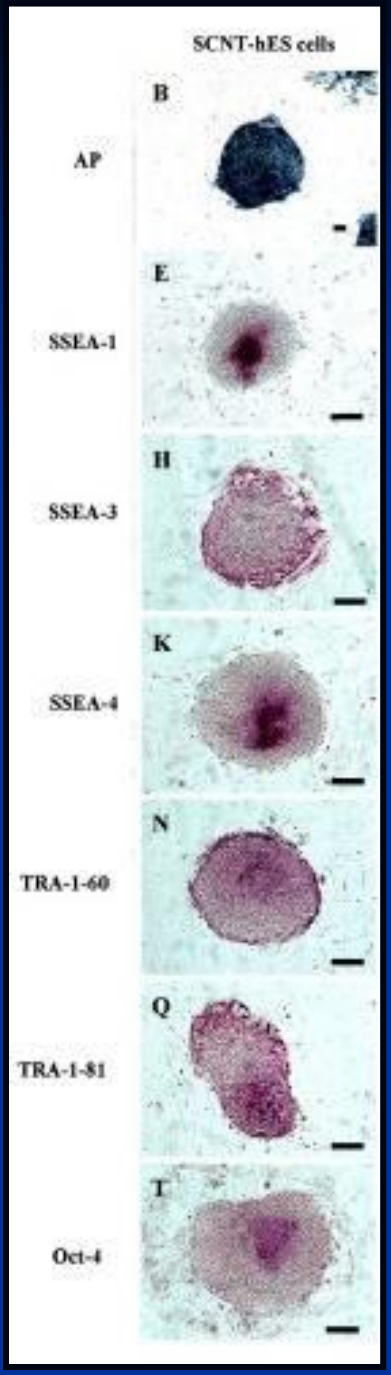
(somatic cell nuclear transfer, SCNT)



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.**

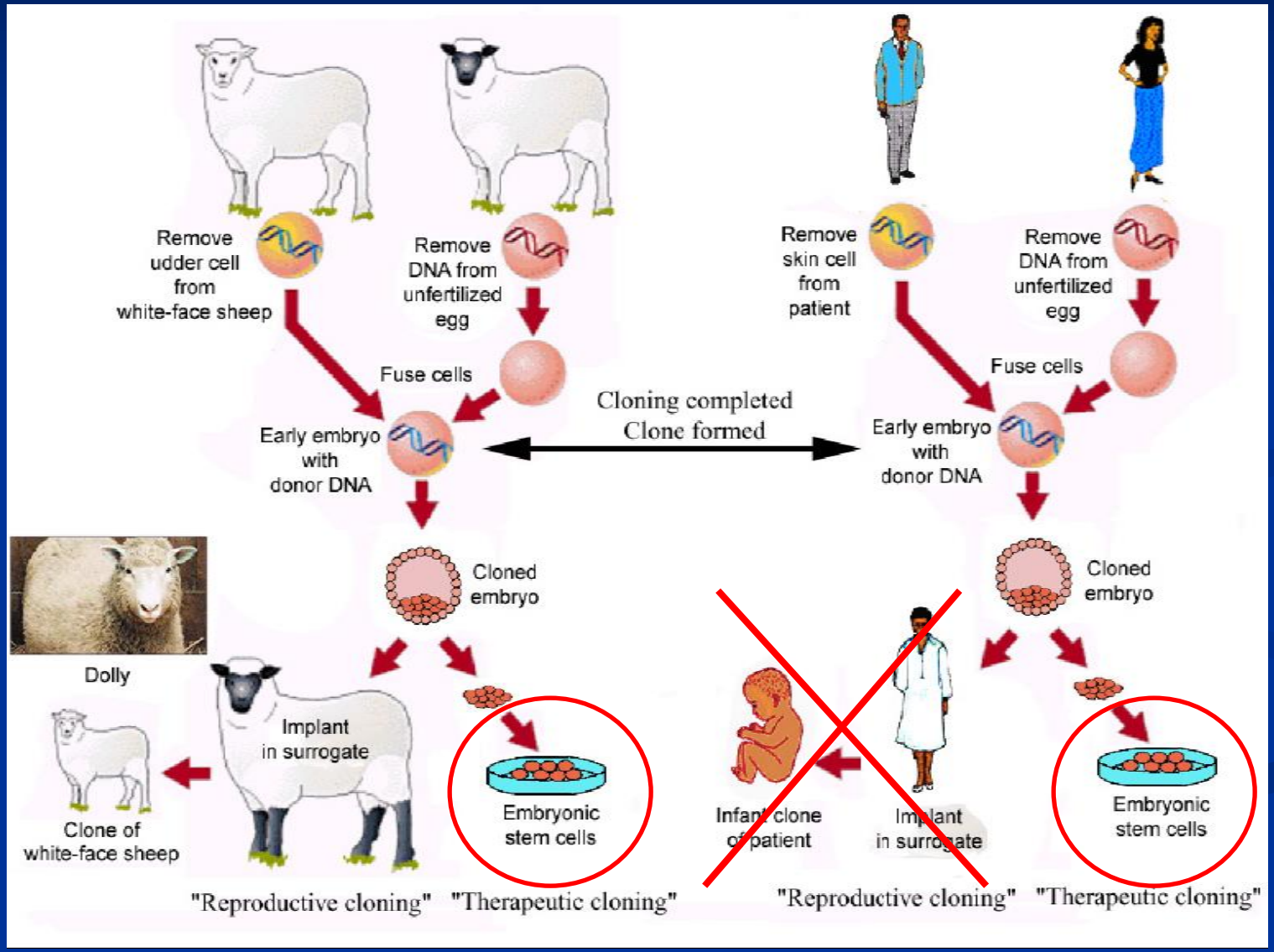
Cloning

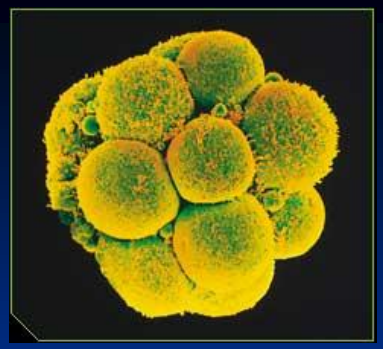
Reproductive cloning is a technology used to generate an animal that has the same nuclear DNA as another currently or previously existing animal.

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

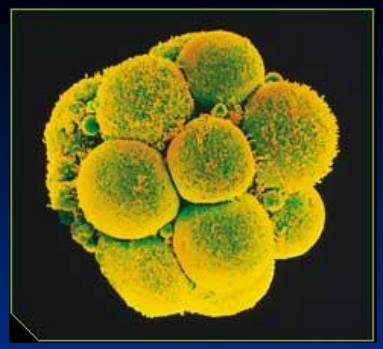




Embryo three days after fertilization

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.

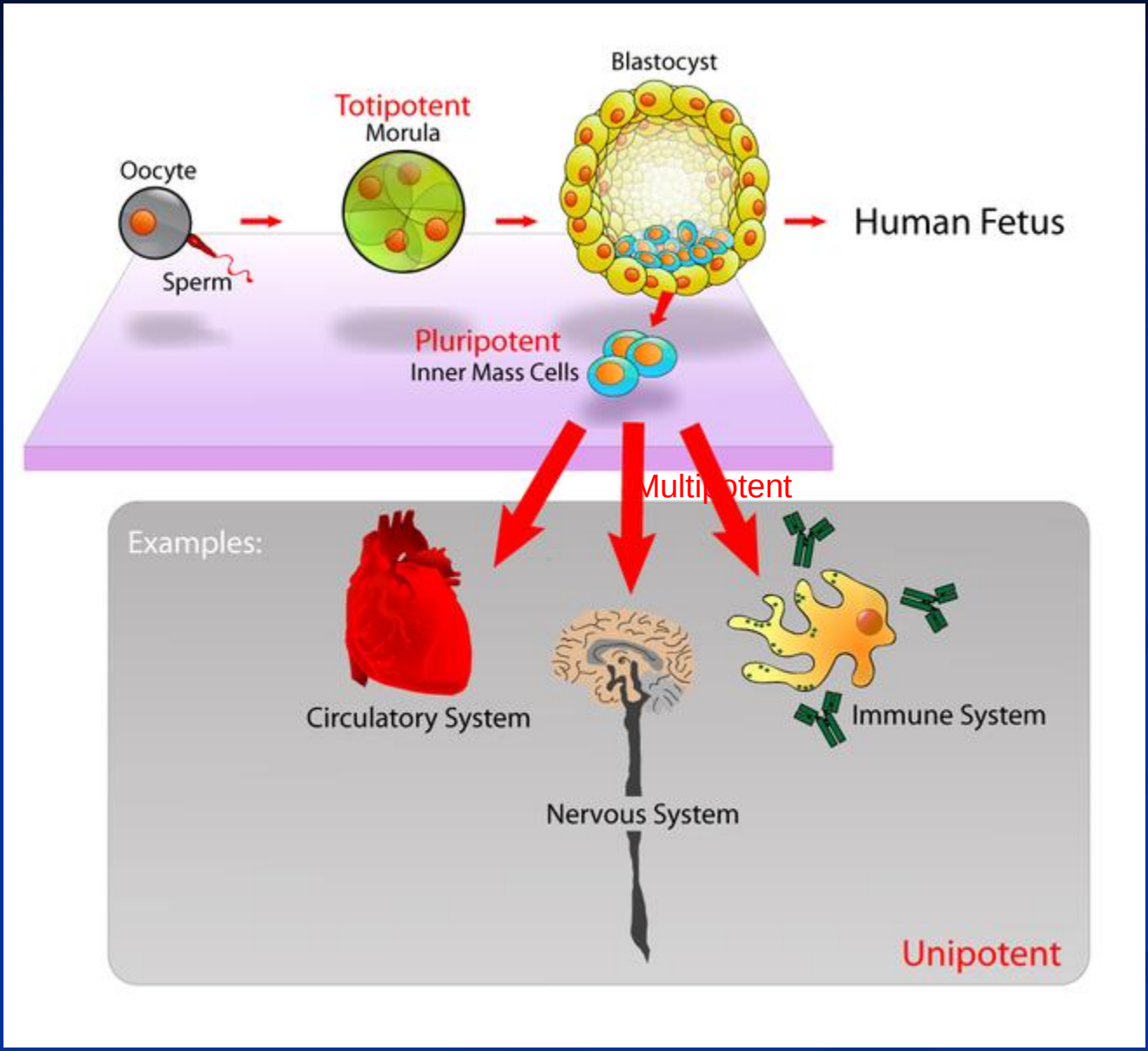


Stem cells

- 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.


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

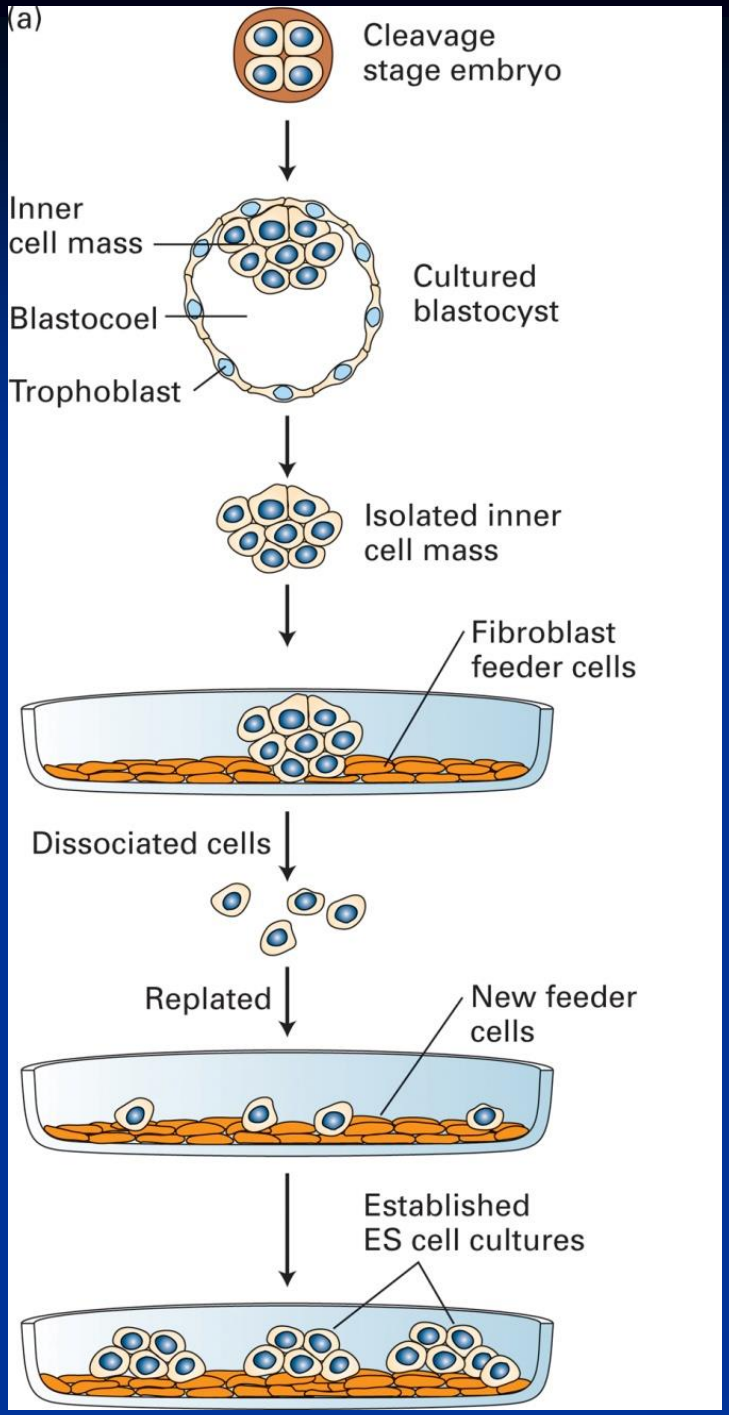
Embryonic Stem Cell Culture

Stem cells are extracted 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.

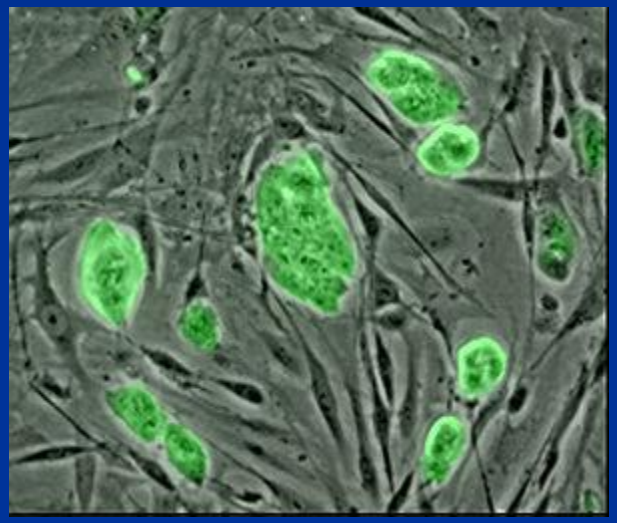
The research aims to induce these cells to generate healthy tissue needed by patients.

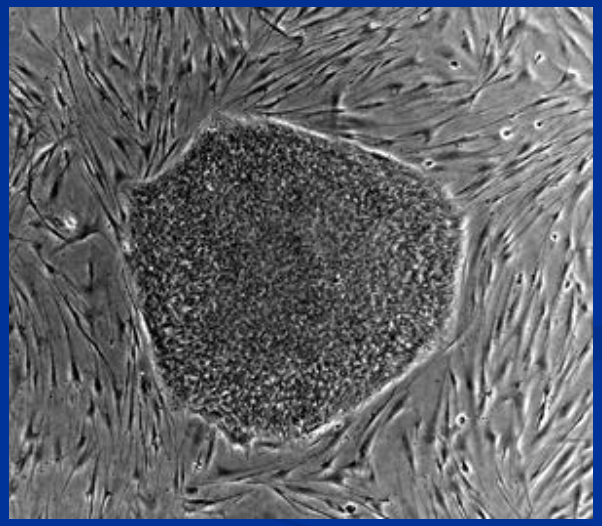
Embryonic stem cells can be maintained in culture



Embryonic stem cells in culture

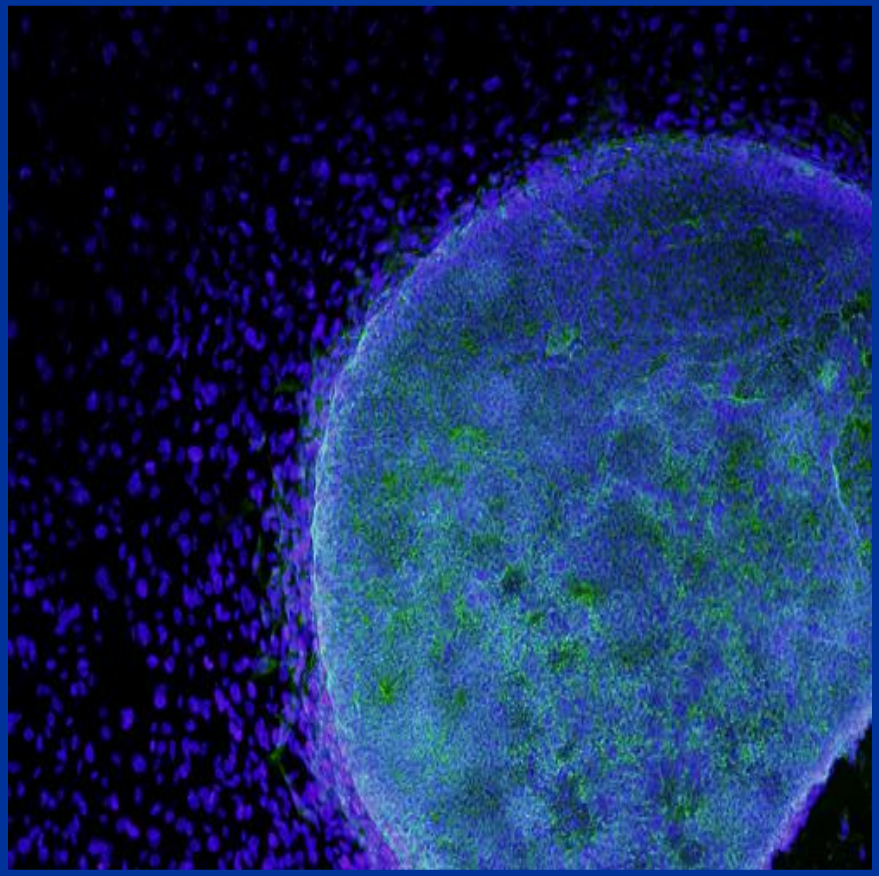
Mouse ES cells are grown on a layer of gelatin and require the presence of Leukemia Inhibitory Factor (LIF)

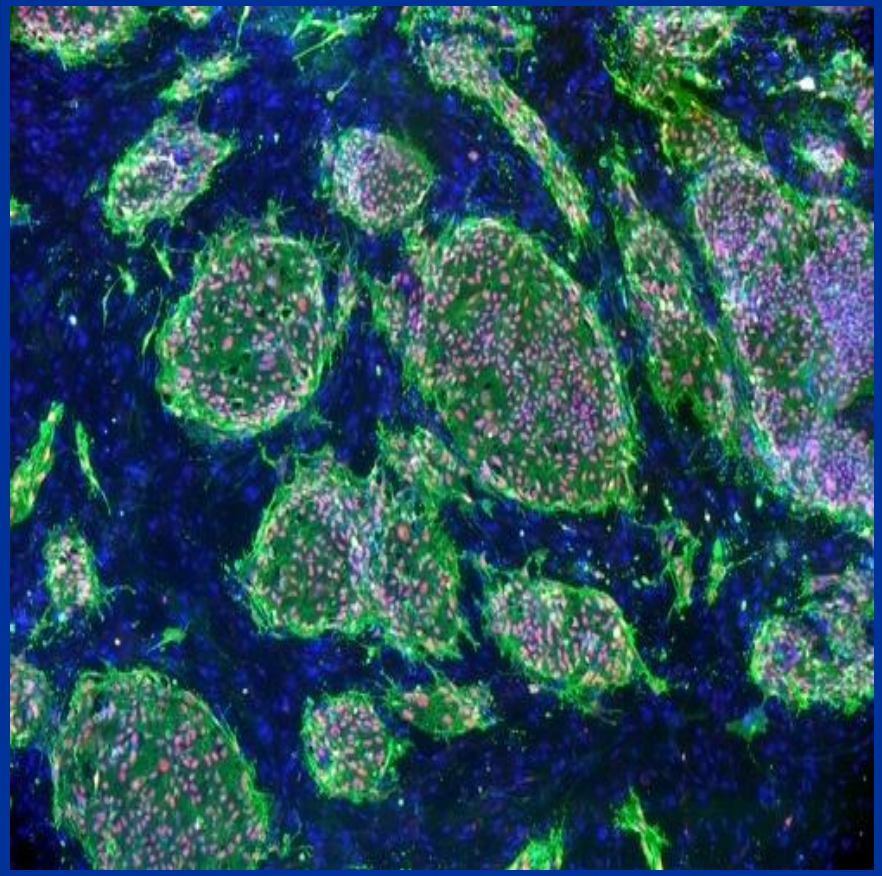




Human ES cells are grown on a feeder layer of mouse embryonic fibroblasts and require the presence of basic Fibroblast Growth Factor (bFGF or FGF-2)

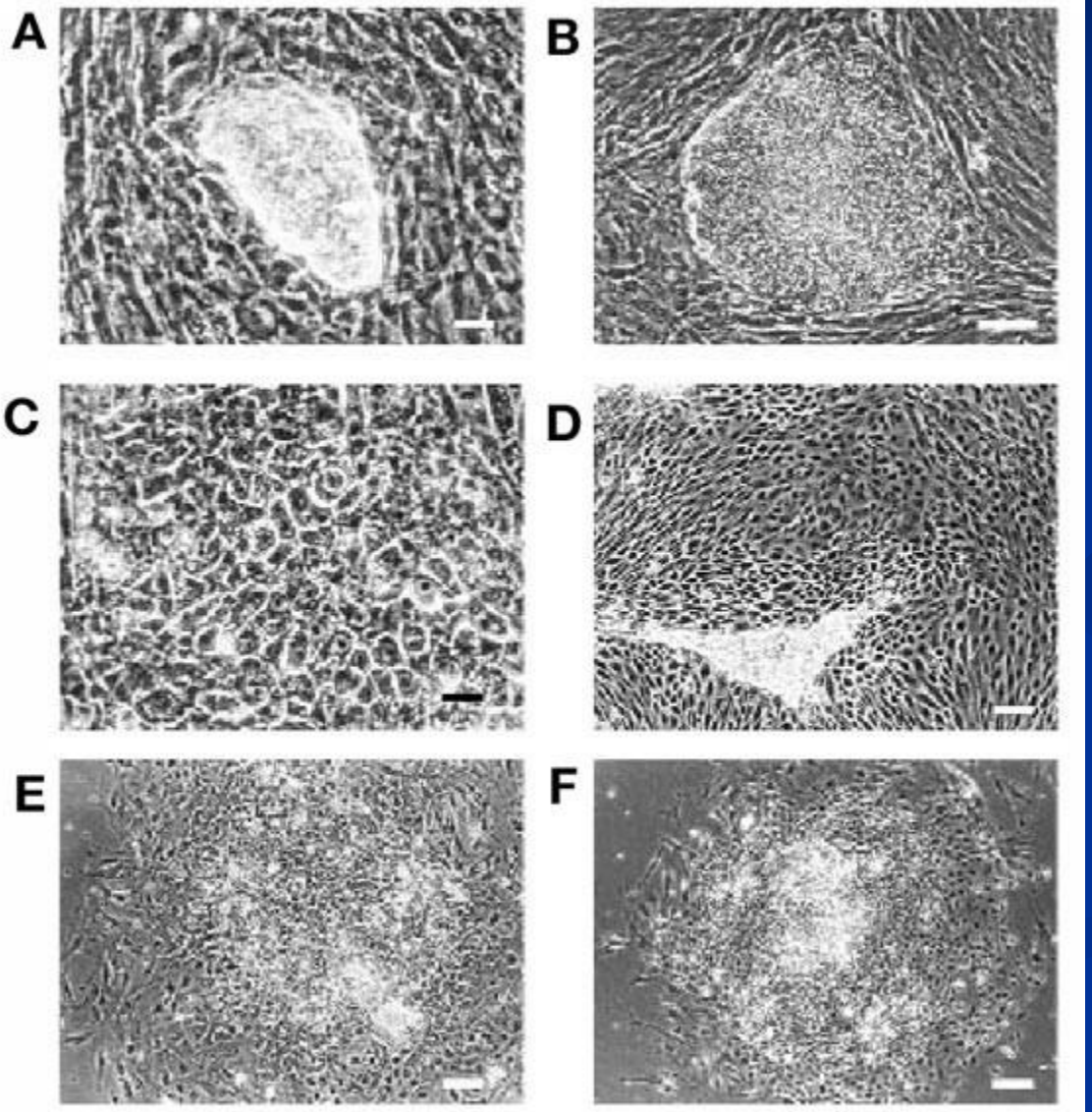
Fluorescent imaging of embryonic stem cell colonies.





Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro

Nature Biotechnology **volume 18**, pages 399–404 (2000)

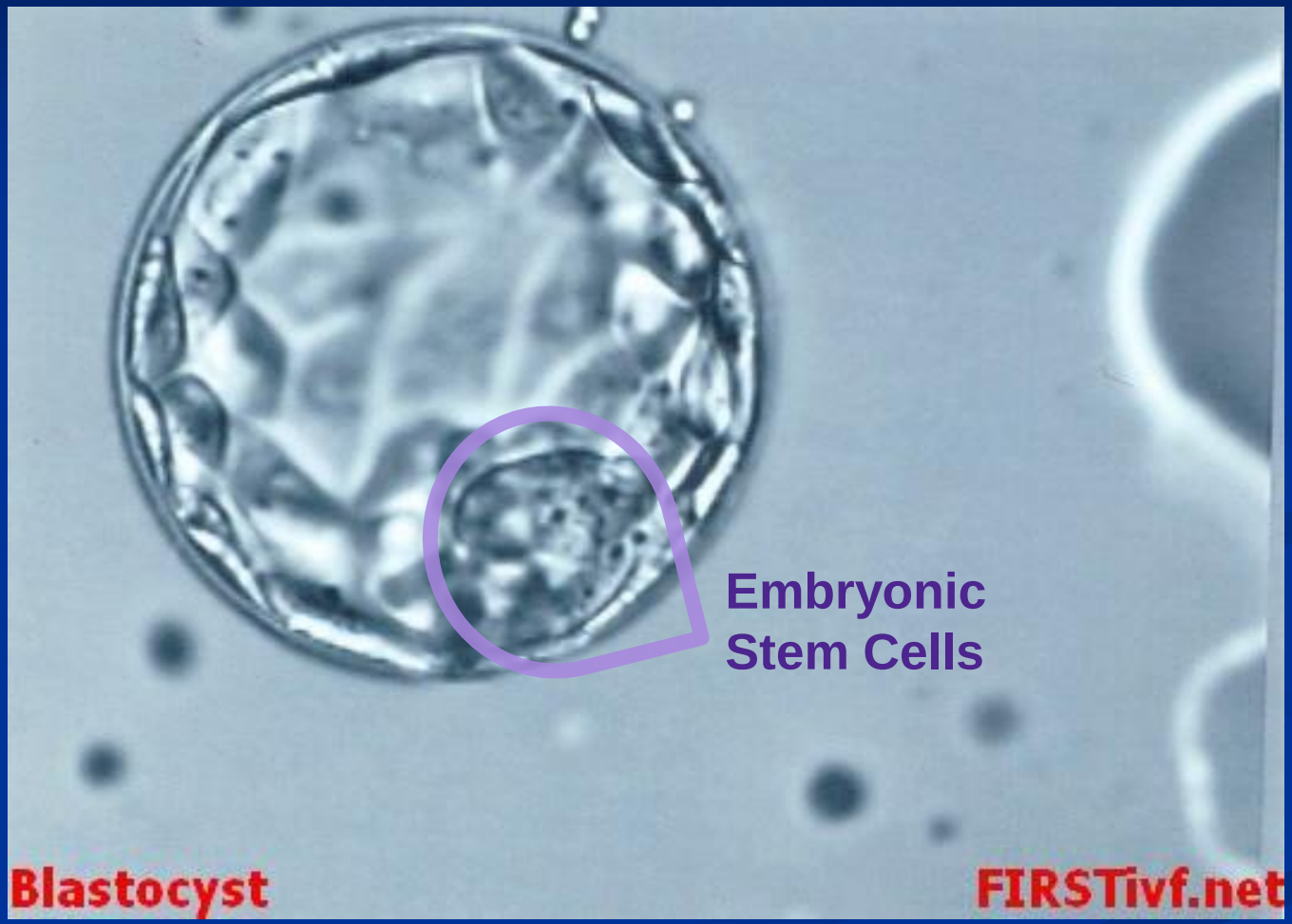


We describe the derivation of pluripotent embryonic stem (ES) cells from human blastocysts. Two diploid ES cell lines have been cultivated in vitro for extended periods while maintaining expression of markers characteristic of pluripotent primate cells. Human ES cells express the transcription factor Oct-4, essential for development of pluripotential cells in the mouse. When grafted into SCID mice, both lines give rise to teratomas containing derivatives of all three embryonic germ layers. Both cell lines differentiate in vitro into extraembryonic and somatic cell lineages. Neural progenitor cells may be isolated from differentiating ES cell cultures and induced to form mature neurons.

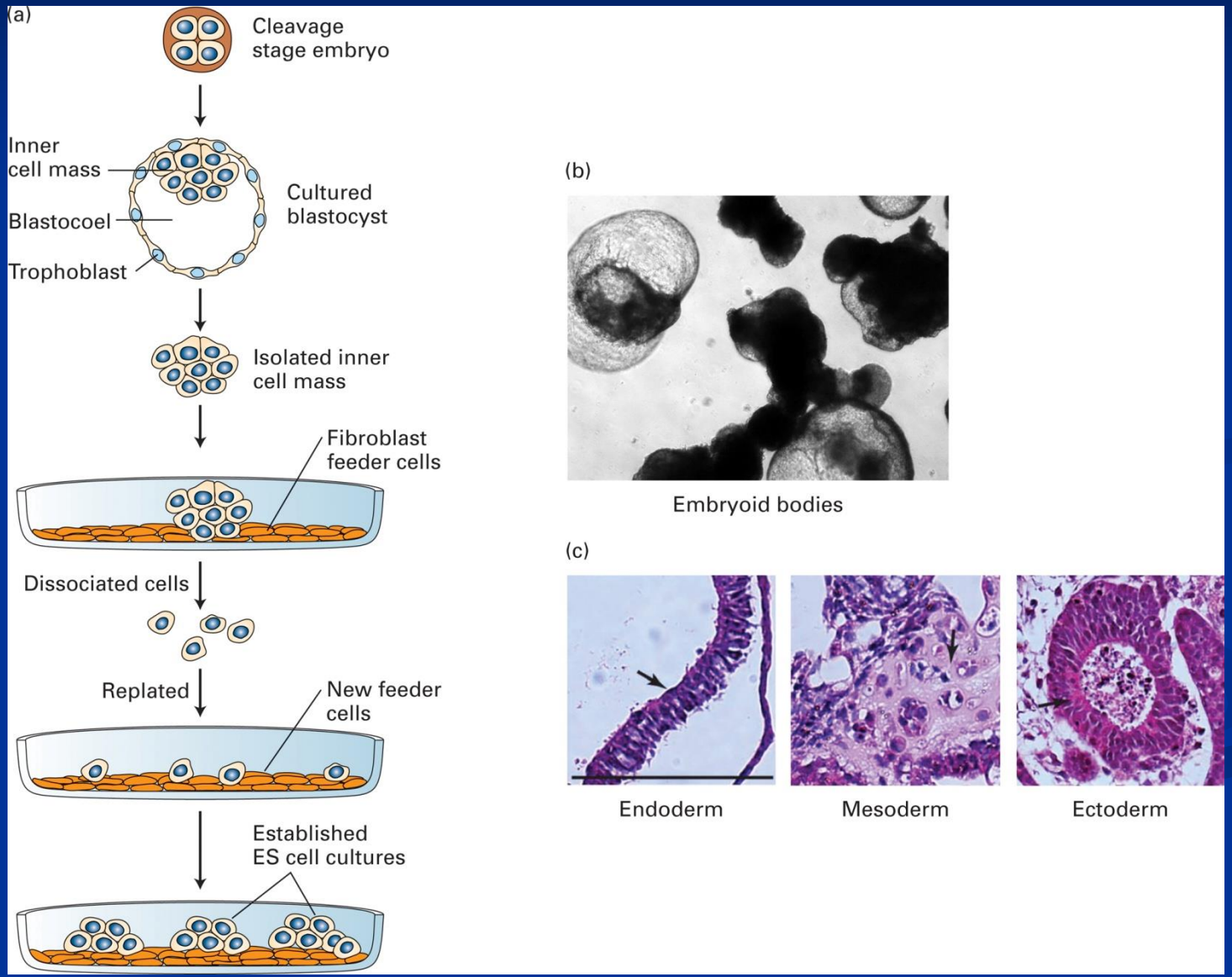
Sources of Embryonic Stem Cells

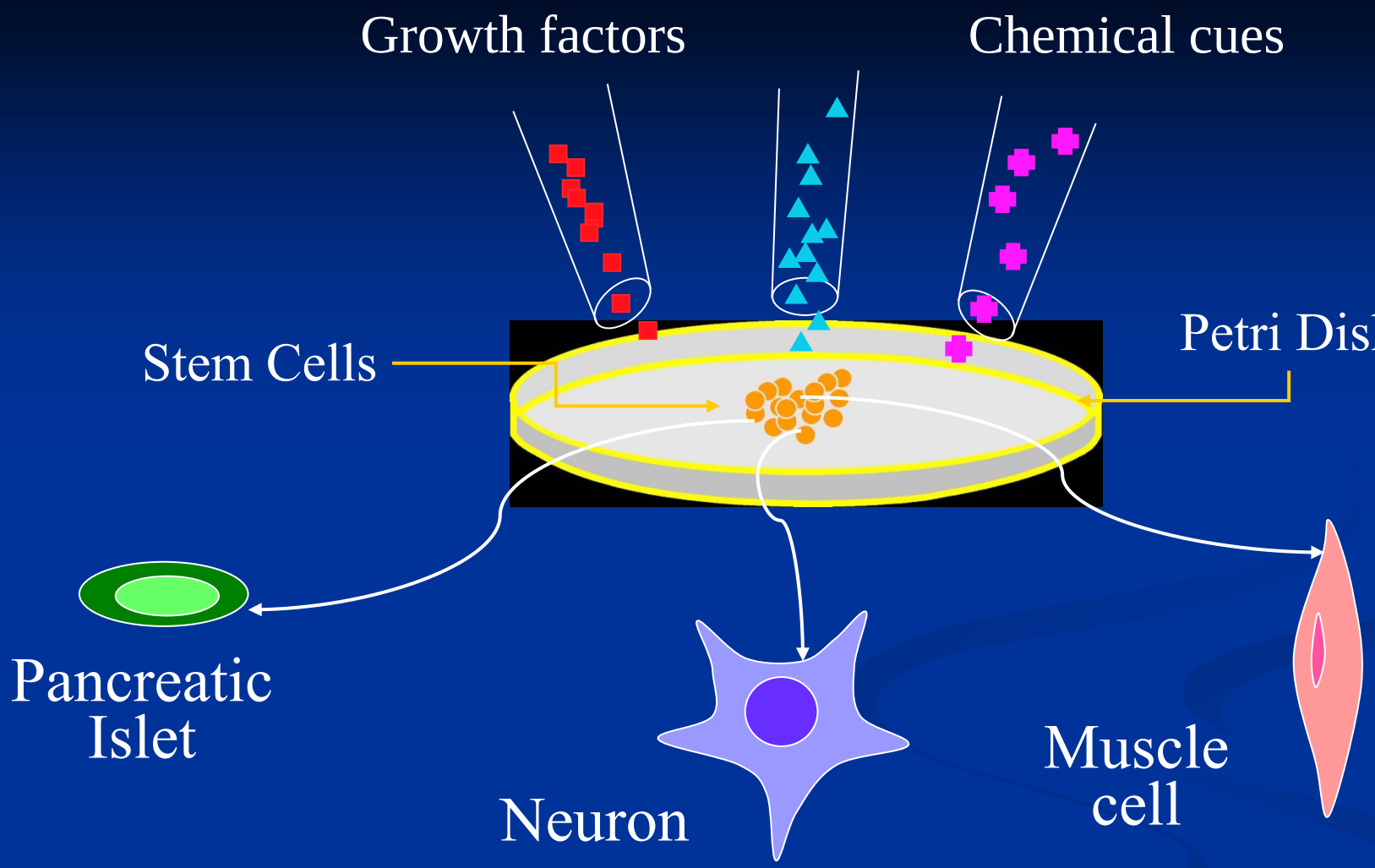
- Embryonic stem cell lines
- Excess embryos from IVF clinics

Intra-Cytoplasmic Sperm Injection

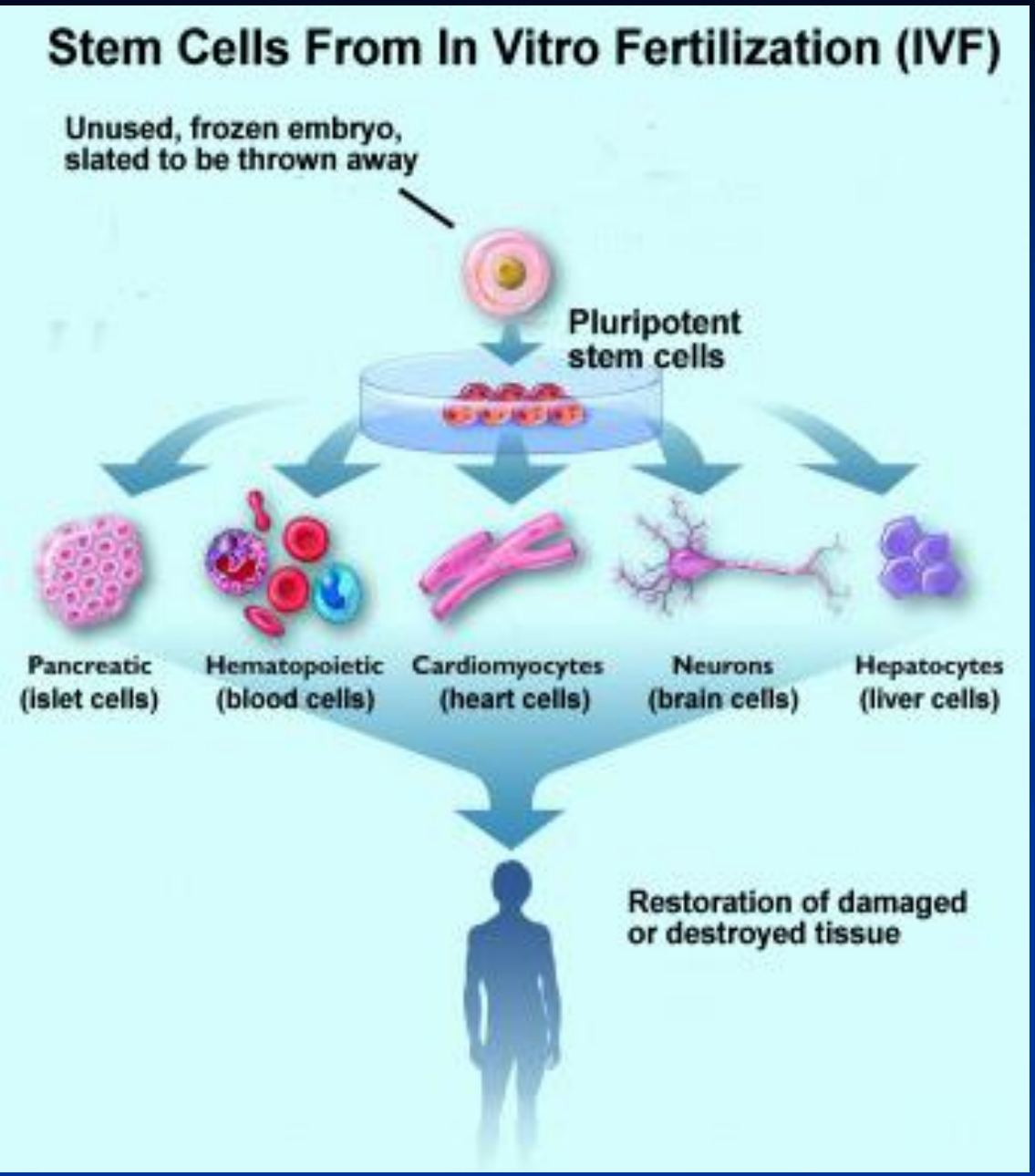

Embryonic
Stem Cells
Blastocyst
FIRSTivf.net

Embryonic stem (ES) cells can be maintained in culture and can form differentiated cell types.





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: “Multi potent”

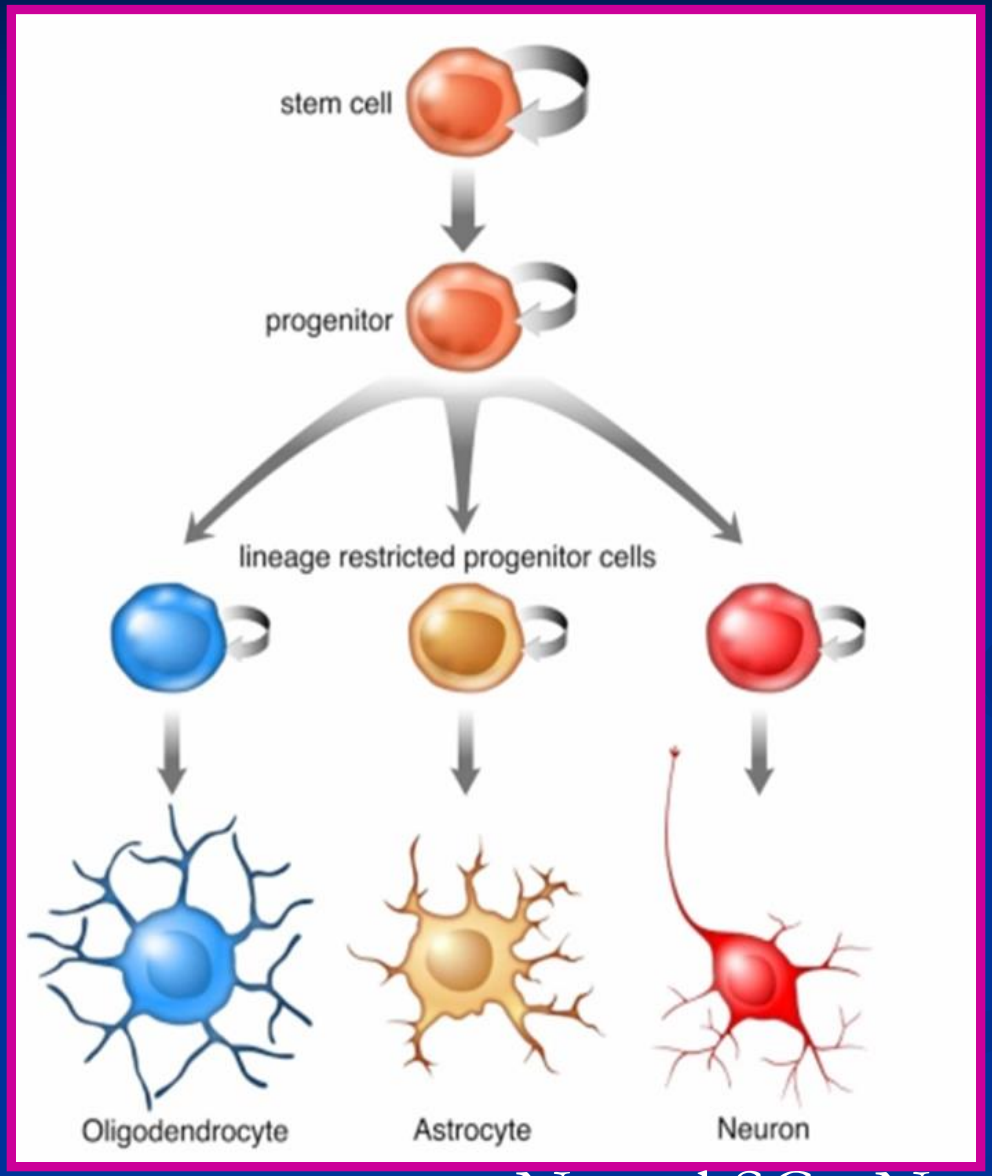
- Adult stem cells are rare: 1 in 10,000 to 15,000 cells in the bone marrow is a hematopoietic stem cell (**HSC**)

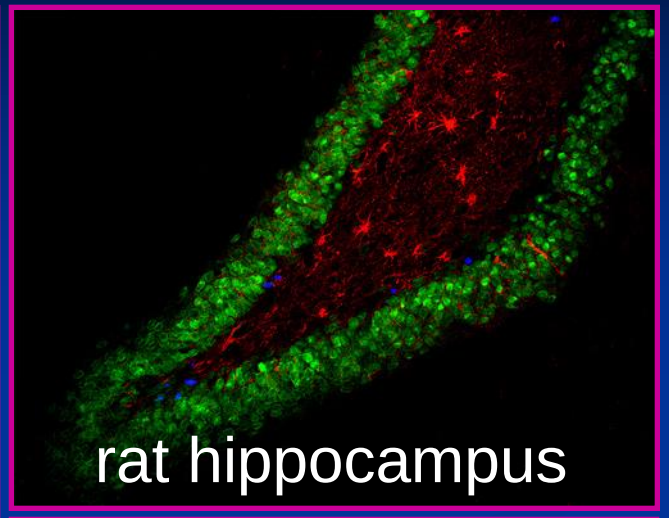
- Primary functions:
  - to maintain homeostasis
  - with limitations, to replace cells that die because of injury or disease

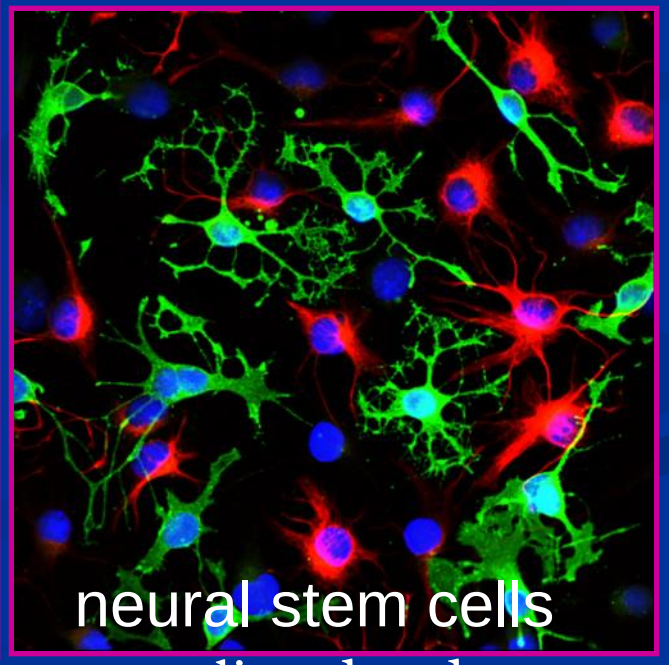
- Dispersed in tissues throughout the mature organism and behave very differently, depending on their local environment.
  - HSCs are constantly being generated in the bone marrow where they differentiate into mature types of blood cells (replace blood cells)
  - Stem cells in the small intestine are 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,



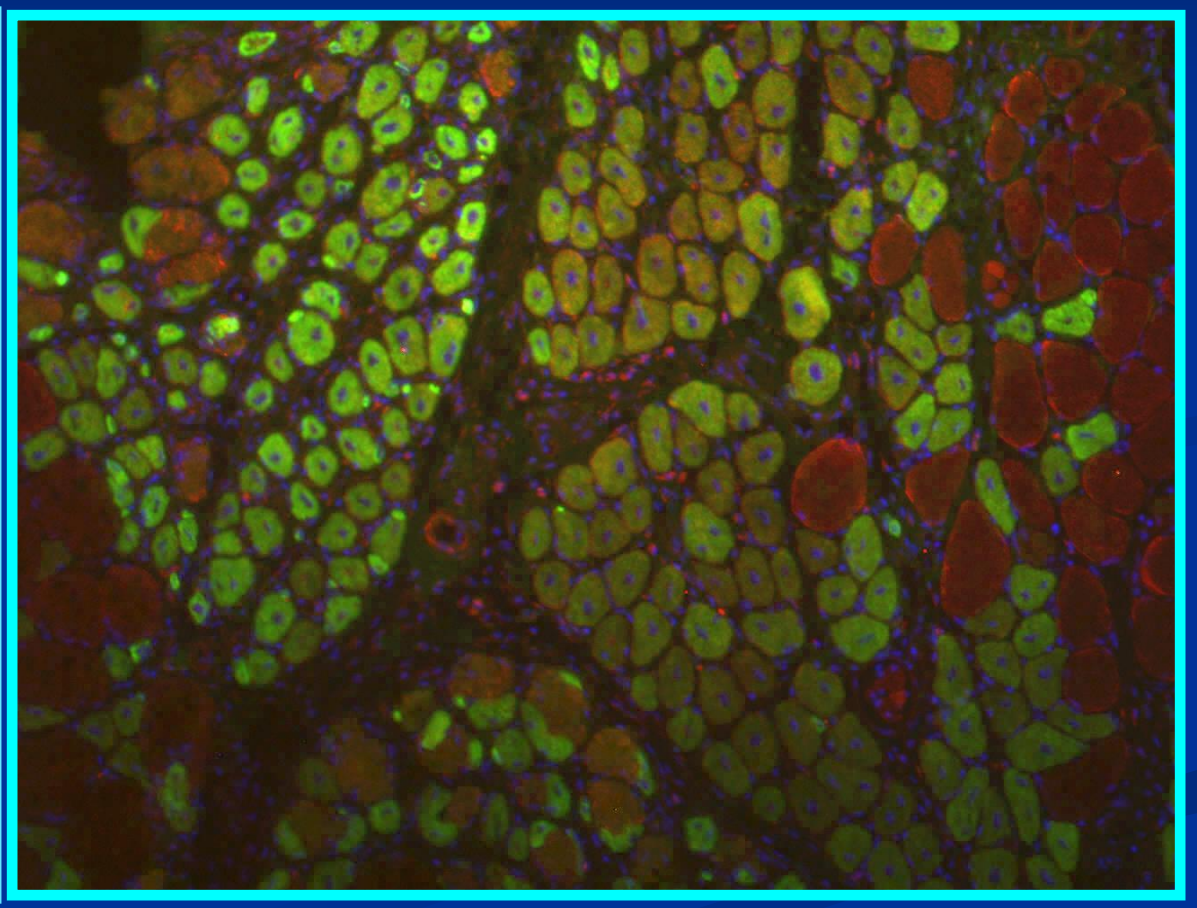




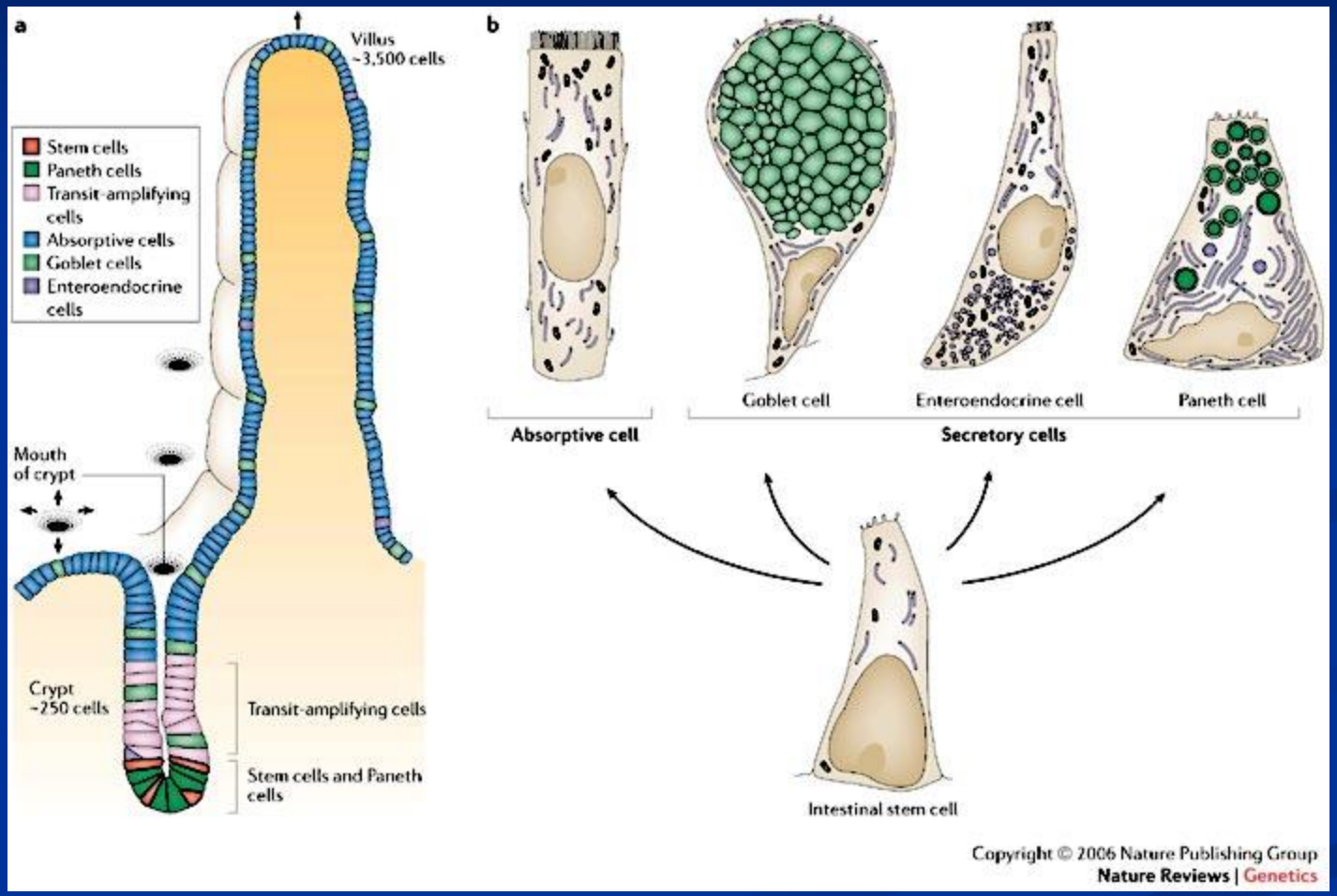
Neural SCs, Neurons, oligodendrocytes,

Stem cells in mature skeletal muscle:

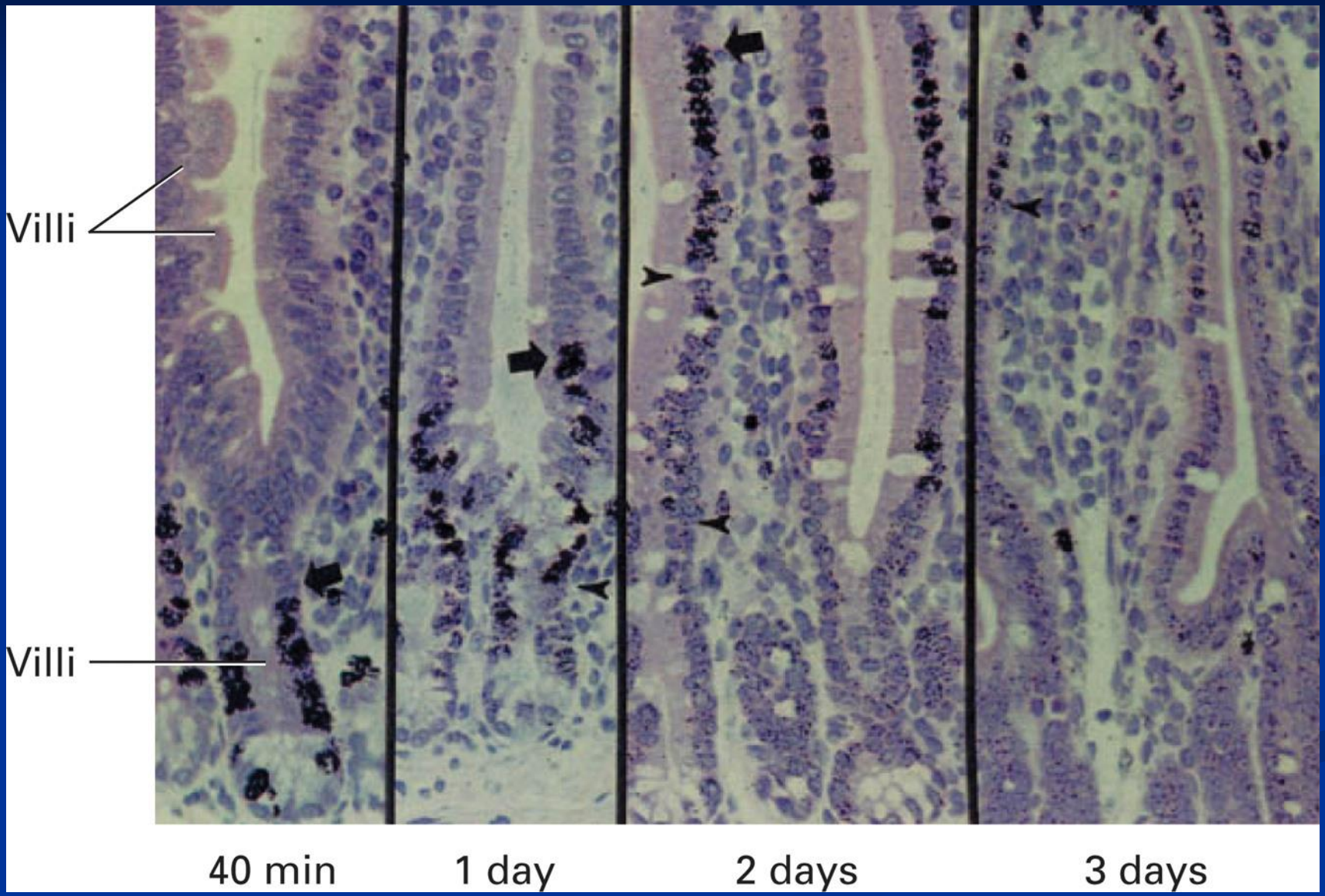
Healthy + new muscle fibers, Muscle SCs



Stem Cells in the Intestine



Regeneration of the intestinal epithelium from stem cells can be demonstrated in pulse-chase experiments.



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

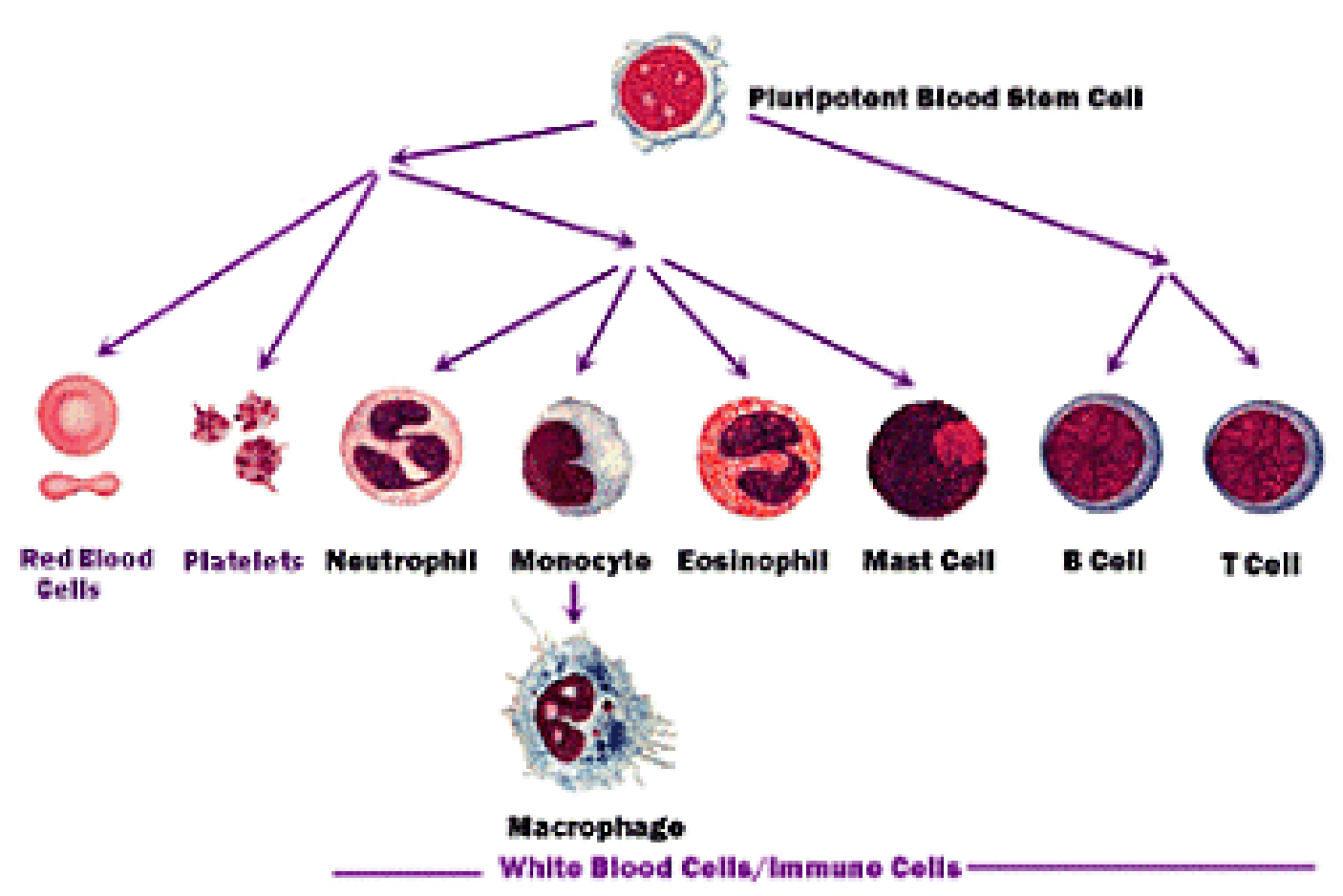
Evidence that Some Adult Stem Cells show Pluripotent Capacity

Present in adult tissues as minute sub-populations in certain stem cell niches:

Bone marrow derived mesenchymal stem cells

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
- Henderson Family Marks Anniversary of Transplant by Touring CRYO-CELL
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- CRYO-CELL Earns Accreditation by the American Association of Blood Banks
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by Michael E. Trigg, M.D.

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Why cord blood banking

Why PBKM

How to do it

PL 13:00 2017-11-24

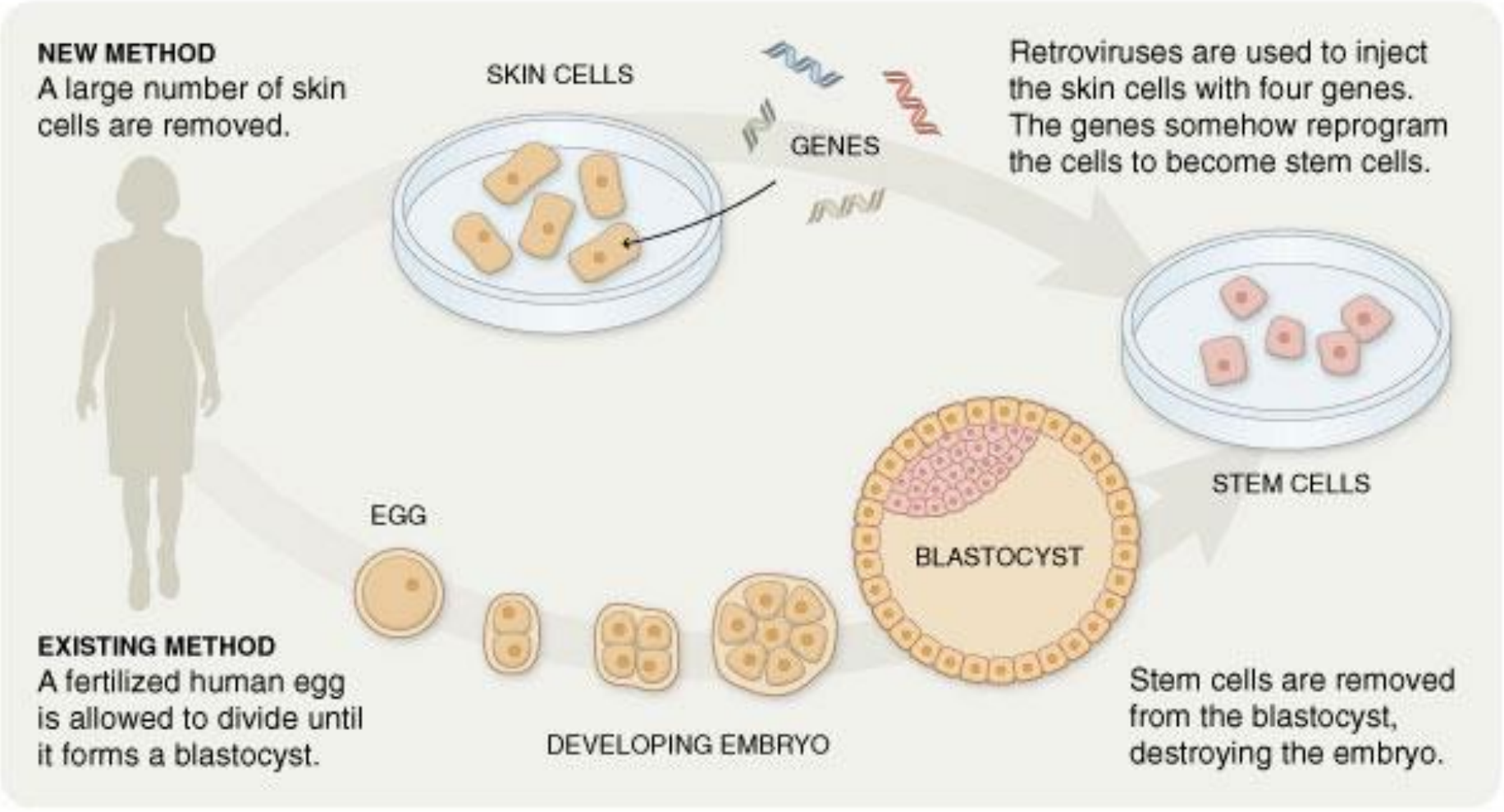
Induced Pluripotent Stem (iPS) Cells

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

Shinya Yamanaka

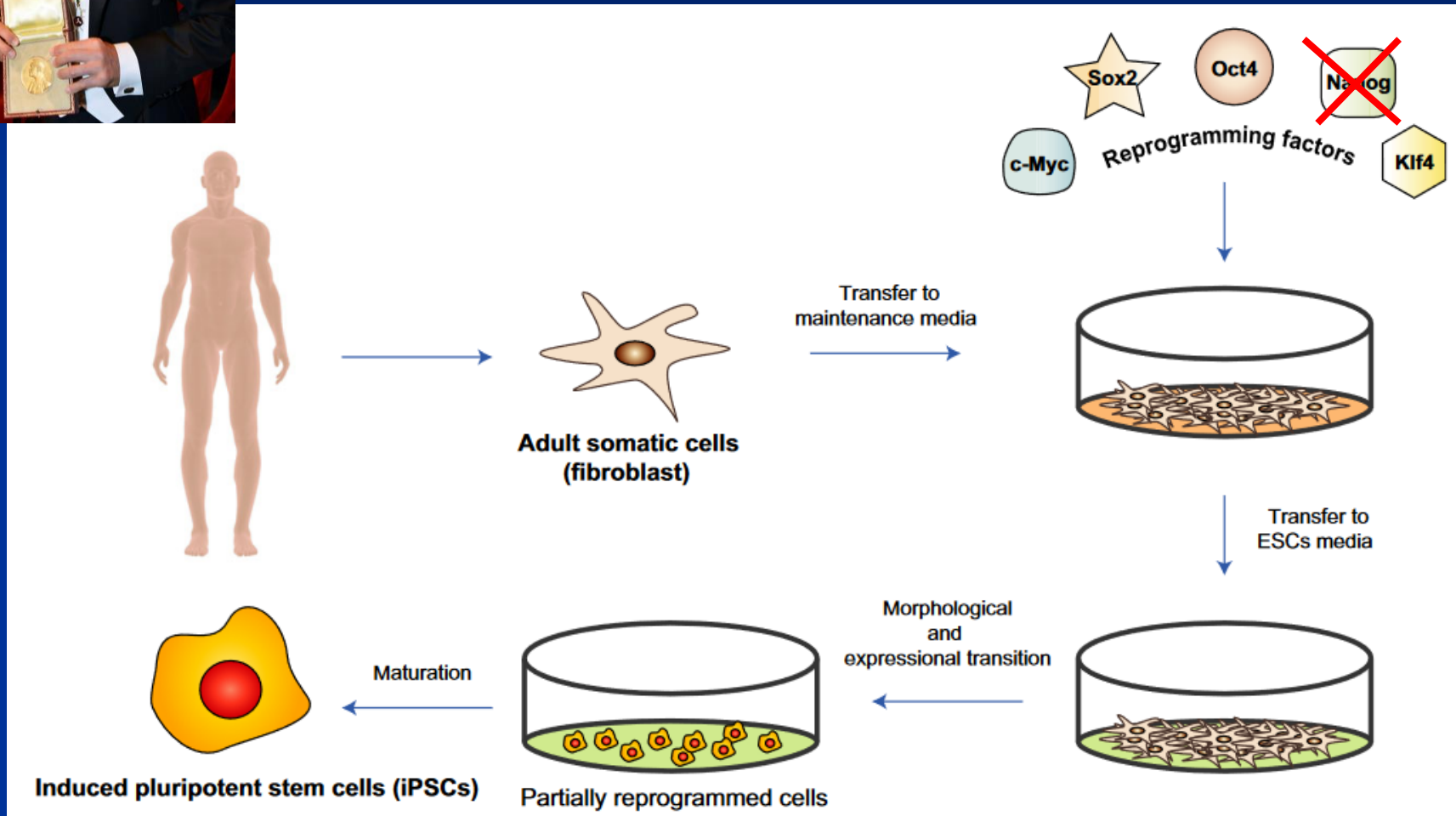
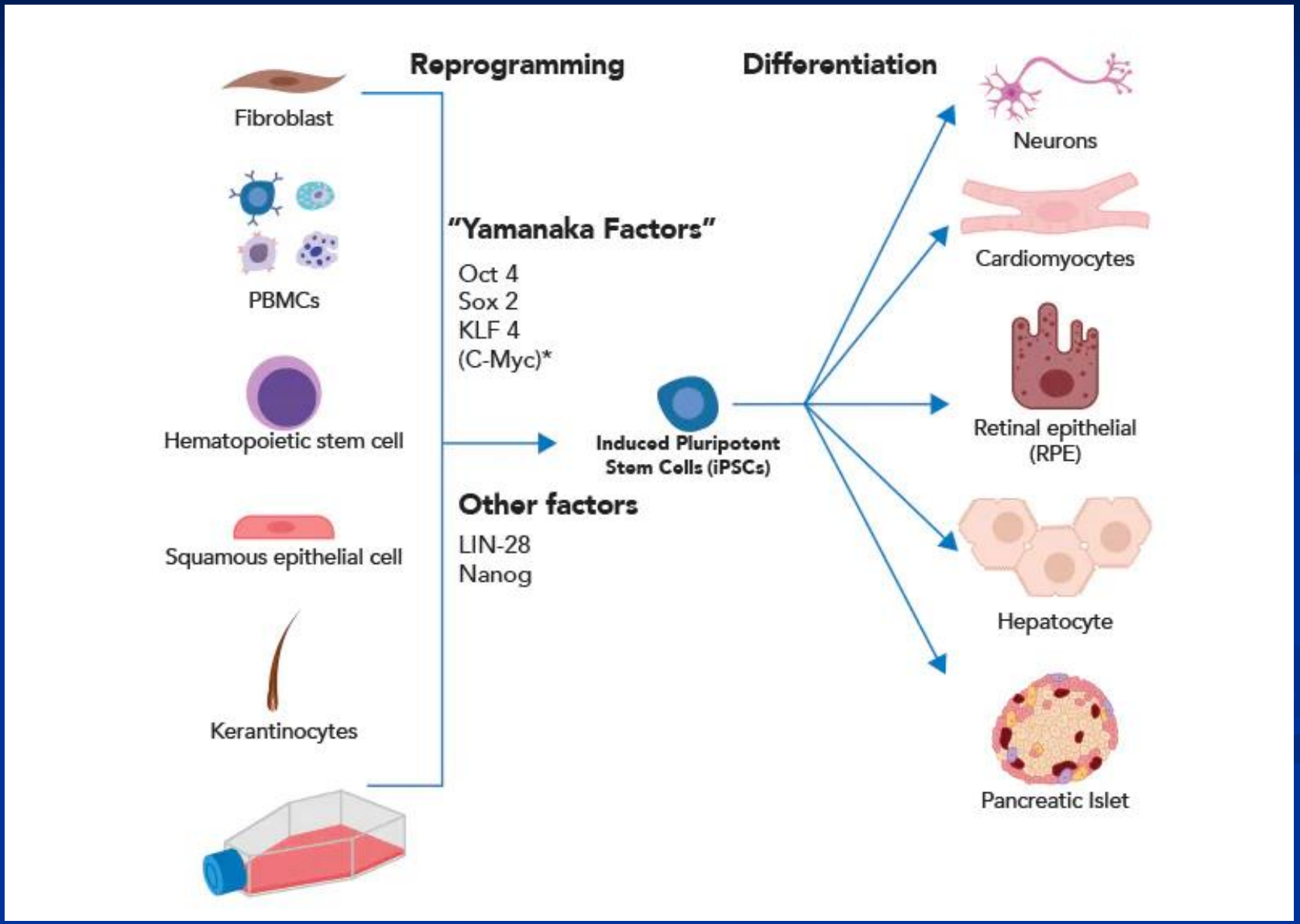
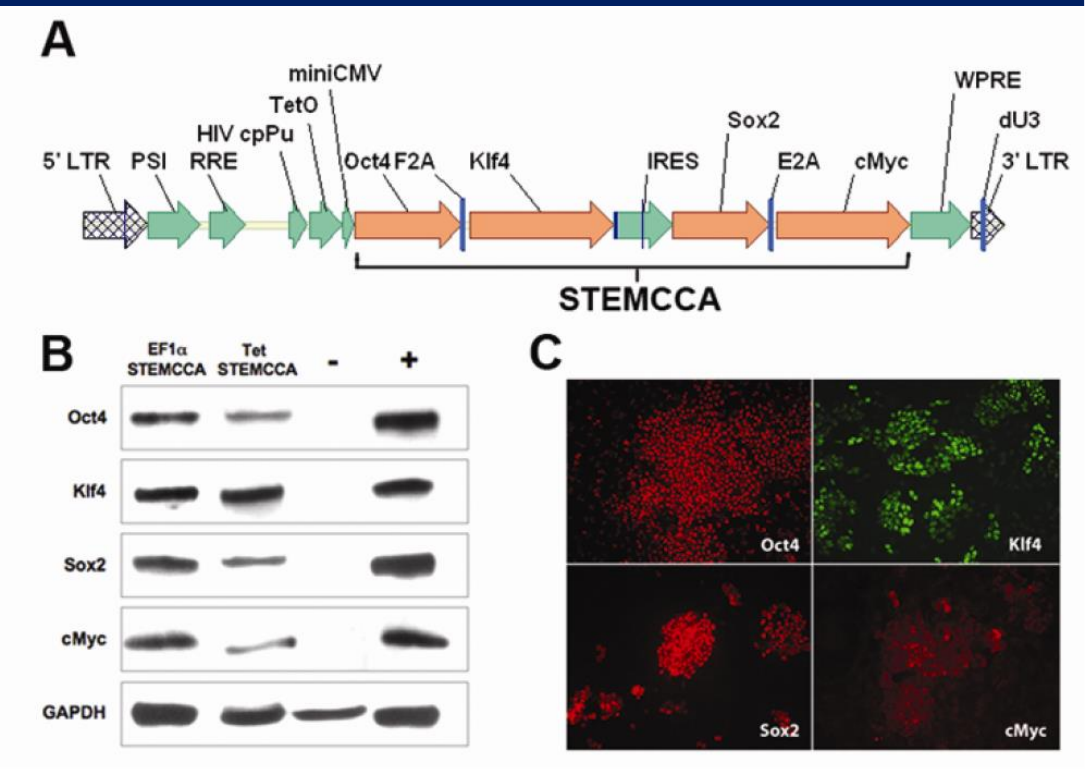


Figure 1. Reprogramming adult somatic cells into induced pluripotent stem cells (iPSCs) through ectopic expression of reprogramming factors. Forced expression of these pluripotency factors resets the epigenetic and transcriptional profile of the specialized cells and reverts them back to their embryonic state.

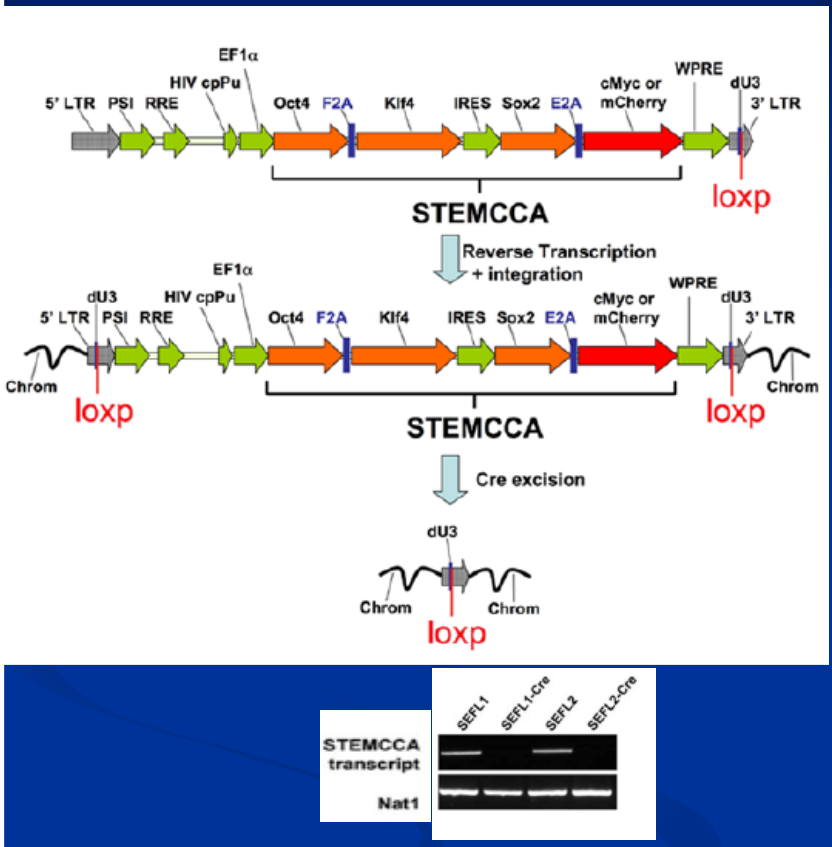
Nuclear reprogramming



STEMCCA: Single Vector Delivery of 4 Transcription Factors



TetOn/TetOff Inducible Promoter



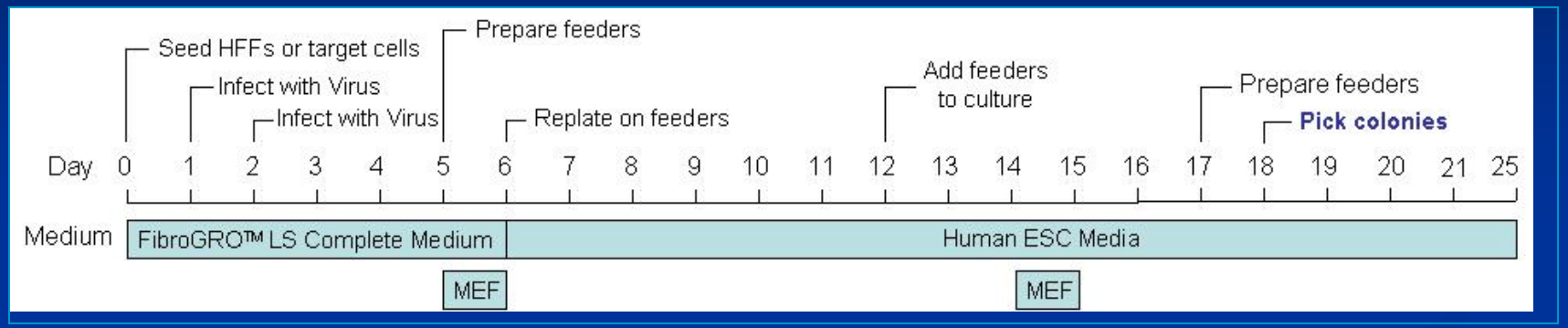
Constitutive Promoter/loxP-Flanked

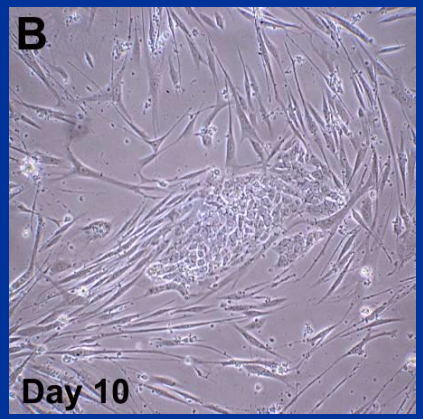
Sommer, C.A.; et al. 2009. Stem Cells 27(3): 543-549.

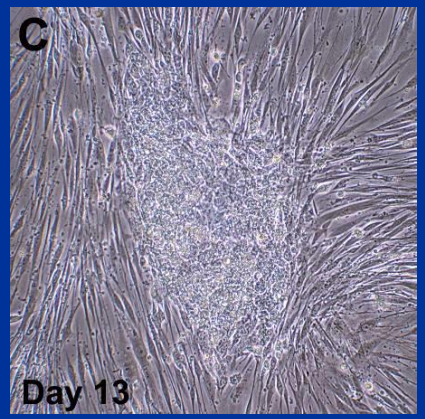
Sommer, C.A.; et al. 2010. Stem Cells 28(1): 64-74.

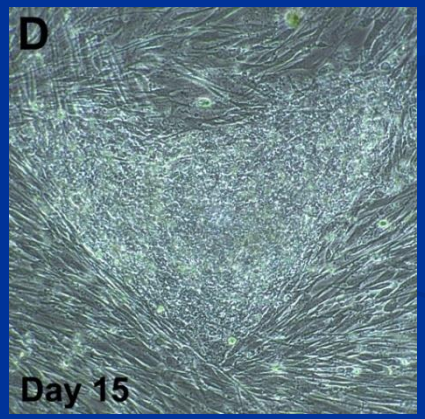
Time Course of Human iPS Colony Formation

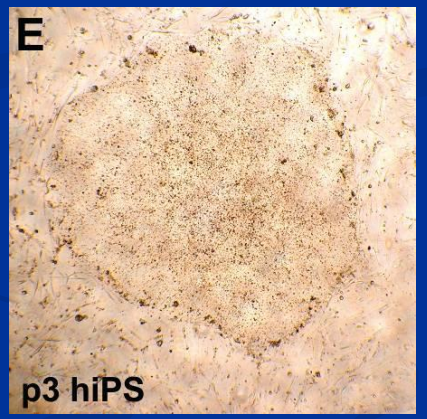
using mouse STEMCCA lentivirus











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)

Limitations of iPSCs

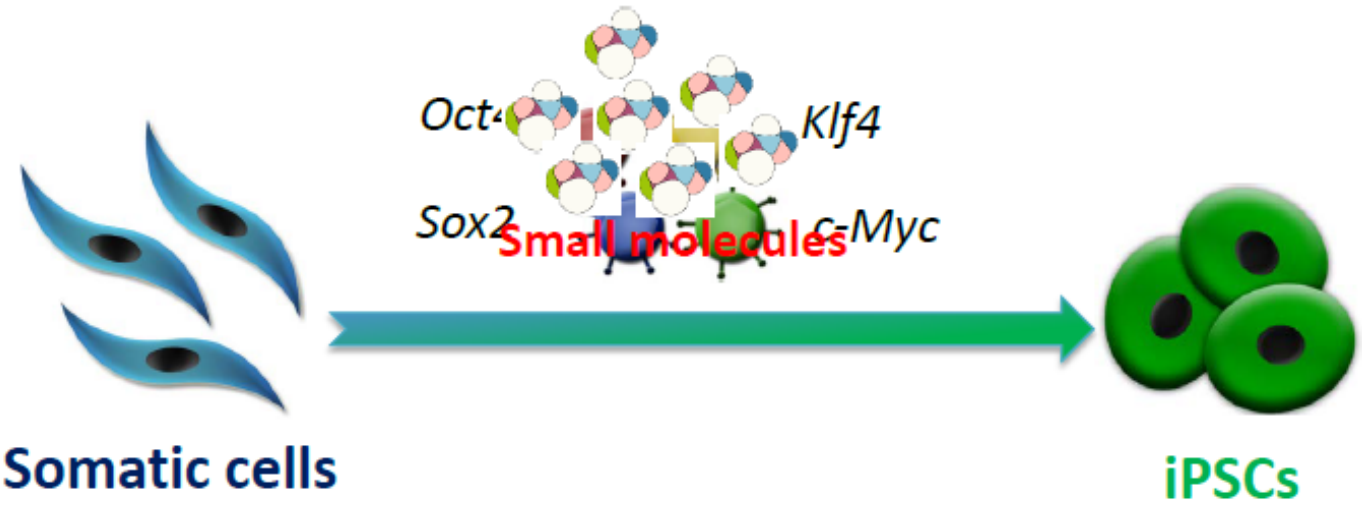
During somatic cell reprogramming to iPSCs, there are many genetic and epigenetic changes that occur.

With current reprogramming technologies, the epigenetic landscape of the somatic cells is often incompletely or aberrantly modified.

This phenomenon, known as epigenetic memory, often biases the iPSCs to differentiate toward their cell of origin.

Grand Challenge:

Can somatic reprogramming be achieved by using only small molecules?

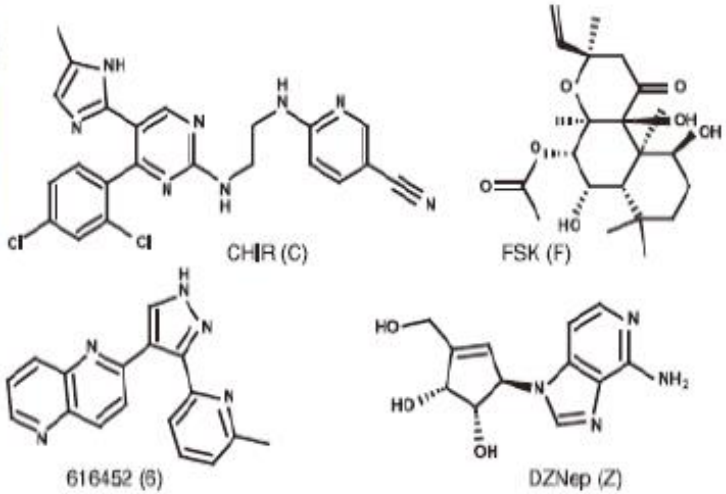


Researchers have been striving to achieve a new way to reprogram somatic cells to iPS cells without the addition of extra genes.

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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News & Comment > News > 2014 > September > Article

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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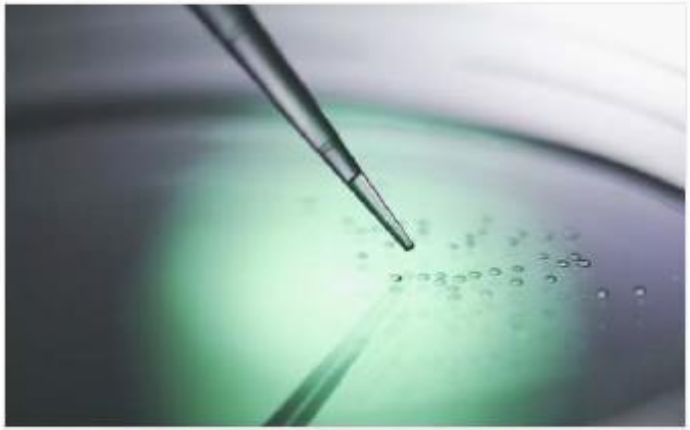


Andrew Brookes/Corbis

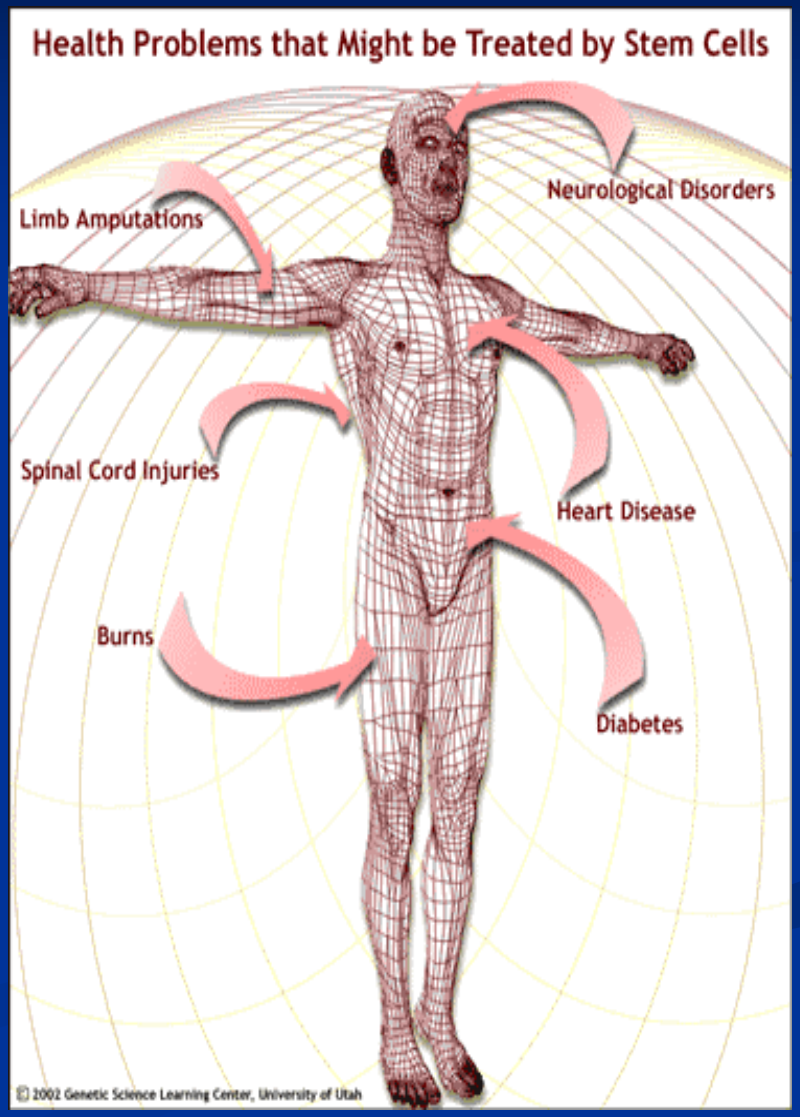
Applications of Stem Cells

- Disease

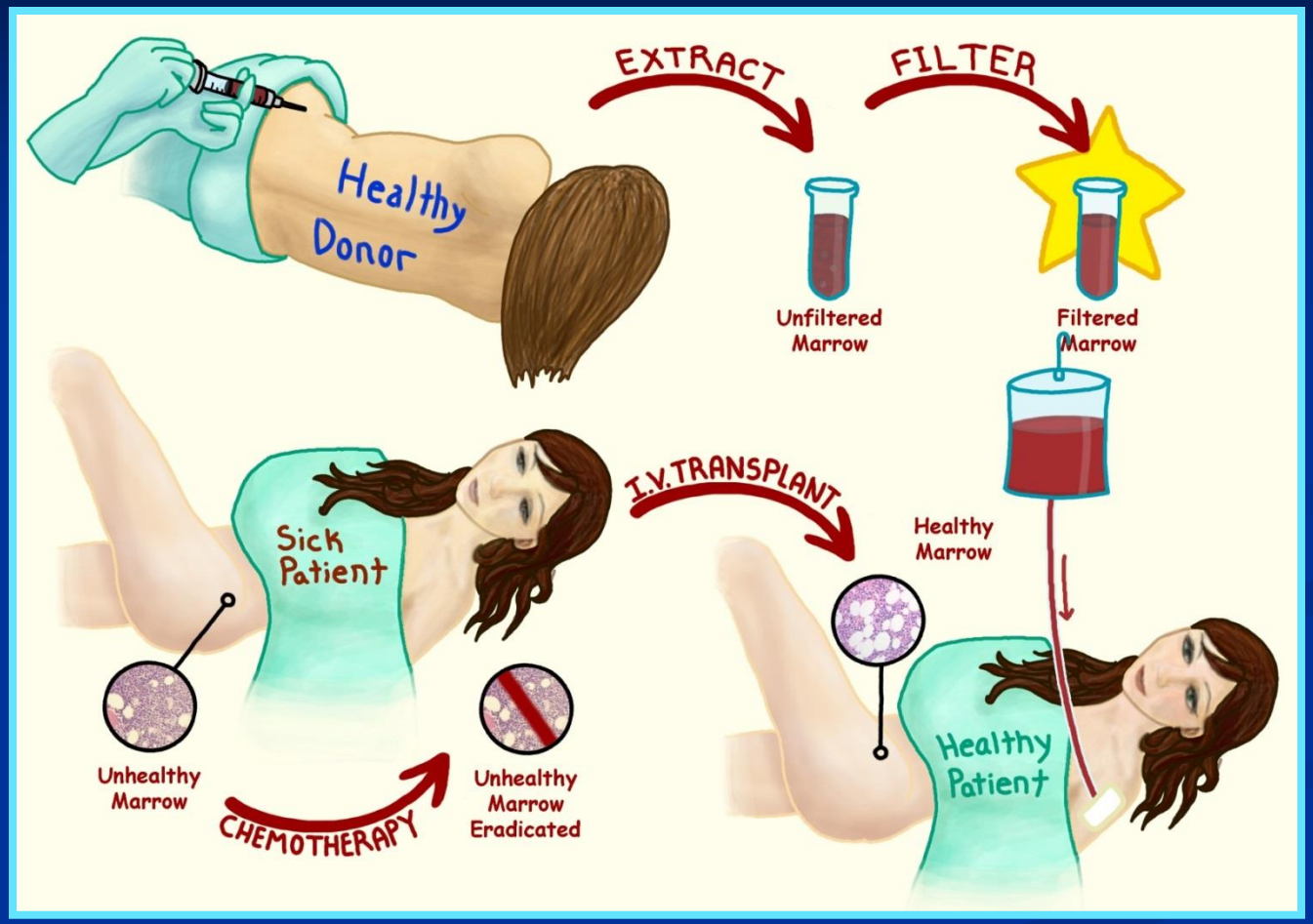
  Blood cancer, Diabetes, Spinal cord injury, Parkinson's disease, heart disease

- Genetic based Disease

  Cystic fibrosis, Huntington's



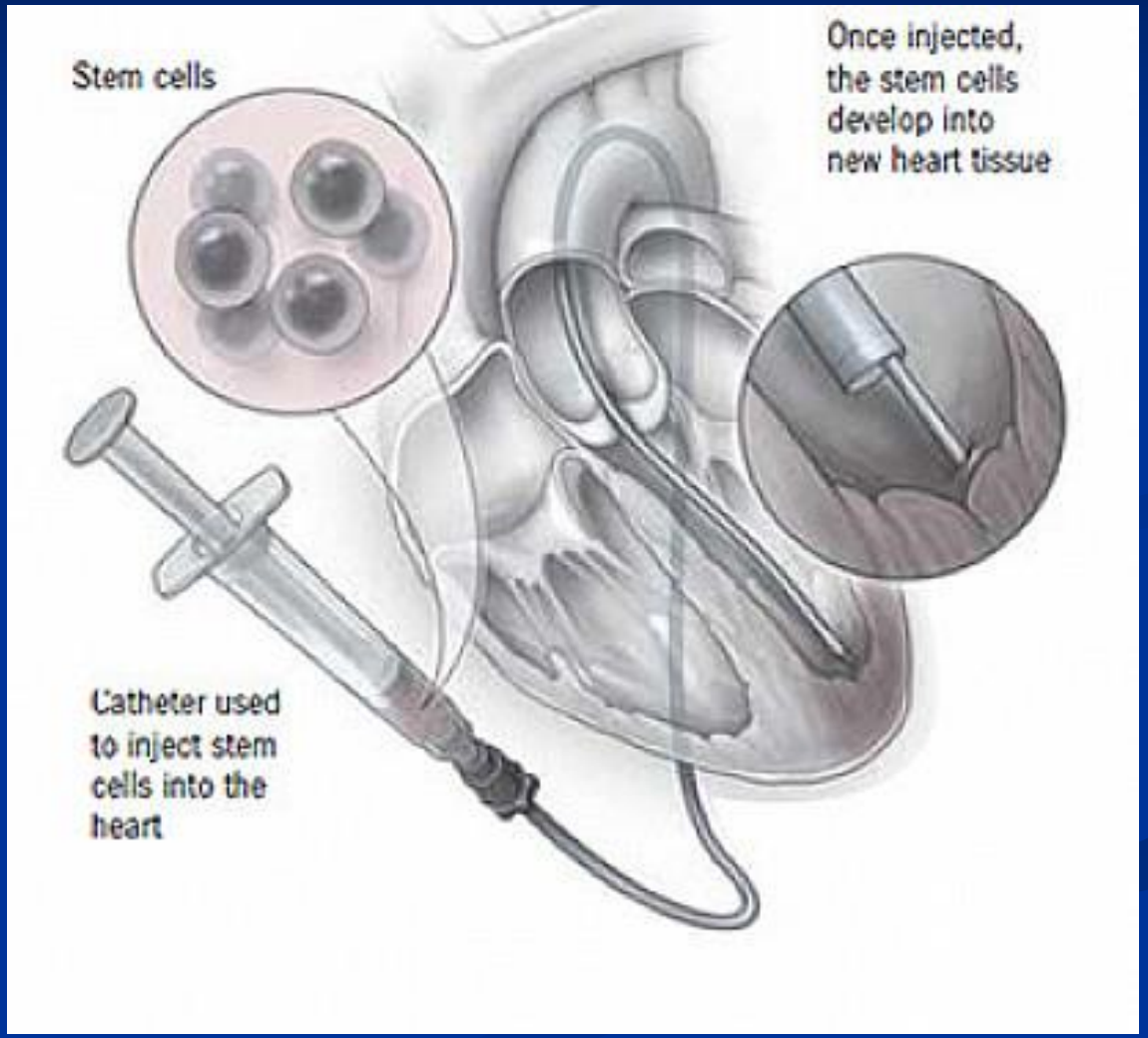
Bone marrow transplant:



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

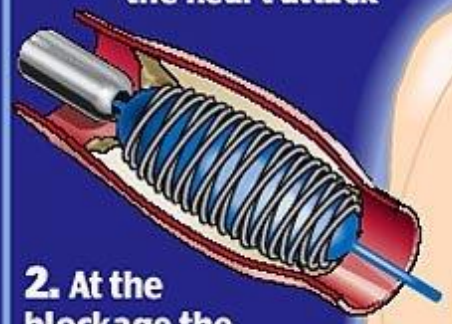
Stem cell therapy for the heart

Several different types of approaches are being used to repair damaged heart muscle with stem cells. **The stem cells, which are often taken from bone marrow, may be inserted into the heart using a catheter**. Once in place, stem cells help regenerate damaged heart tissue.

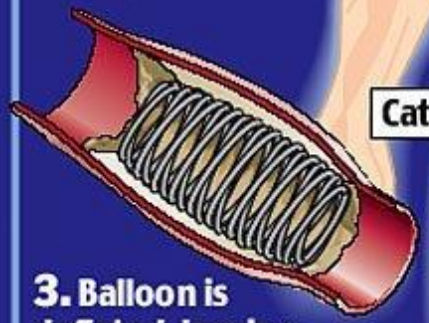


HOW IT WORKS

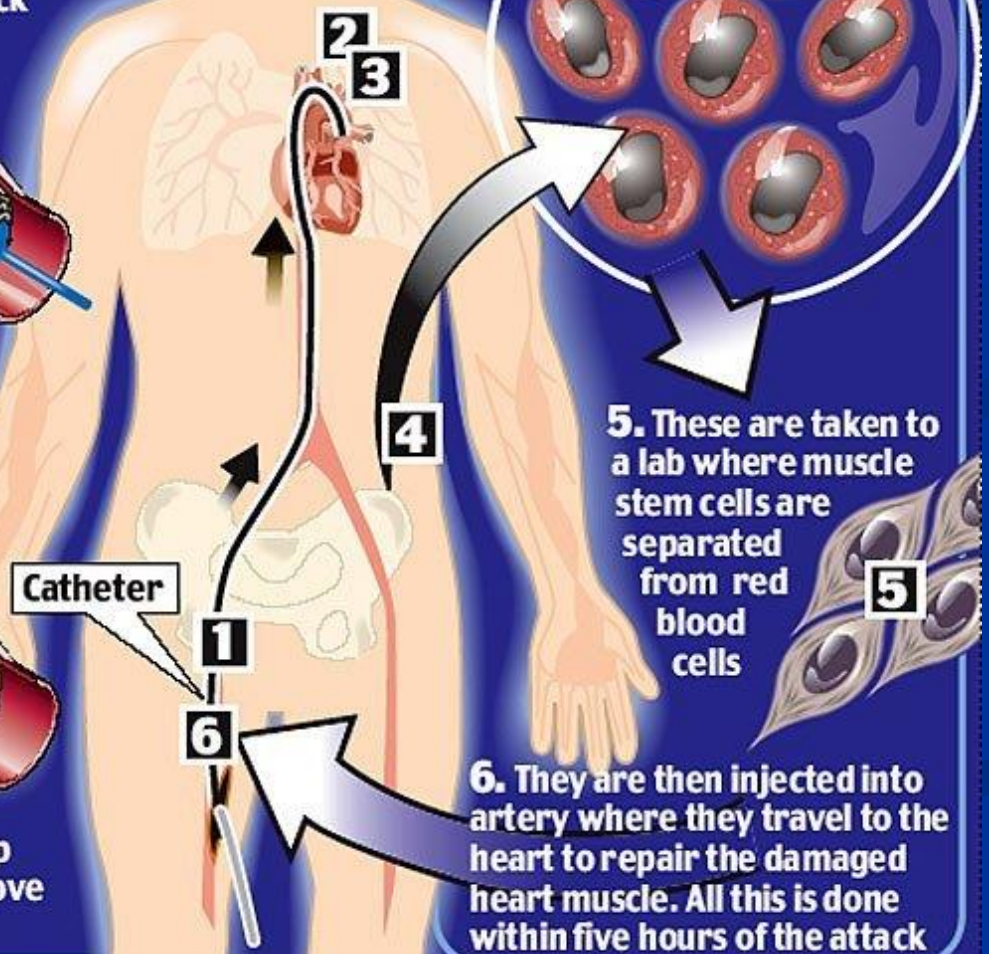
Suspected heart attack patient is referred to cardiology department to have angioplasty **1.** This is when a balloon and stent in a catheter are fed through arteries to the blockage that caused the heart attack



2. At the blockage the balloon is inflated, opening up the stent



3. Balloon is deflated, leaving stent in place to keep artery open to improve flow of blood



4. Cells are removed from bone marrow in the patient's hip under local anaesthetic

5. These are taken to a lab where muscle stem cells are separated from red blood cells

6. They are then injected into artery where they travel to the heart to repair the damaged heart muscle. All this is done within five hours of the attack

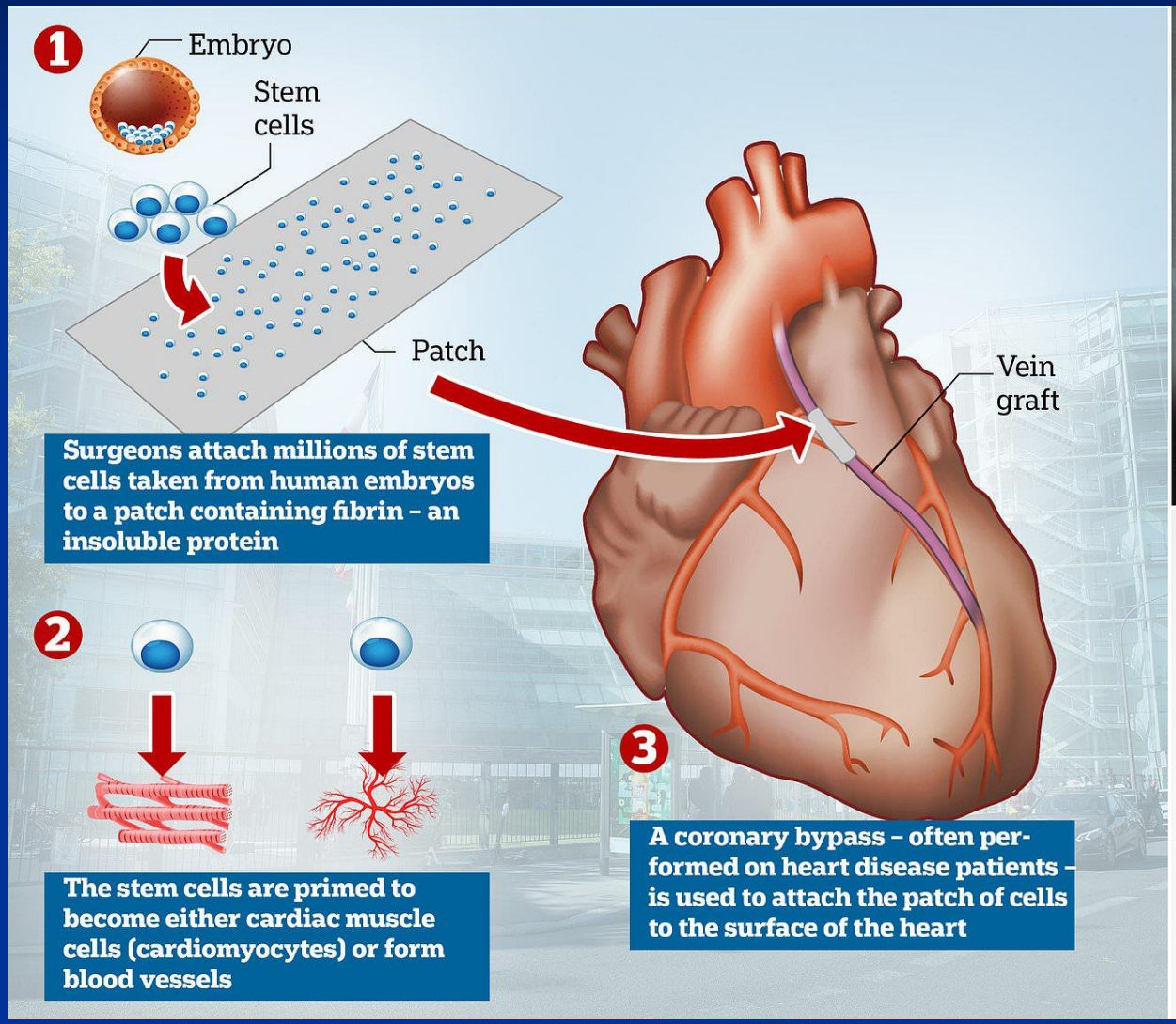
Experimental model system

Heart muscle cells beating in a petri dish!

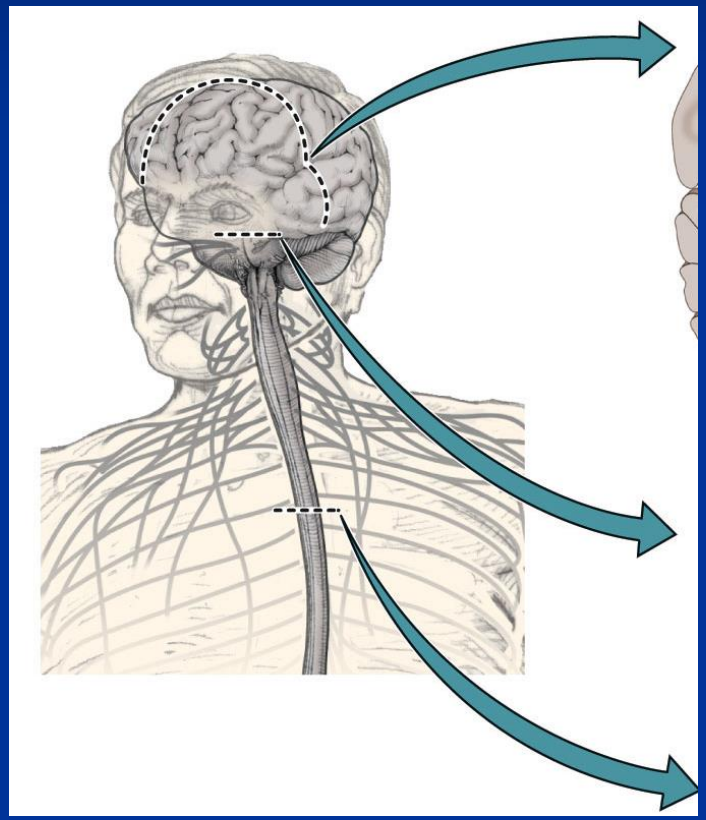
from mouse embryonic stem cells

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

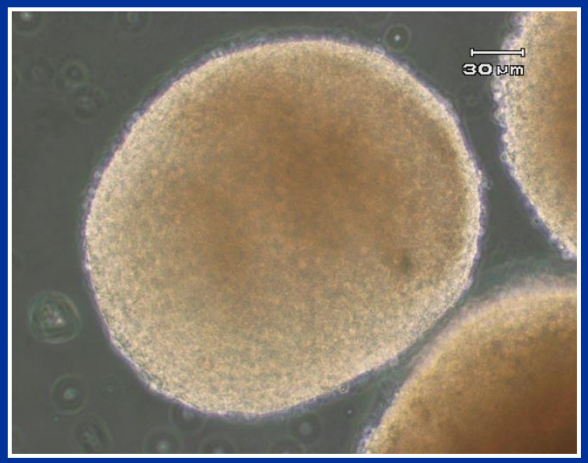
- Brain and spinal cord injury and stroke (loss of nerve cells and myelin-forming oligodendrocytes).

- 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).

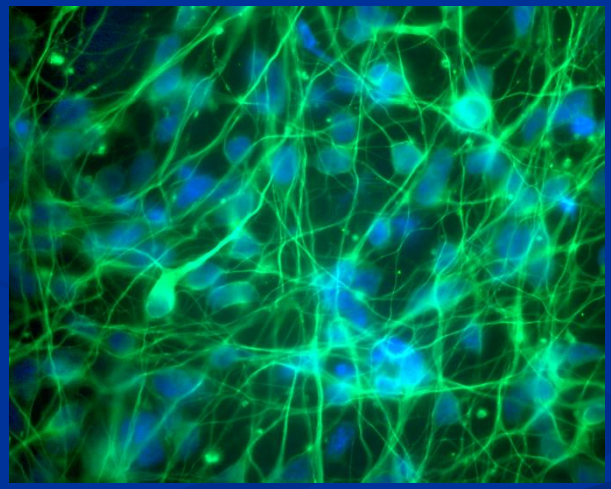
- The vision: To use stem cells to restore the cells that are lost as a result of injury or neurodegenerative diseases.

Make stem cells into nerve cells

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



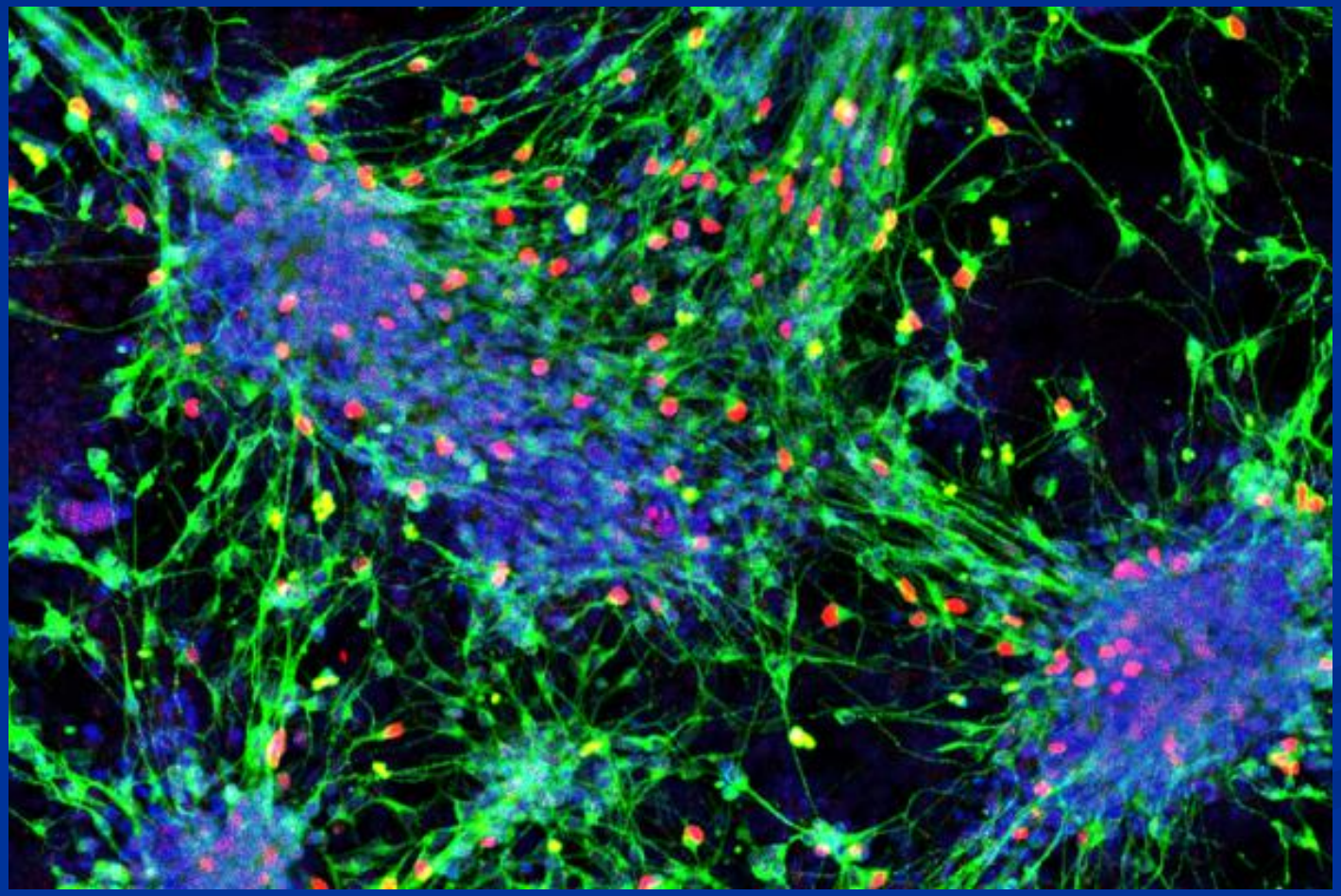
Stem cells differentiated into neurons



In vitro atlas of dorsal spinal interneurons reveals Wnt signaling as a critical regulator of progenitor expansion

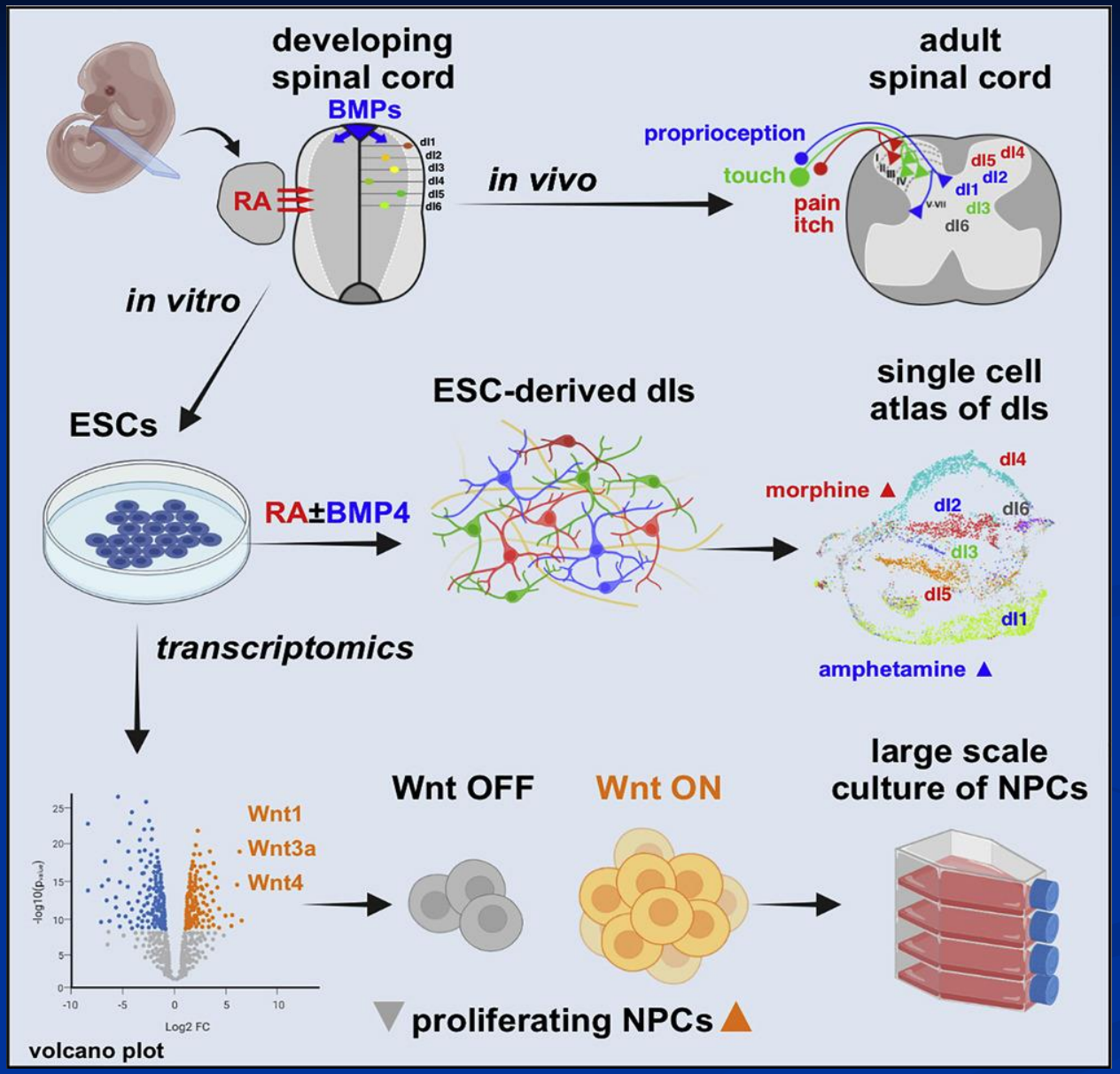
Sandeep Gupta, Riki Kawaguchi, Eric Heinrichs, Salena Gallardo, Stephanie Castellanos, Igor Mandric, Bennett G. Novitch and Samantha J. Butler

Tuesday, July 19, 2022
Published in Cell Reports



Scientists develop blueprint for turning stem cells into sensory interneurons

The findings represent an important step toward cell therapies to restore sensation in people with spinal cord injuries



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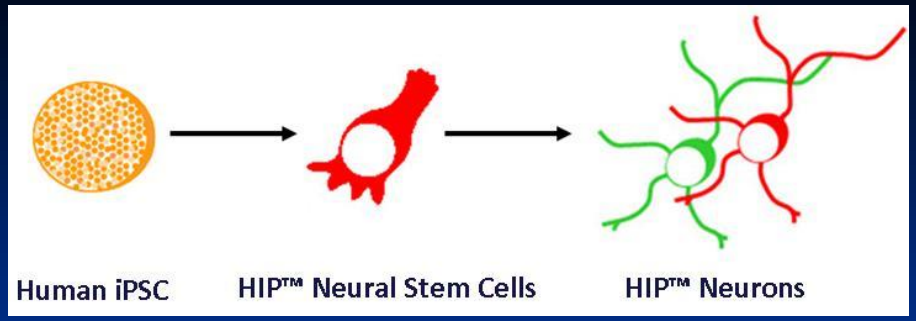
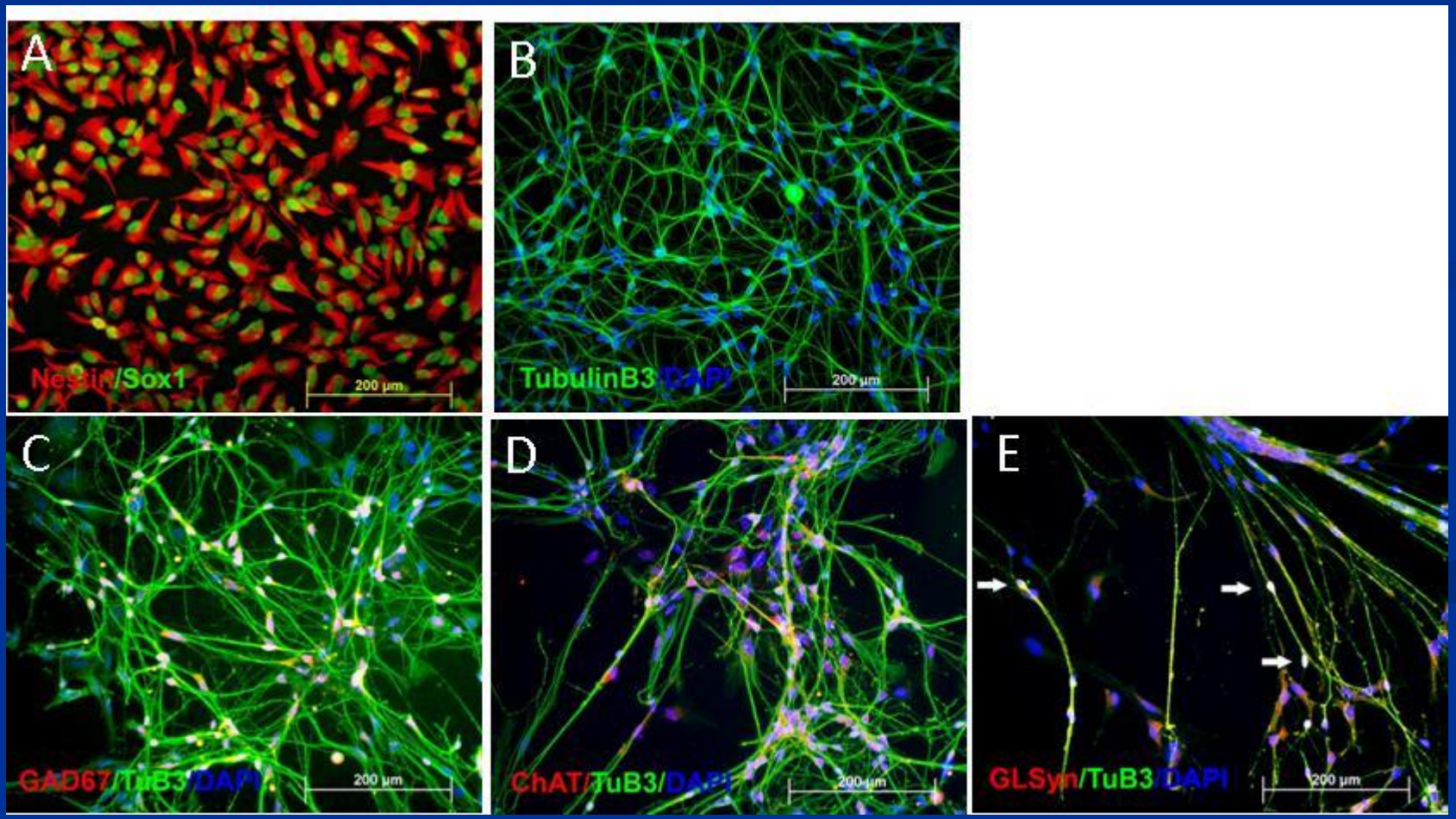


MTI-GlobalStem

Human iPSC-Derived Neural Cells



Christopher Reeve

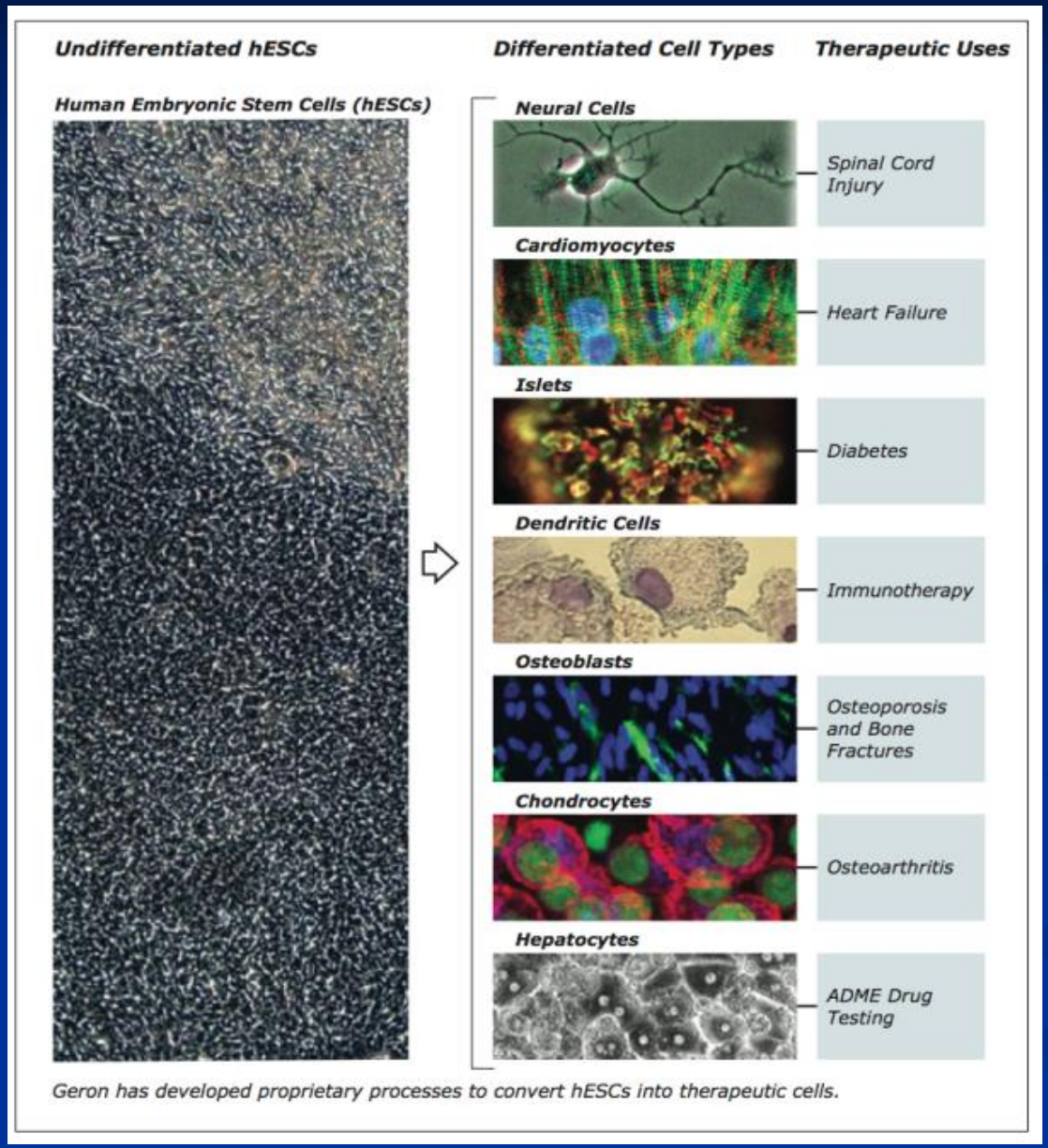
1952-2004

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

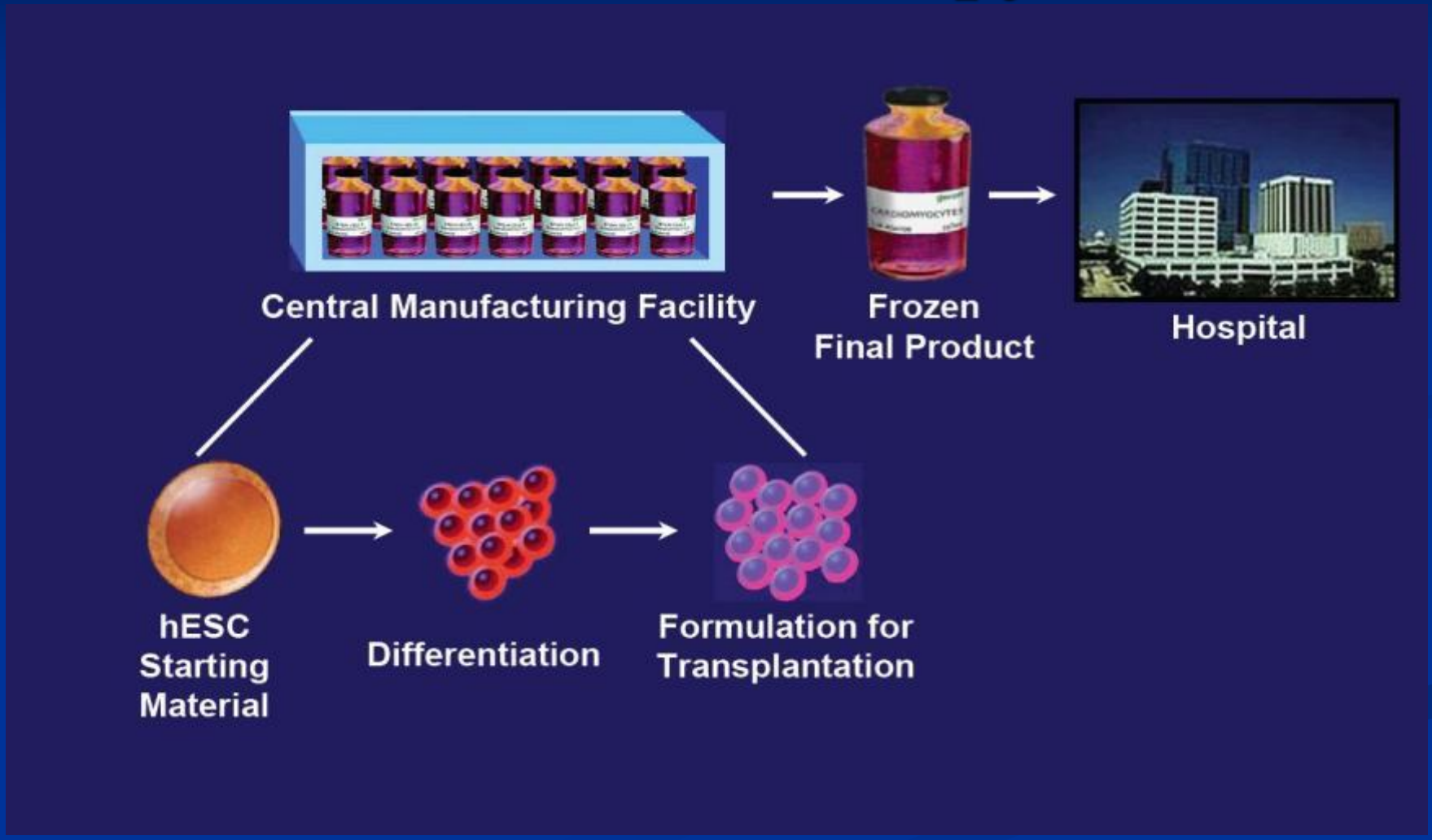
Geron Stem Cell Therapy

•Tom Okarma - Geron

Human Embryonic Stem Cells

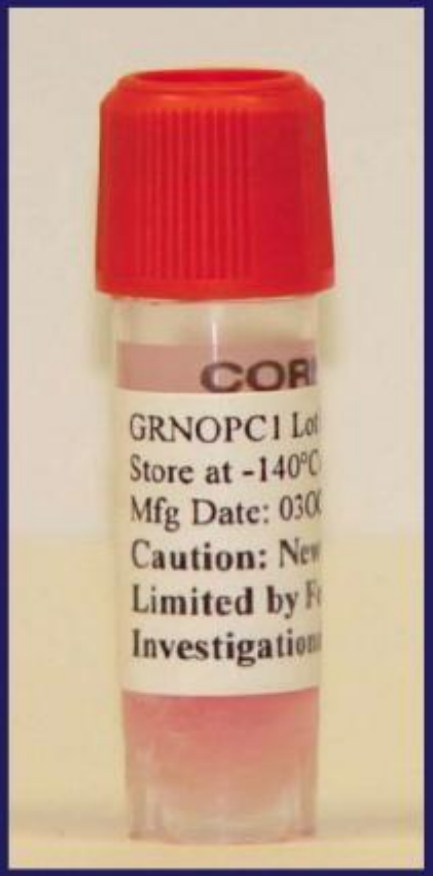


Human Embryonic Stem Cell (hESC) Based Therapy



•Tom Okarma - Geron

Geron Oligodendrocyte Progenitor Cells GRNOPC1



GRNOPC1

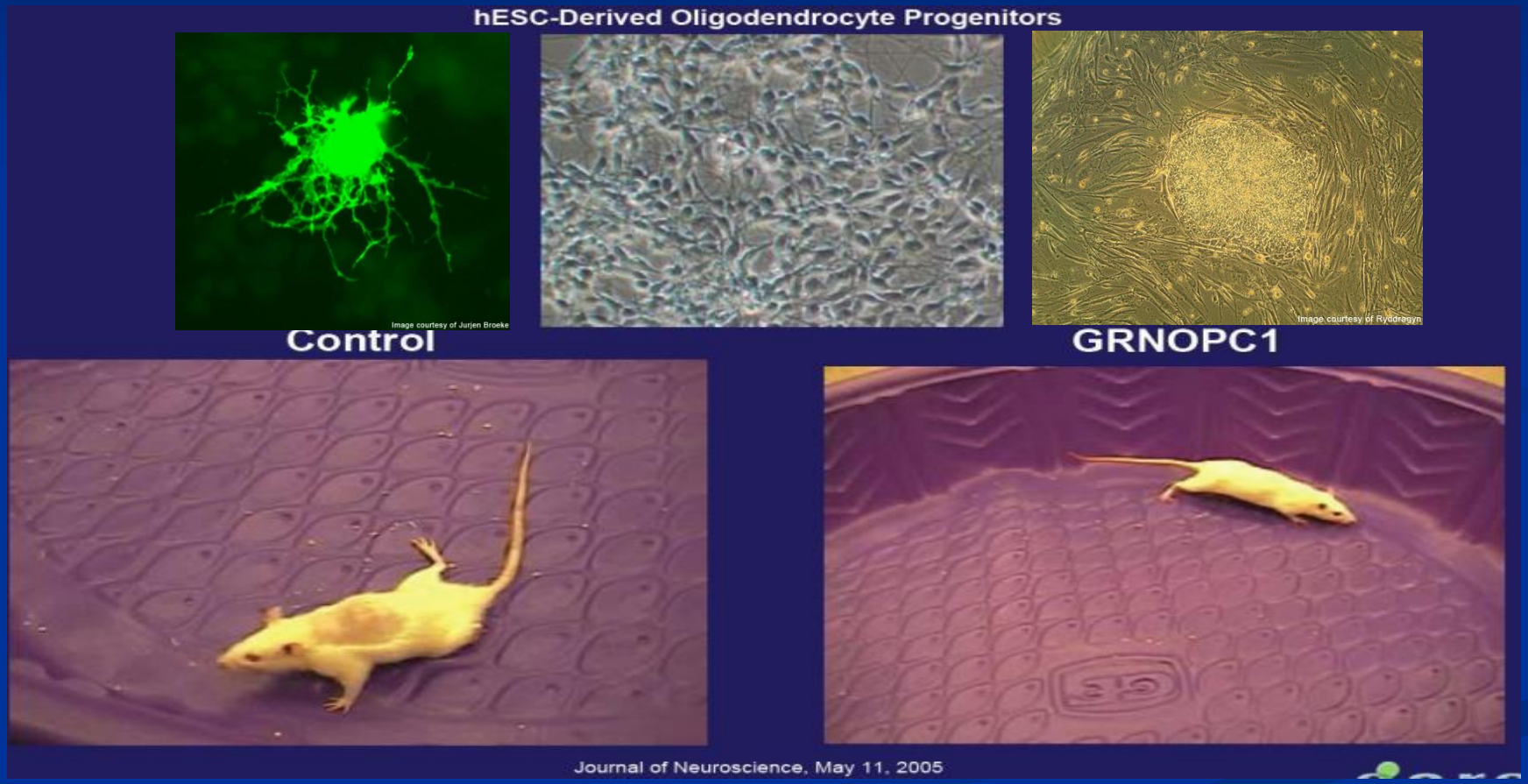
- Cryopreserved Allogeneic Cell Population
- Derived from Human Embryonic Stem Cells
- Characterized Composition of Cells
- Contain Oligodendrocyte Progenitor Cells
- Produces Neurotrophic Factors
- Induces Myelination of Denuded Axons

Intended Application

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

•Tom Okarma - Geron

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

Properties of GRNOP1

24 Studies

1977 Rodents

858 Injected with GRNOPC1

5 x 10^9 OPC1 Tested in Studies

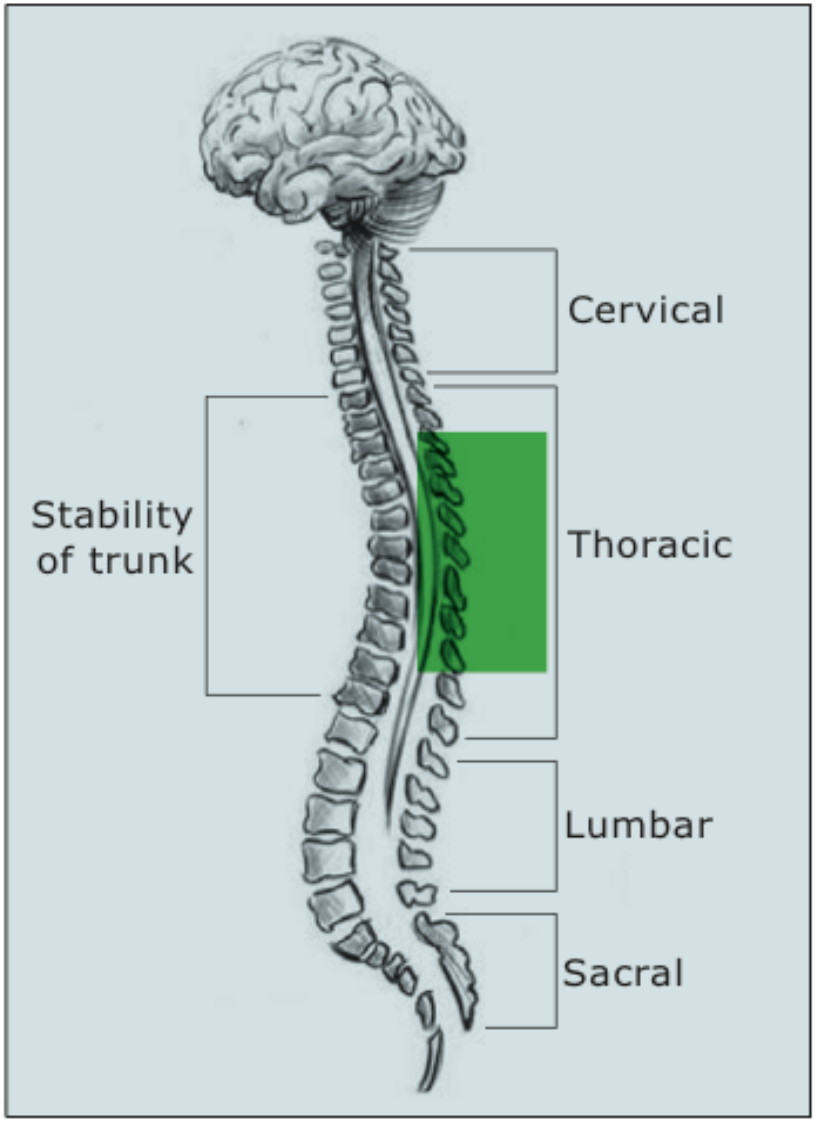
GRNOPC1

- 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

•Tom Okarma - Geron

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

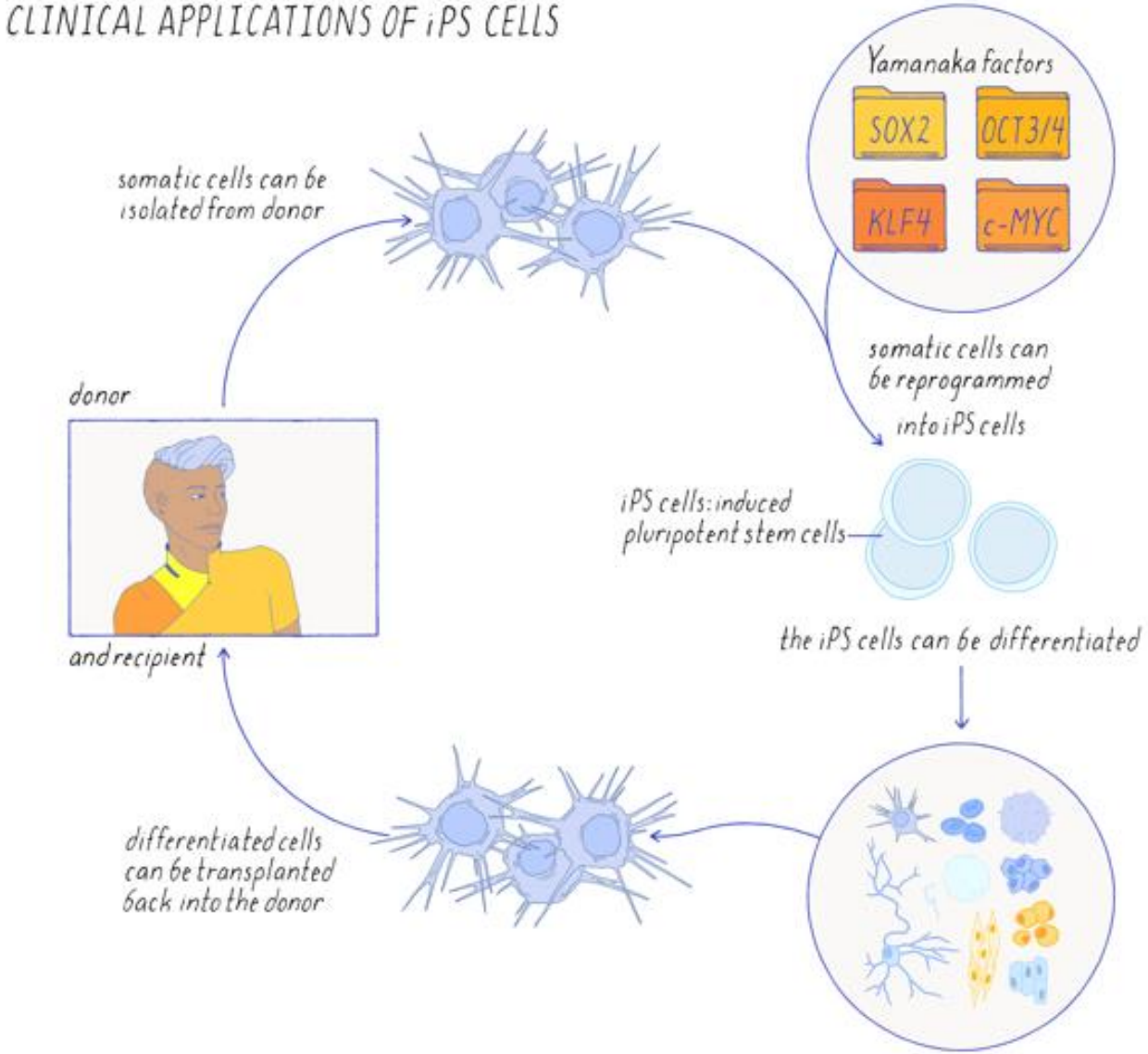
Trial Sponsor (Location)	Disease Target	Cell Therapy	No. Patients	Phase
City of Hope (CA, USA)	recurrent high grade gliomas	*E. Coli* CD-expressing neural stem cells	24	phase 1
	recurrent high grade gliomas	carboxylesterase-expressing neural stem cells	53	phase I
Neuralstem Inc. (MD, USA)	ALS	fetal-derived 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

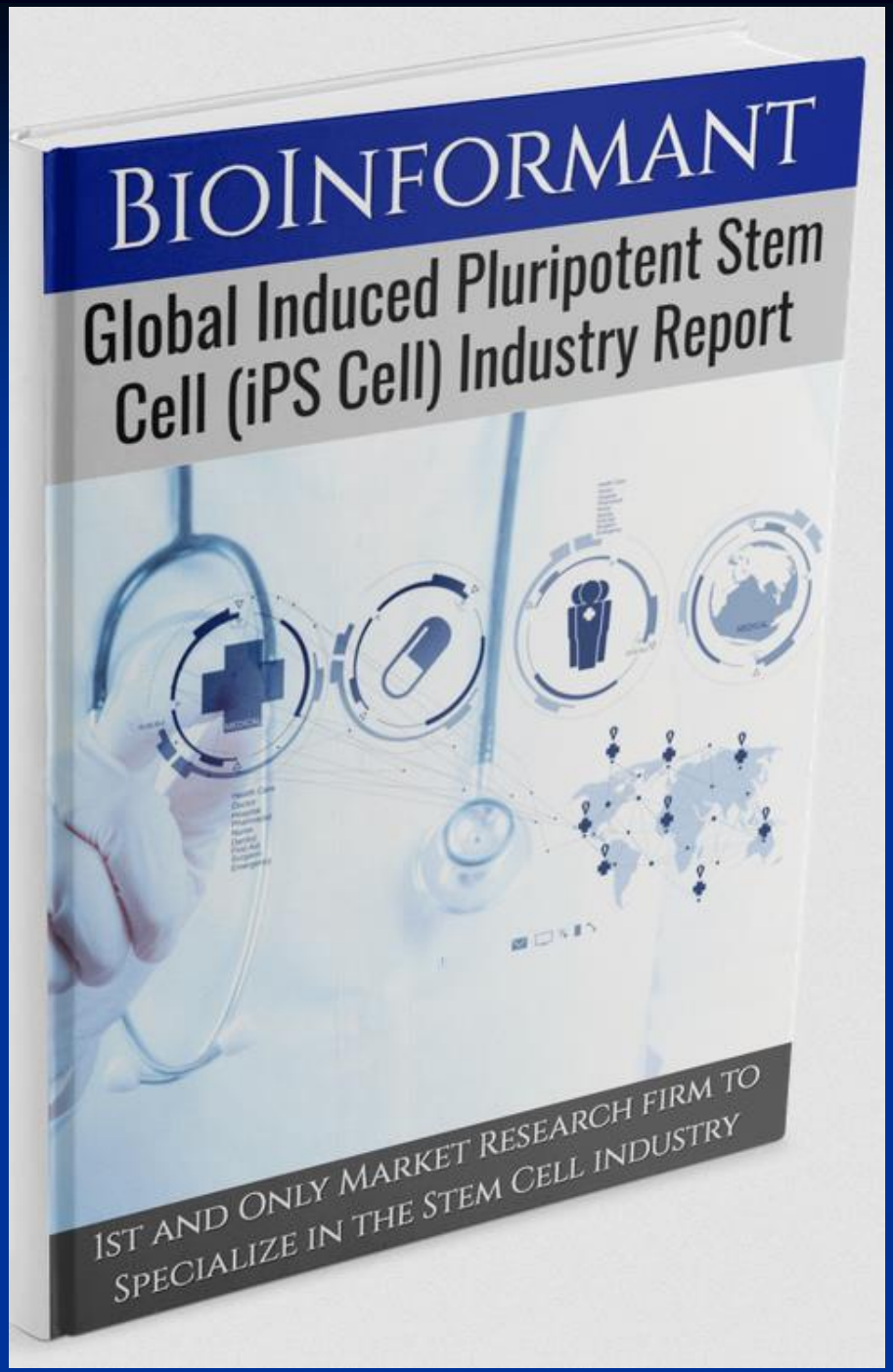
Amyotrophic lateral sclerosis

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


CLINICAL APPLICATIONS OF iPS CELLS
Yamanaka factors
SOX2
OCT3/4
KLF4
c-MYC
somatic cells can be isolated from donor
somatic cells can be reprogrammed into iPS cells
donor
and recipient
iPS cells: induced pluripotent stem cells
the iPS cells can be differentiated
differentiated cells can be transplanted back into the donor



Global Induced Pluripotent Stem Cell (iPSC) Industry Report, 2022

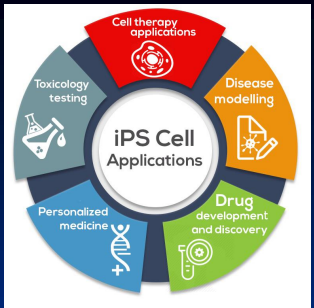
Global Induced Pluripotent Stem Cell (iPSC) Industry Report, 2022

iPS Cell Commercialization

Today, methods of commercializing iPSCs include:

- **Cell Therapy:** iPSCs are being explored in a diverse range of cell therapy applications for the purpose of reversing injury or disease.
- **Disease Modelling:** By generating iPSCs from patients with disorders of interest and differentiating them into disease-specific cells, iPSCs can effectively create disease models "in a dish."
- **Drug Development and Discovery:** iPSCs have the potential to transform drug discovery by providing physiologically relevant cells for compound identification, target validation, compound screening, and tool discovery.
- **Personalized Medicine:** The use of techniques such as CRISPR enable precise, directed creation of knock-outs and knock-ins (including single base changes) in many cell types. Pairing iPSCs with genome editing technologies is adding a new dimension to personalized medicine.
- **Toxicology Testing:** iPSCs can be used for toxicology screening, which is the use of stem cells or their derivatives (tissue-specific cells) to assess the safety of compounds or drugs within living cells.

Other applications of iPSCs include their use as research products, as well as their integration into 3D bioprinting, tissue engineering, and clean meat production. Technology allowing for the mass-production and differentiation of iPSCs in industrial-scale bioreactors is also advancing at breakneck speed.



Global Induced Pluripotent Stem Cell (iPSC) Industry Report, 2022

2013 was a landmark year because it saw the first cellular therapy involving the transplant of iPSCs into humans initiated at the RIKEN Center in Kobe, Japan. Led by Dr. Masayo Takahashi, it investigated the safety of iPSC-derived cell sheets in patients with macular degeneration. Vision Loss

Graft versus Host Disease

In another world first, Cynata Therapeutics received approval in 2016 to launch the first formal clinical trial of an allogeneic iPSC-derived cell product (CYP-001) for the treatment of GvHD. CYP-001 is a iPSC-derived MSC product. In this historic trial, CYP-001 met its clinical endpoints and produced positive safety and efficacy data for the treatment of steroid-resistant acute GvHD.

mesenchymoangioblast

Given this early success, Cynata is advancing its iPSC-derived MSCs into Phase 2 trials for the severe complications associated with COVID-19, as well as GvHD and critical limb ischemia (CLI). It is also undertaking an impressive Phase 3 trial that will utilize Cynata's iPSC-derived MSC product, CYP-004, in 440 patients with osteoarthritis (OA). This trial represents the world's first Phase 3 clinical trial involving an iPSC-derived cell therapeutic product and the largest one ever completed.

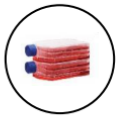
About Cynata Therapeutics (ASX: CYP)

Cynata Therapeutics Limited (ASX: CYP) is an Australian clinical-stage stem cell and regenerative medicine company focused on the development of therapies based on Cymerus™, a proprietary therapeutic stem cell platform technology.

Cymerus™ overcomes the challenges of other production methods by using induced pluripotent stem cells (iPSCs) and a precursor cell known as mesenchymoangioblast (MCA) to achieve economic manufacture of cell therapy products, including mesenchymal stem cells (MSCs), at commercial scale without the limitation of multiple donors.

cynata therapeutics

The current conventional manufacturing process is ***sub optimal***

Cells donated	Conventional process		MSCs
Cells from multiple donors	MSC isolation	MSC expansion	Cellular therapies administered
			
Substantial variability in starting material	Limited quantity of MSCs produced per donation	Large number of MSCs required	

Cynata's Cymerus iPSC-derived process ***optimises manufacturing for scalability***

Cells donated	Cynata's patented process: Cymerus			MSCs
✓ ***Completed***				
Cells from **one** donor + iPSC Derivation	iPSC Expansion	Precursor cells	Generation of MCA Colonies + Differentiation into MSCs	Cellular therapies administered
			 	
Avoids inter-donor variability	Effectively limitless expansion	Minimal MSC culture expansion		
CONSISTENCY	***QUANTITY***	***QUALITY***		***EFFICIENCY***

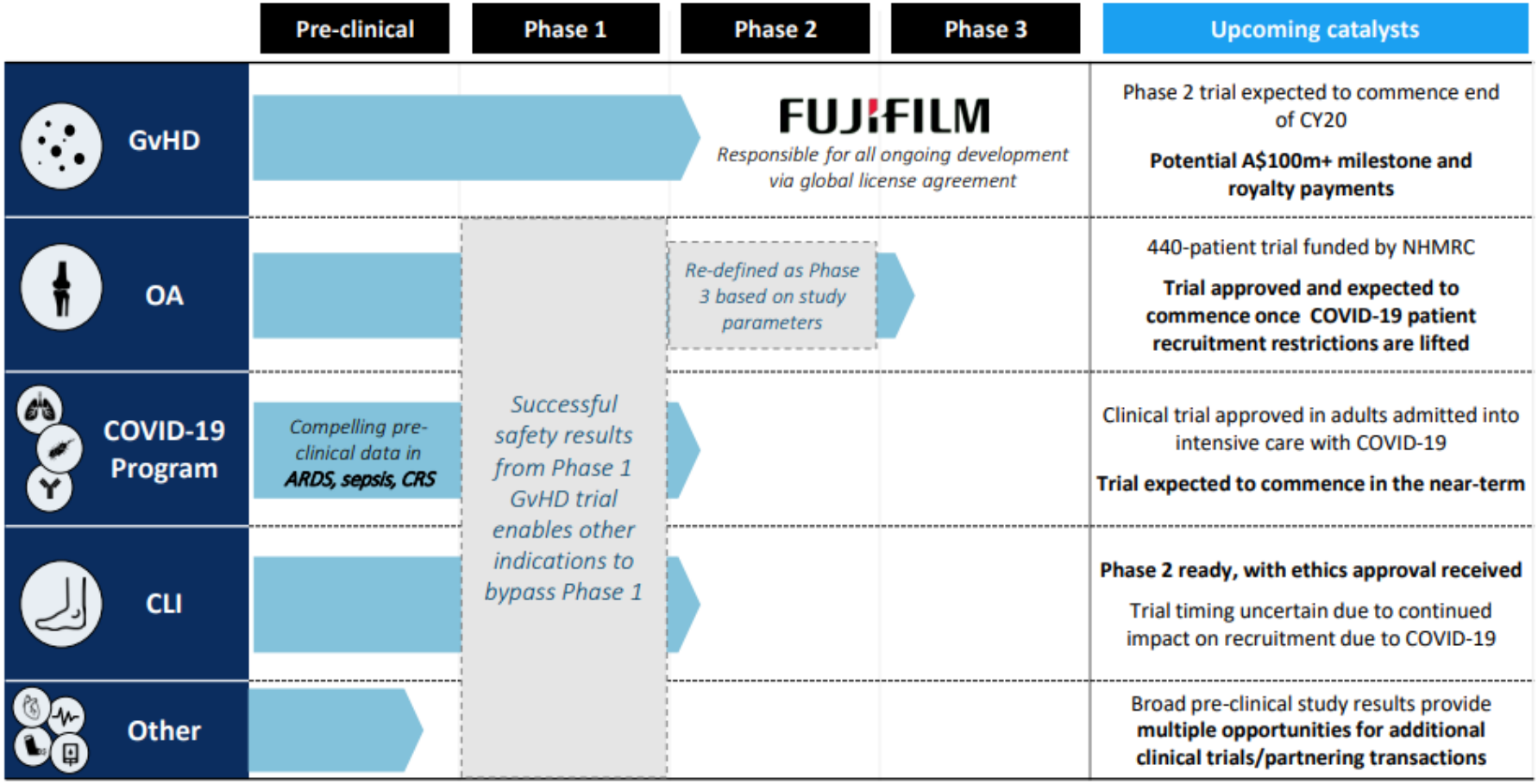
cynata therapeutics

Summary

- Remestemcel-L comprises ex-vivo cultured adult human mesenchymal stromal cells (ce-MSCs) isolated from bone marrow of healthy adult donors and formulated in Plasma-Lyte A, Human Serum Albumin (HSA) Solution and Dimethyl sulfoxide (DMSO).
- Remestemcel-L is a frozen cell suspension stored in a cryogenic vial.
- The manufacturing process for remestemcel-L involves:
  - Donor screening process
  - Capturing cells from the bone marrow of a healthy donor, which are expanded in a pharmaceutical-grade manufacturing facility
  - Three stage process of 1) generation of a donor cell bank, 2) finalization of drug substance and the finished product and 3) secondary packaging and distribution to the customer
- One donation of bone marrow can manufacture enough Drug Product (DP) to treat more than 500 patients.
- Remestemcel-L stored at ≤ -135°C in liquid nitrogen vapor phase has a 48-month shelf-life.
- The product is thawed and resuspended in Plasma-Lyte A prior to intravenous administration.
- Remestemcel-L is an off-the-shelf product available when needed to treat patients.

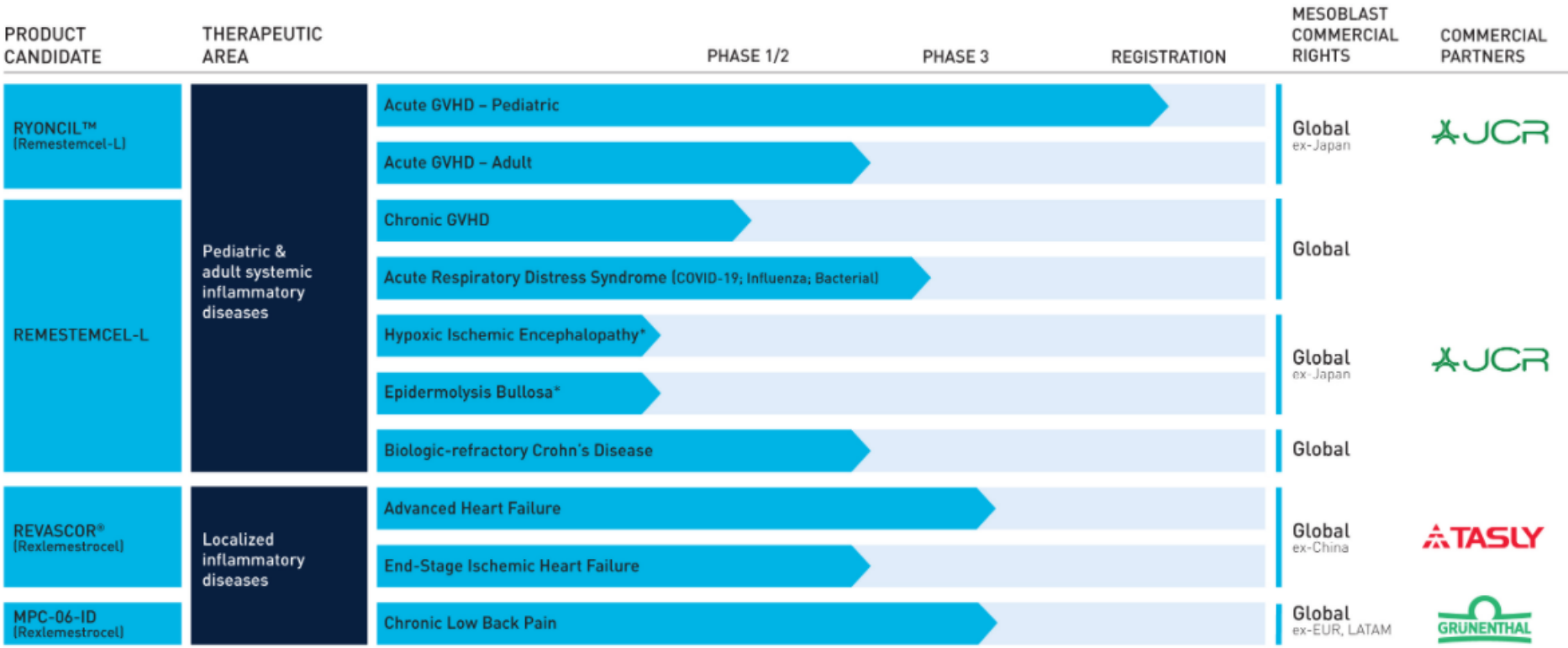
Broad, advanced development pipeline with multipl

cynata therapeutics



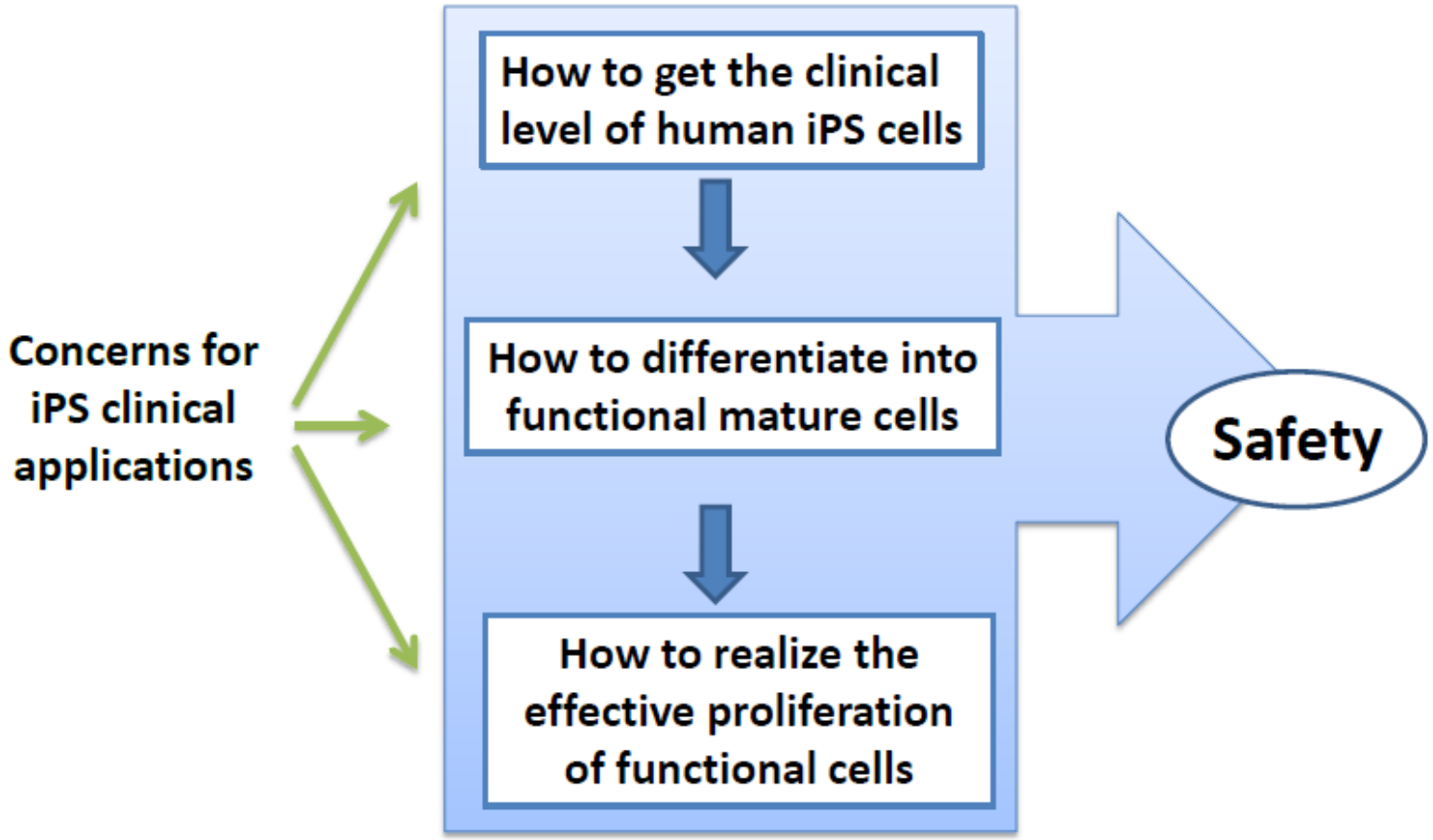
cynata therapeutics

Phase 3 Product Candidates

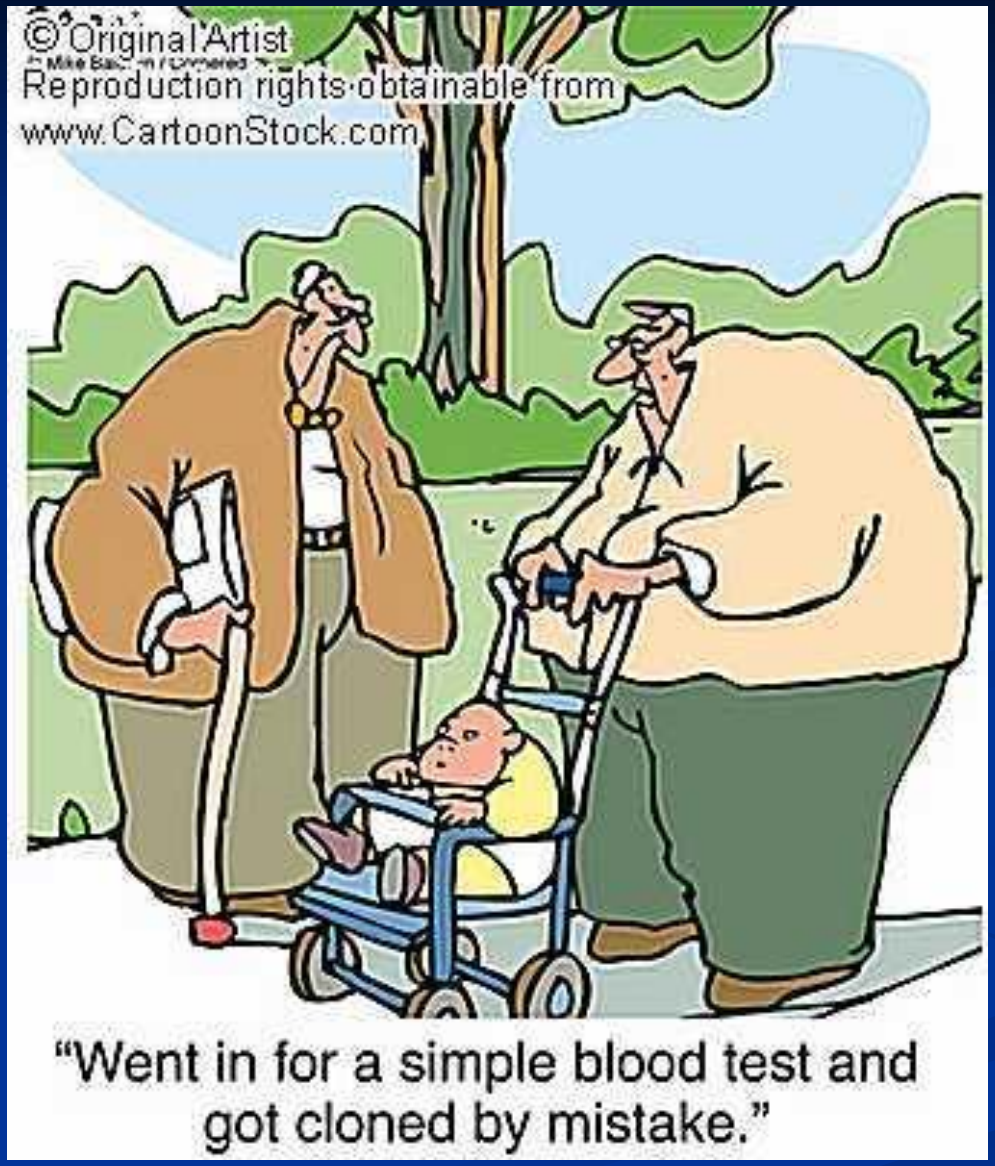


*Mesoblast has the right to use data generated by JCR Pharmaceuticals Co Ltd in Japan to support its development and commercialization plans for remestemcel-L in the US and other major healthcare markets, including for GVHD, HIE and EB.
This chart is figurative and does not purport to show individual trial progress within a clinical program.

The Concerns Regarding the Clinical Applications of iPSCs



Transgenes increase the risk of dangerous mutations or cancer, gene genetic maniplation limited the clinical application.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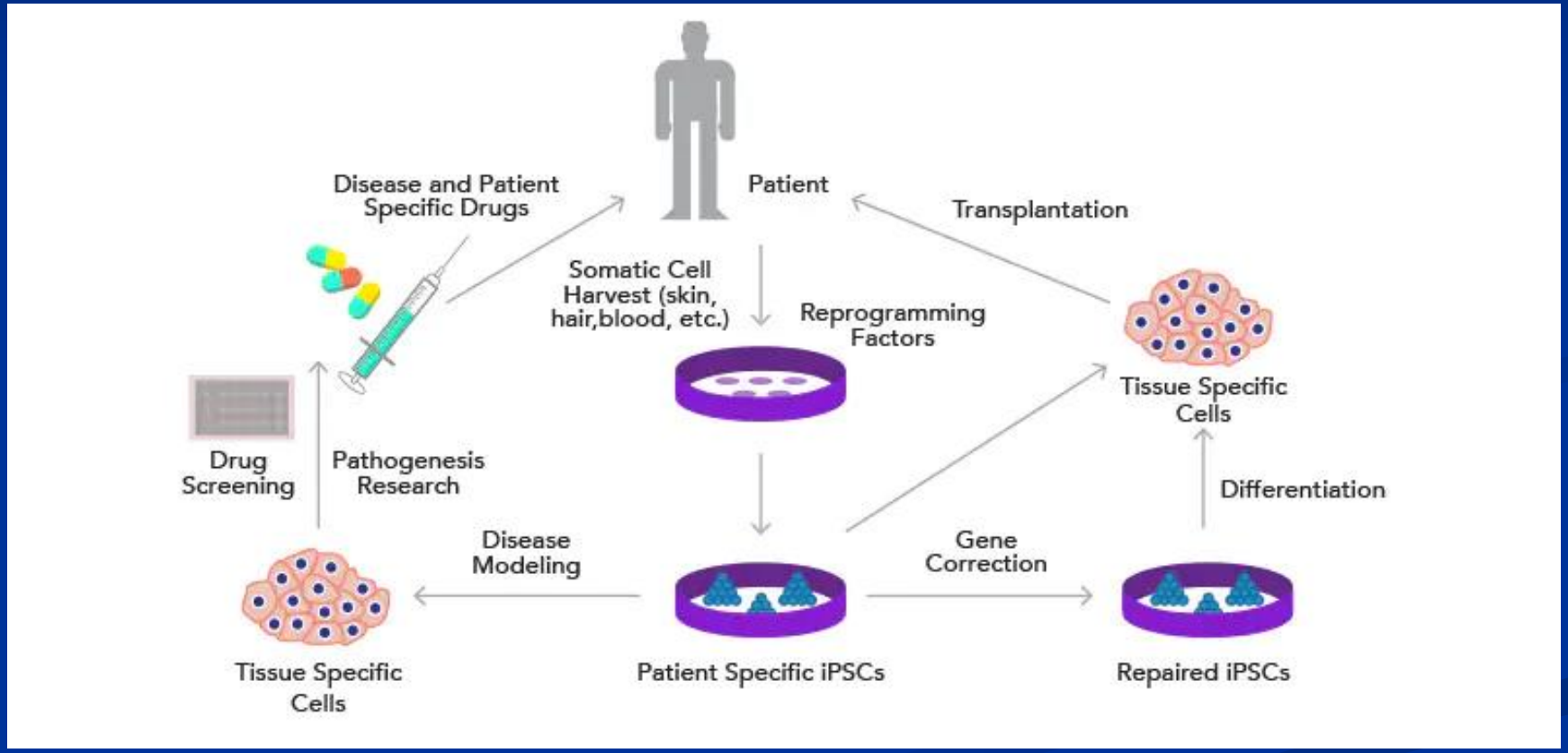
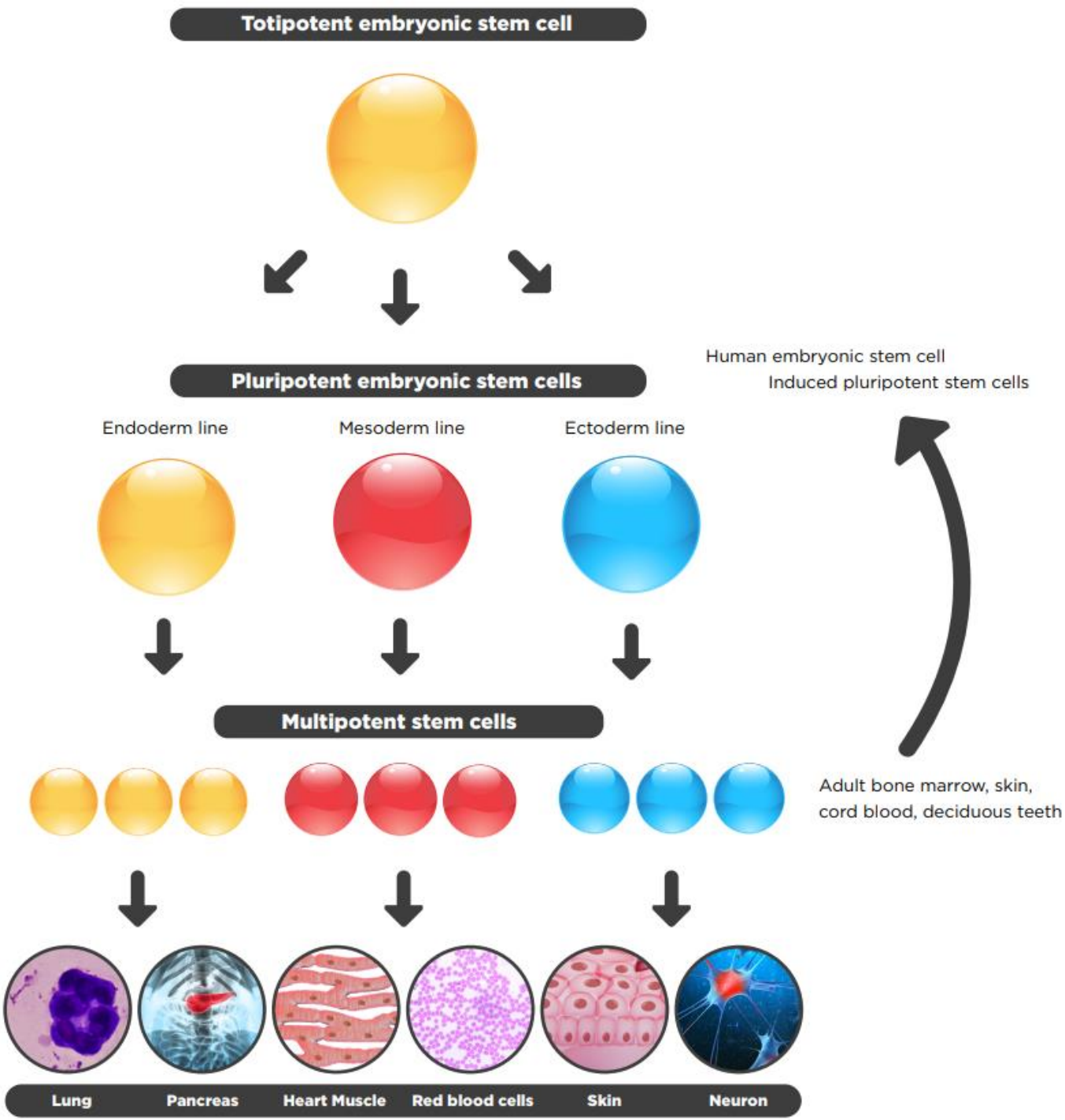
"Went in for a simple blood test and got cloned by mistake."


Patient
Disease and Patient Specific Drugs
Transplantation
Somatic Cell Harvest (skin, hair,blood, etc.)
Reprogramming Factors
Tissue Specific Cells
Drug Screening
Pathogenesis Research
Differentiation
Disease Modeling
Gene Correction
Tissue Specific Cells
Patient Specific iPSCs
Repaired iPSCs

Cynata's lead product candidate CYP-001 met all clinical endpoints and demonstrated positive safety and efficacy data for the treatment of steroid-resistant acute graft-versus-host disease (GvHD) in a Phase 1 trial. Planning for a Phase 2 clinical trial in GvHD is presently underway. Clinical trials of Cymerus products in osteoarthritis (Phase 3), respiratory failure and diabetic foot ulcers (DFU) are currently ongoing. In addition, Cynata has demonstrated utility of its Cymerus technology in preclinical models of numerous diseases, including the clinical targets mentioned above, as well as critical limb ischaemia, idiopathic pulmonary fibrosis, asthma, heart attack, sepsis, acute respiratory distress syndrome (ARDS) and cytokine release syndrome.



Pluripotent cells can give rise to all of the cell types that make up the body;

Embryonic stem cells are considered pluripotent.

Multipotent cells can develop into more than one cell type, but are more limited than pluripotent cells