

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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by Salman "al-Farsi" Dhia-alDeen, 1999

# Introduction to Pluripotent Stem Cells

**Dr. Mona Elsafadi**



# Introducing...stem cells!

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Parkinson's disease

**IVF**

SCNT

Human eggs

Drug research

Cure

Grow

iPS cells

Stem cells

**Research**

Ethical

**Embryo**

Pluripotent

Leukaemia

Backlash

**treatment**

Cloning

**Hope**

Controversy

**Cord blood**

**ART**

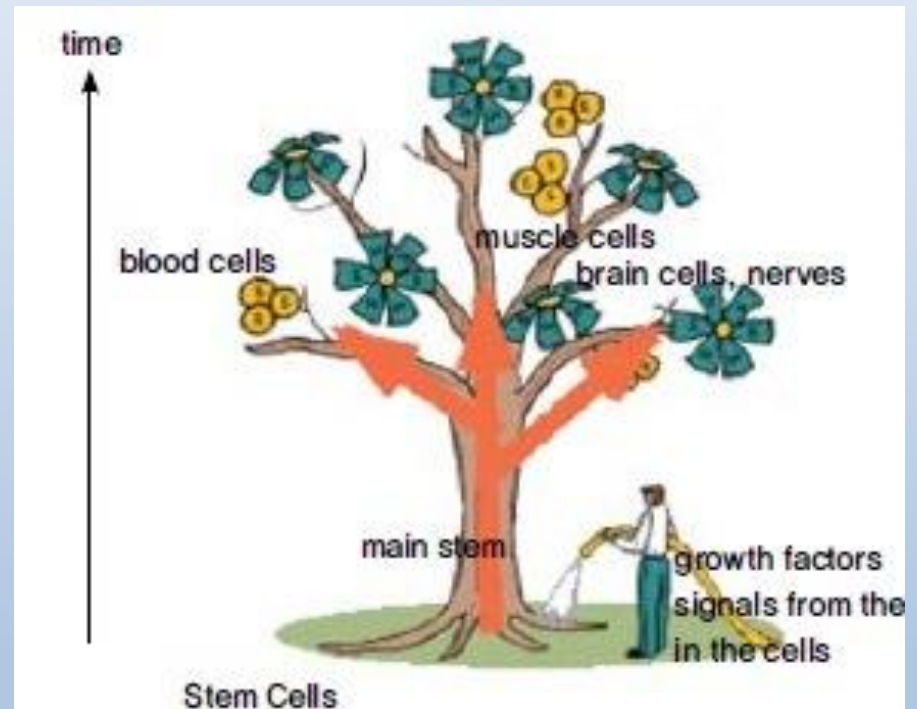
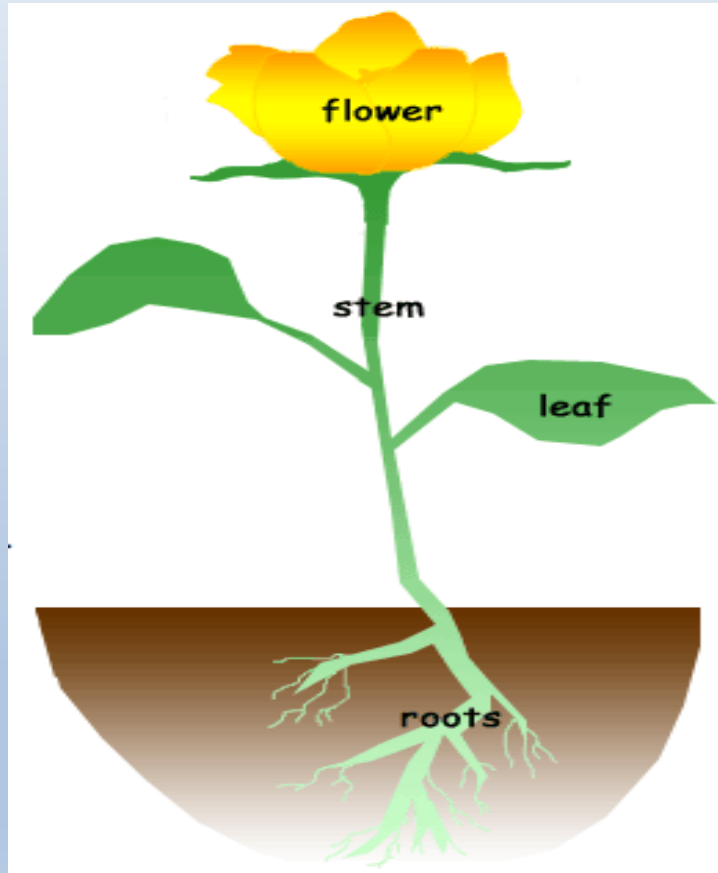
Debate

Therapy

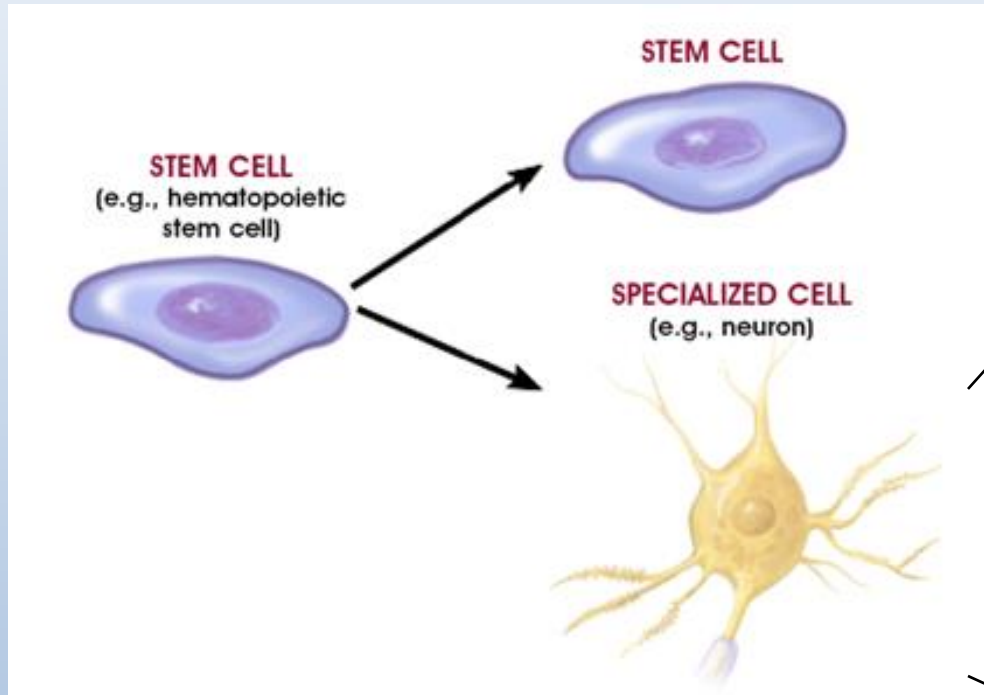
BREAKTHROUGH!

**cure** 

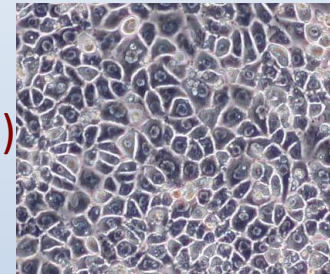
# Stem Cells



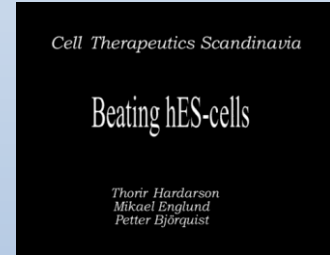
# Unique Characteristics of Stem Cells



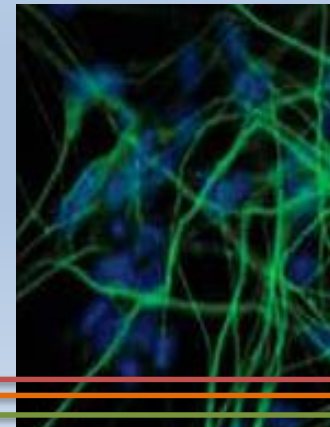
Endoderm  
(hepatocytes)



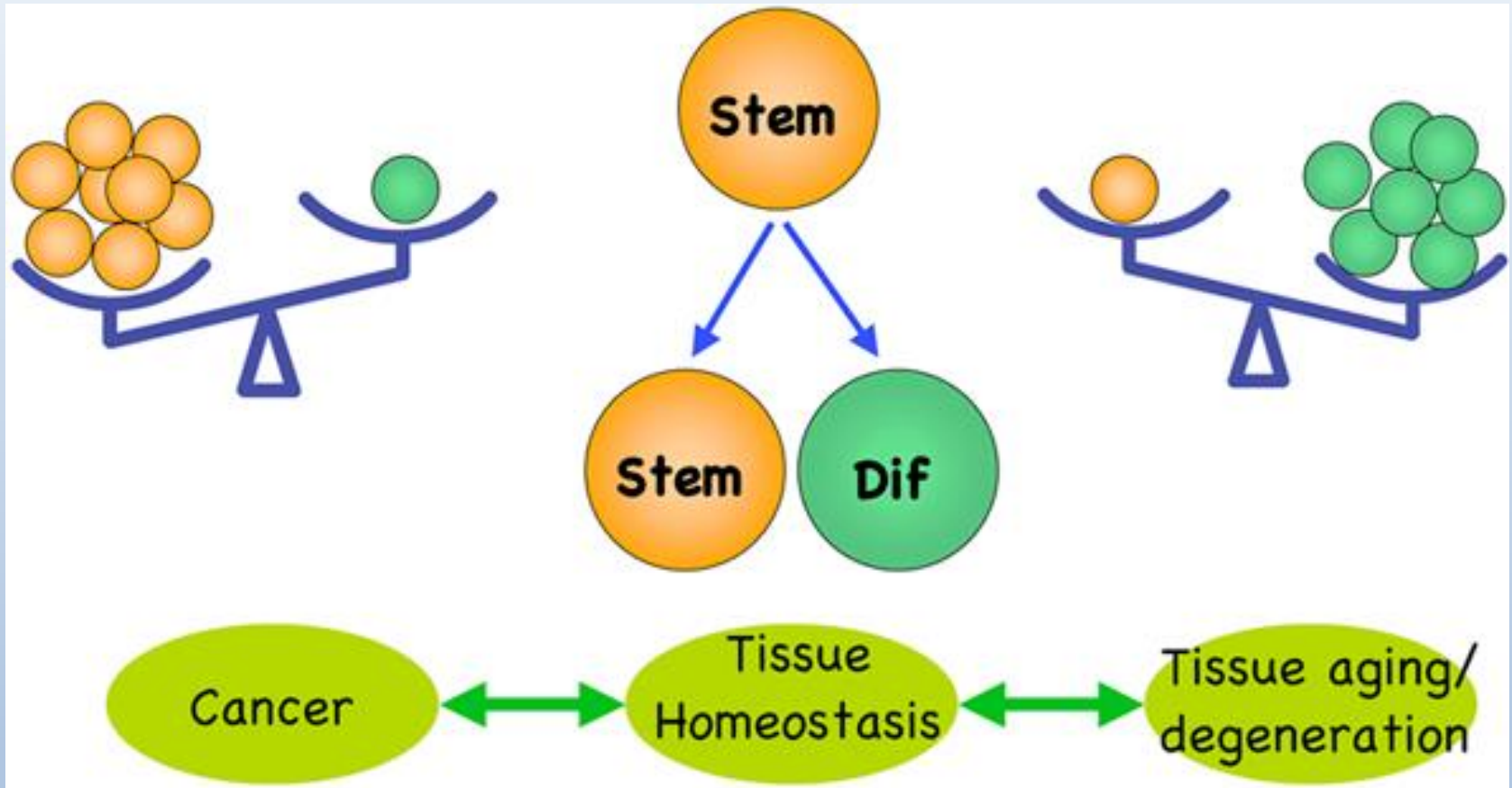
Mesoderm  
(cardiac  
myotubes)



Ectoderm  
(Neurons)



- Unlimited self renewal (Regeneration)
- Differentiation (eg. beating cells of the heart muscles):
  - Internal signals (specific genes)
  - External signals (GF, cytokines)



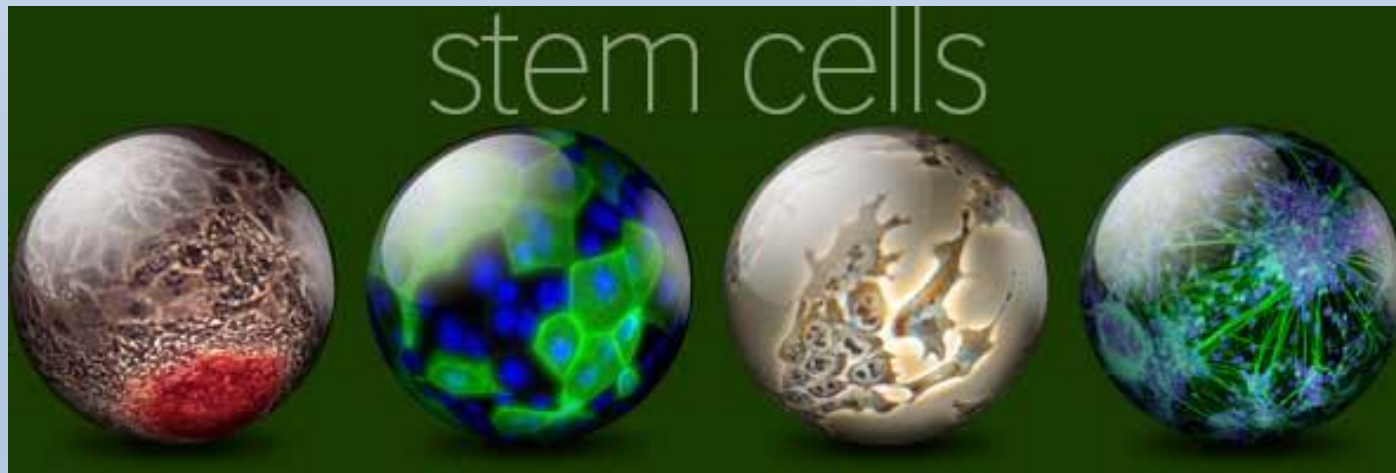
# What are Stem Cells?

A cell that has the ability:

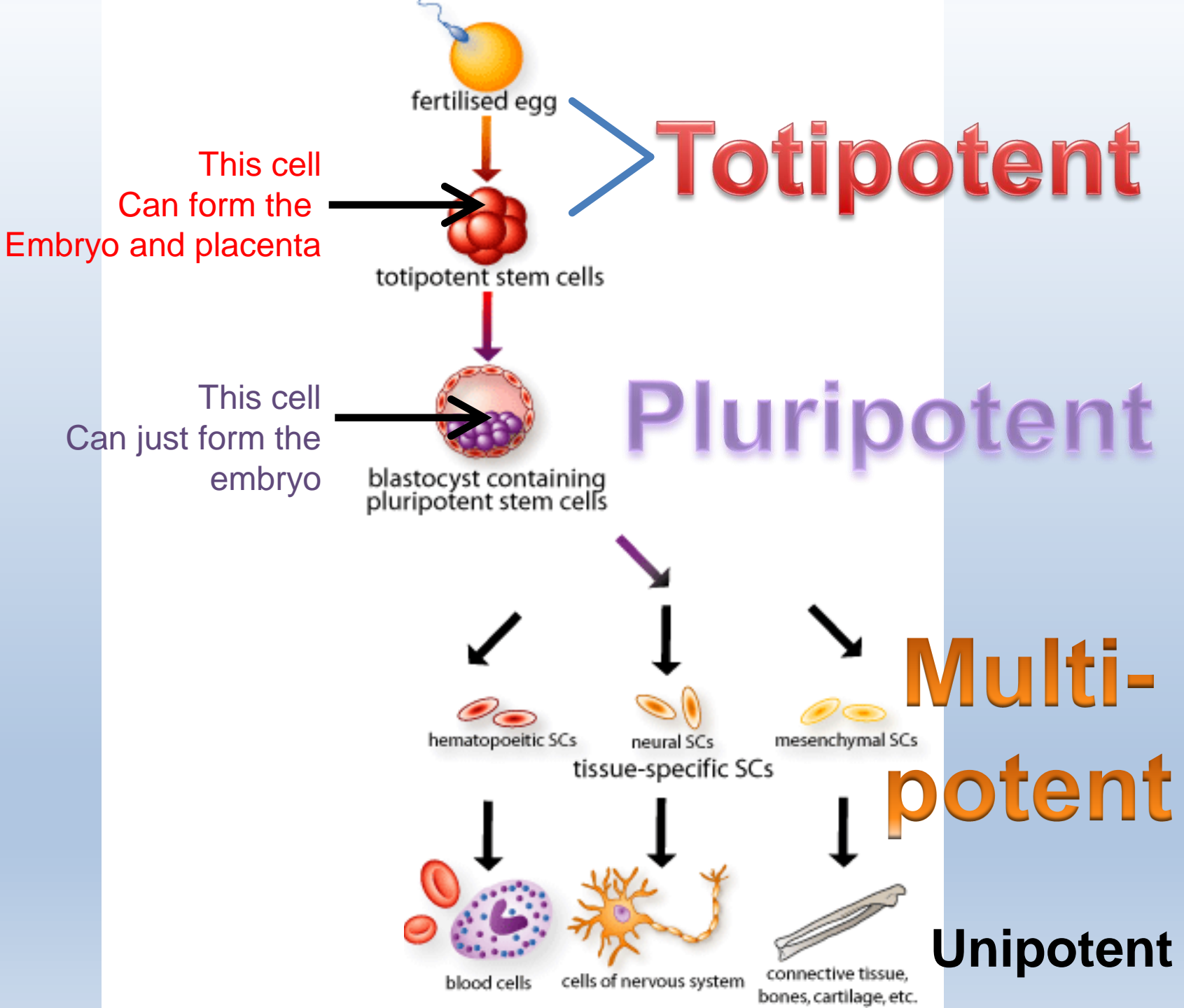
- to continuously divide and give rise to new copy of itself (self-renew)
- and other specialized ( differentiated ) cells/tissues.

**Stem Cell – main function within the body**

Continuous Repair of defective cell types and regeneration of tissues.







# Classification of Stem Cells "1"

## (Potency Based)

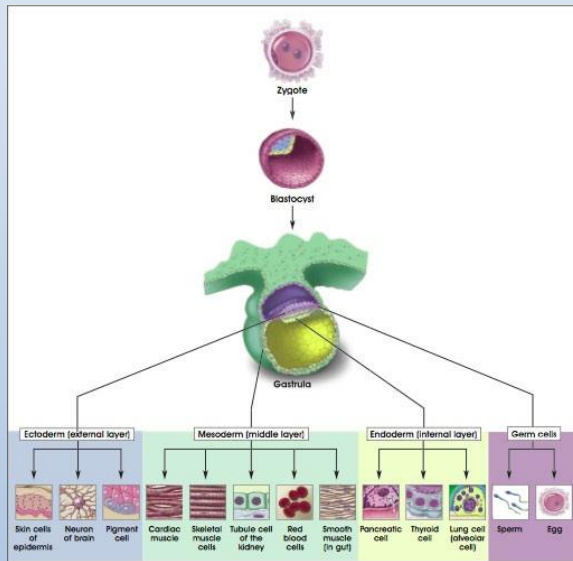
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Potency	Description
Totipotent	1-3 days, differentiate into <b>embryonic</b> and <b>extraembryonic</b> cell types
Pluripotent	Descendants of totipotent cells and differentiate into cells of 3 <b>germ layers</b>
Multipotent	Produce cells of a closely <b>related</b> of cells (e.g. hematopoietic ) <b>family</b> stem cells
Oligopotent	Differentiate into <b>ONLY a few</b> cells, such as lymphoid or myeloid stem cells
Unipotent	Produce <b>ONLY</b> one cell type (e.g. muscle stem cells)

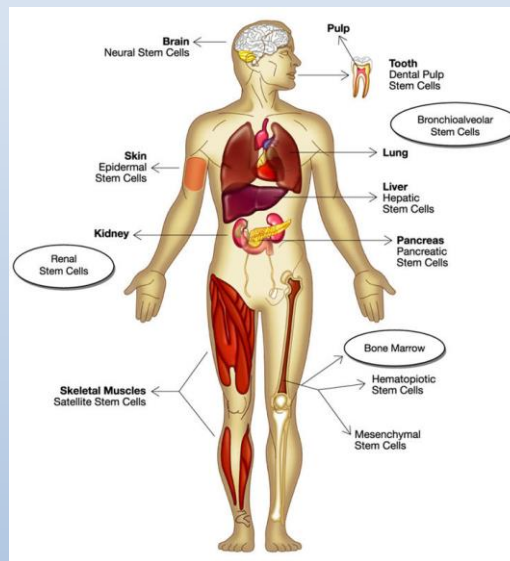
# Classifications of Stem Cells (2)

## (Sourced Based)

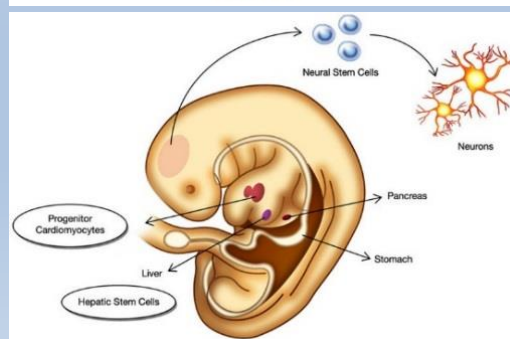
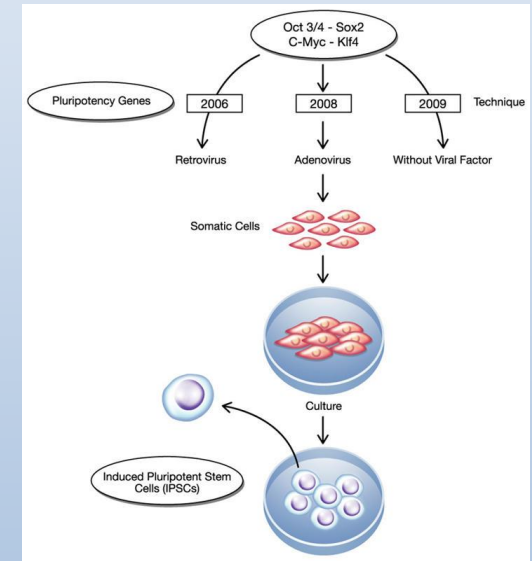
### Embryonic



### Adult (Tissue Specific)



### Induced

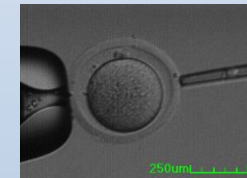


# Sources of Stem Cells



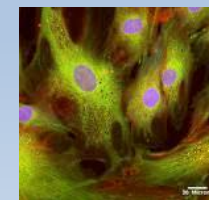
## Embryonic Stem Cells (ESC)

- ★ IVF embryos
- ★ Aborted embryos
- ★ cloned embryos



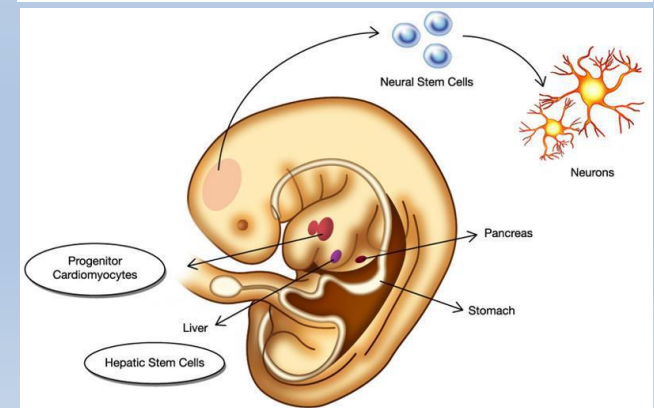
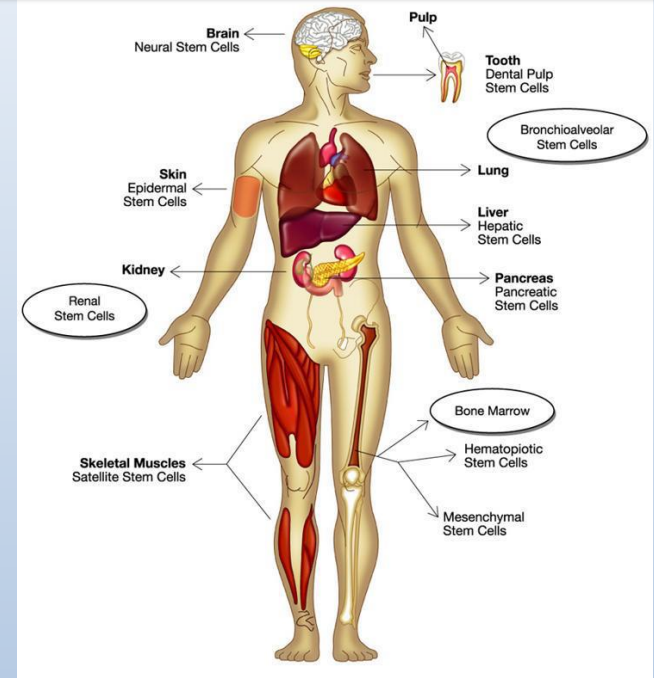
## Adult Stem Cells (ASC):

- ★ Bone Marrow
- ★ Placental Cord
- ★ Mesenchymal Stem cells



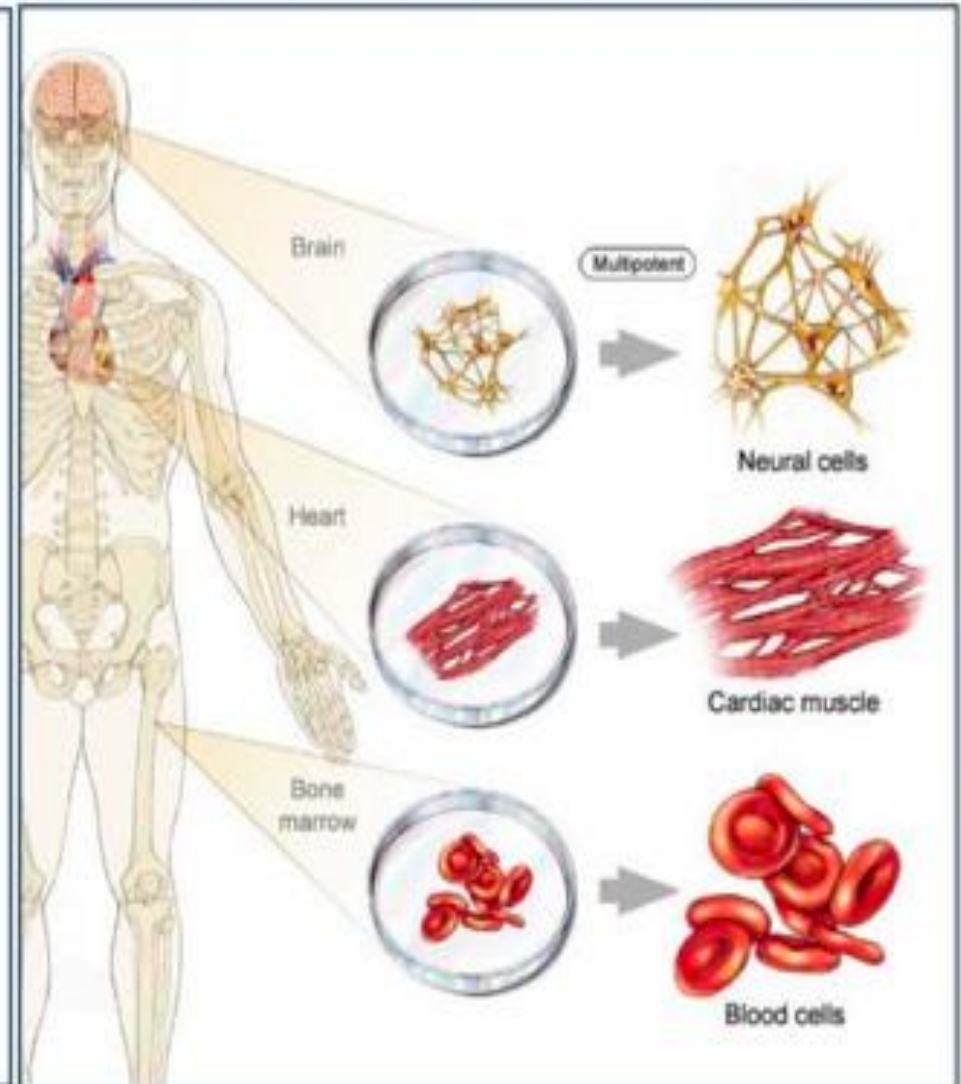
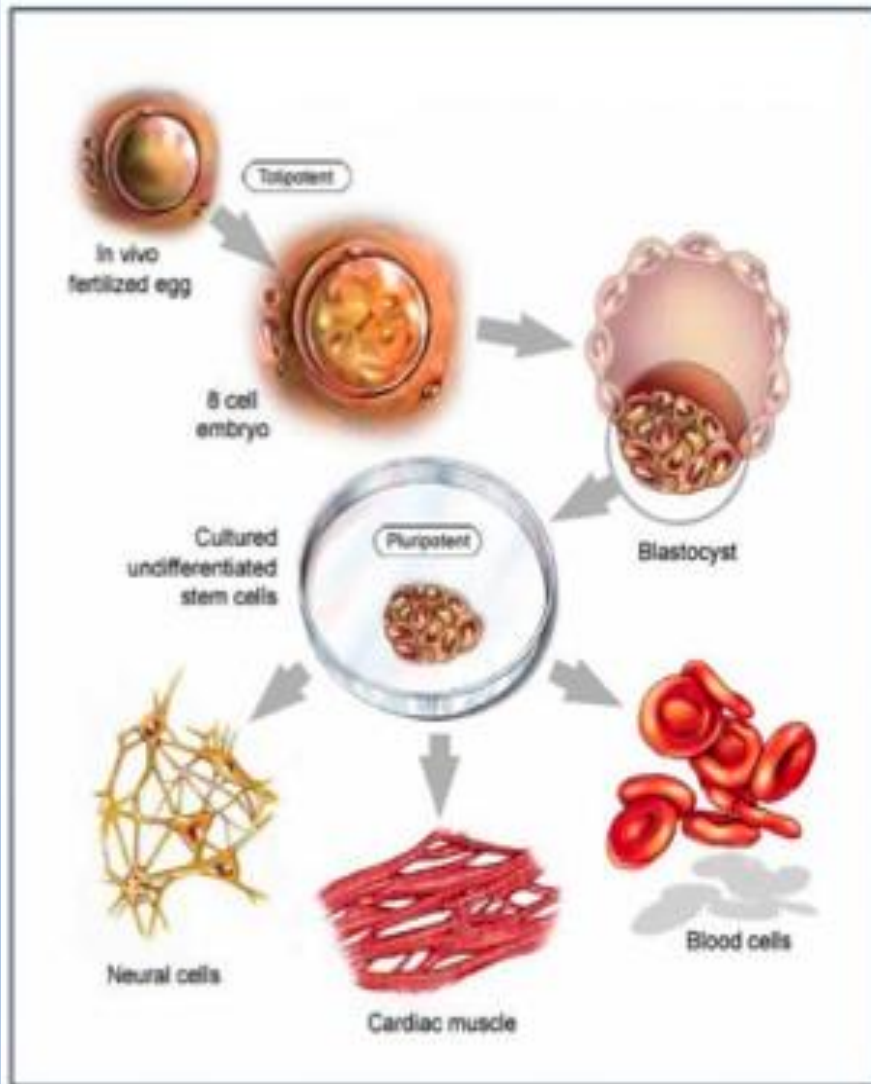
# Adult stem cells (Tissue Specific Stem Cells)

- Found in specific mature body tissues as well as the **umbilical cord** and **placenta** after birth.
- They also can be isolated of **developing embryos' different tissues**



# Embryonic Stem Cells

# Adult Stem Cells

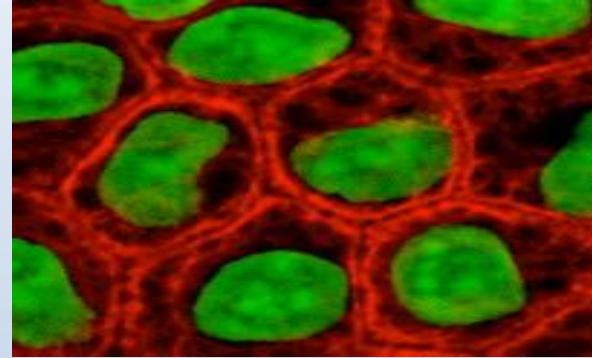


# ESC



- Pluripotent
- large number can be harvested
- May cause immune rejection
- Ethical concerns

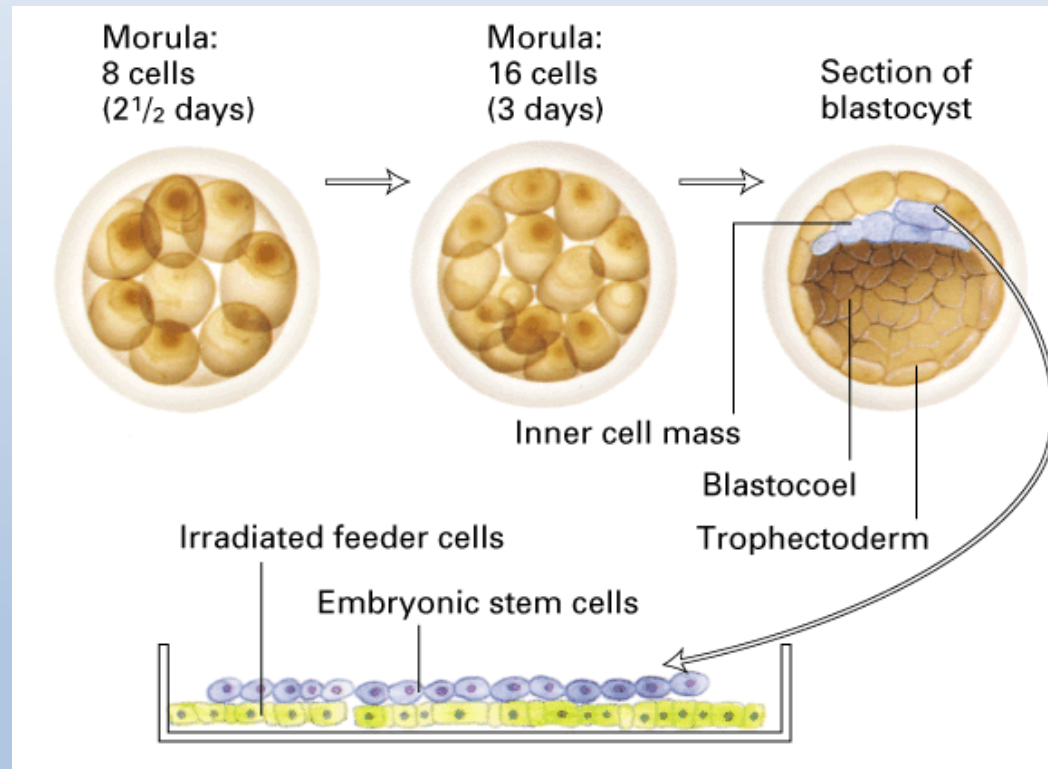
# ASC



- Multipotent
- Limited numbers and more difficult to isolate
- No immune rejection
- No Ethical concerns

# Generation of embryonic stem cells

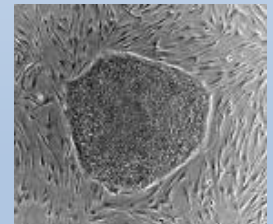
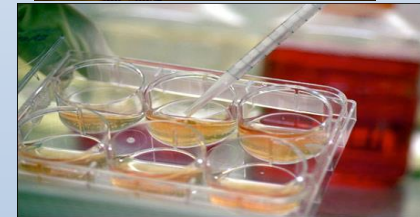
- Embryonic human stem cells were first isolated in 1995 by Dr. James Thomson.
- derived from 4-5 day old embryo (Blastocyst):
  - Trophoblast
  - Blastocoel
  - Inner Cell Mass (ICS)





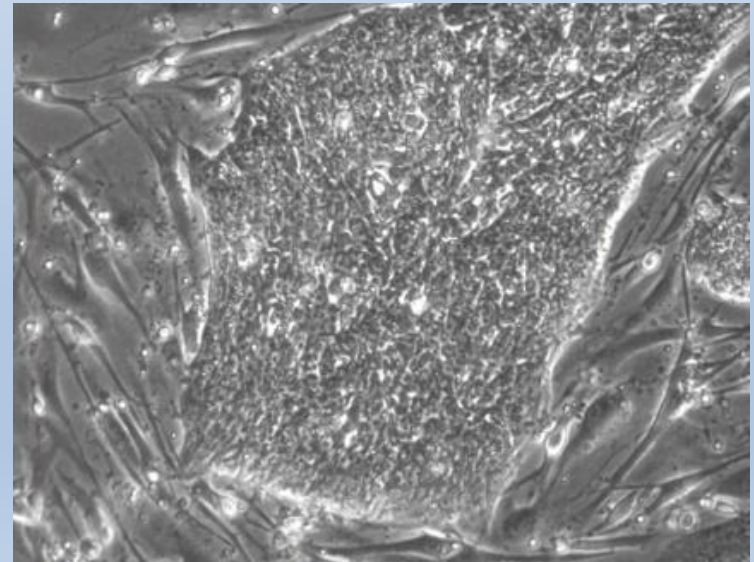
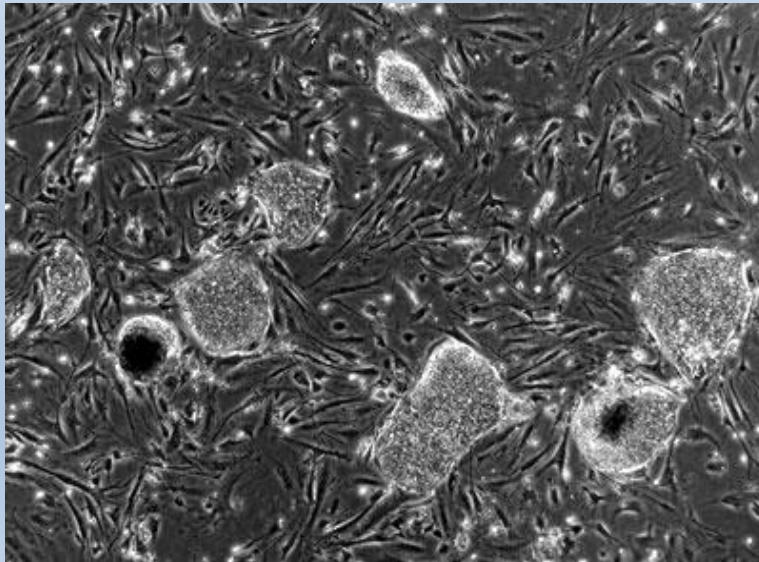
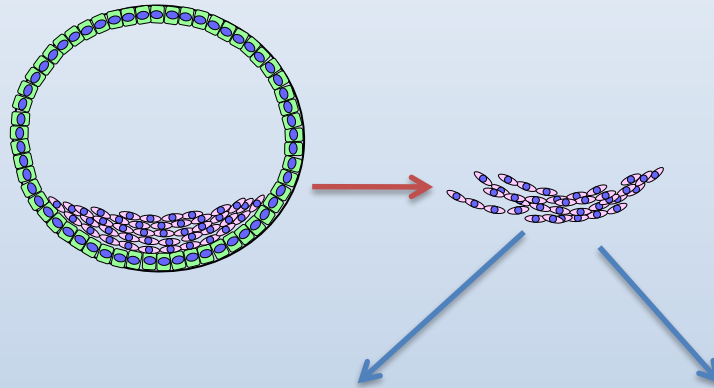
# Generation of embryonic stem cells

- Isolate and transfer of ICS into culture dish in culture media
- Culture at 37c and 5% CO<sub>2</sub>
- Inner surface of culture dish is coated with inactivated MEFs as a feeder layer:
  - provides sticky surface for attachment
  - release nutrients
- Cells divide and spread over the dish
- ESCs are removed gently and plated into several different culture plates.



# Human Embryonic Stem Cell Colony

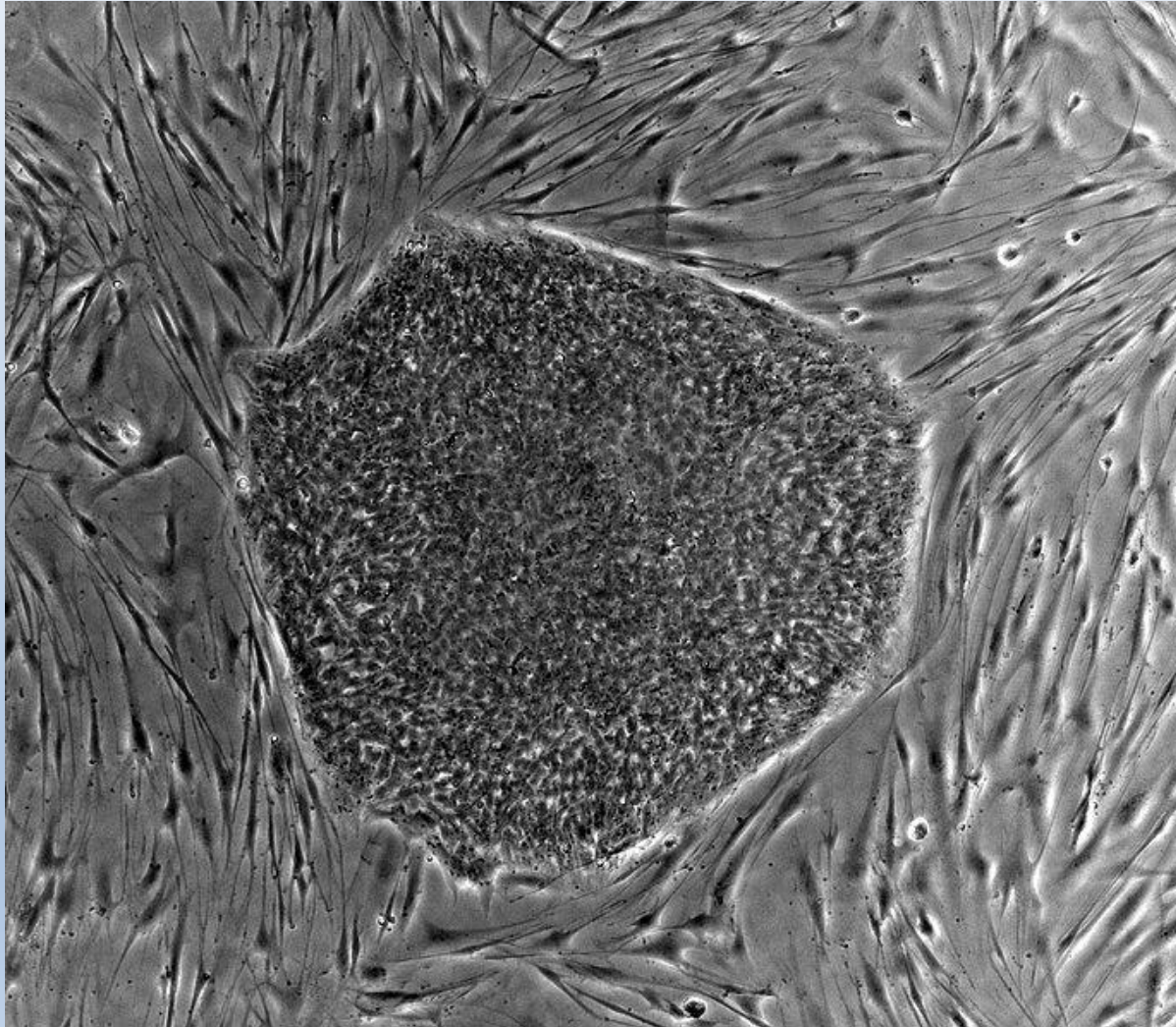
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# Embryonic stem cells in the dish

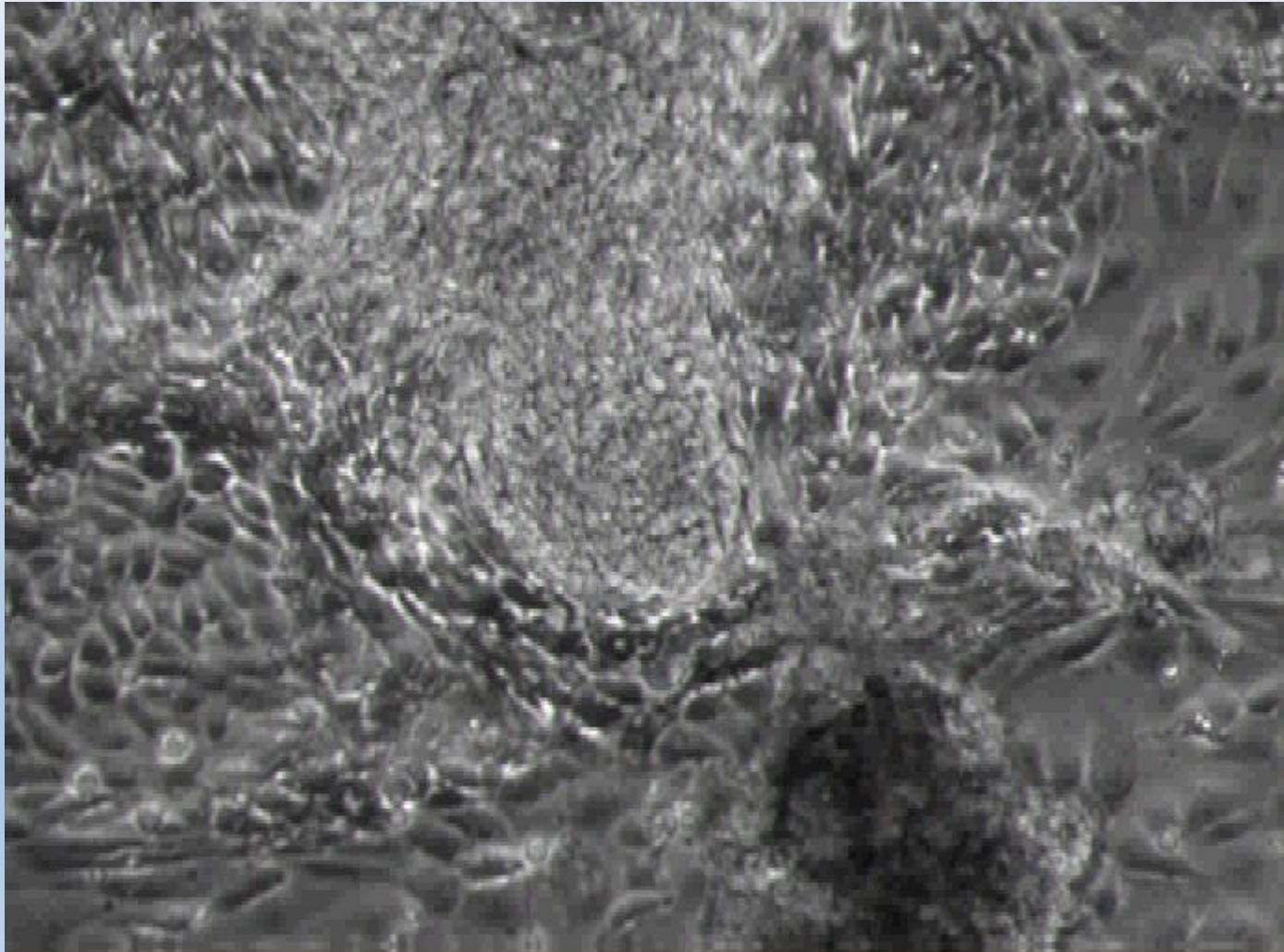
What do cultured ES cells look like?

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# Beating cardiomyocytes derived from hESCs

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# Challenges with Embryonic Stem Cells

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- Abnormalities in chromosome number and structure were found in some human ESC lines.
- Stem cells need to be differentiated to the appropriate cell types *before* they can be used clinically.
- Stem cell development or proliferation must be controlled once placed into patients (risk of teratoma formation).
- The use of mouse “feeder” cells to grow ESC could result in problems due to xenotransplantation.
- Possibility of rejection of stem cell transplants as foreign tissues is very high.

# Somatic Cell Nuclear Transfer SCNT

## CLONING

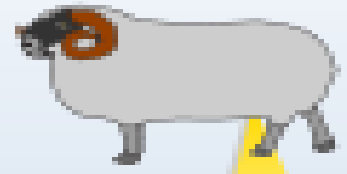


REPRODUCTIVE CLONING

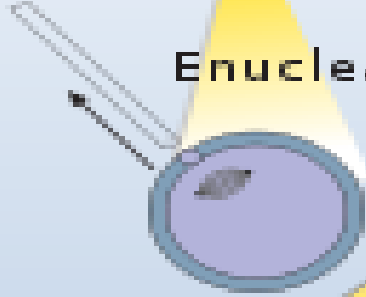
(July 1996 – February 2003)



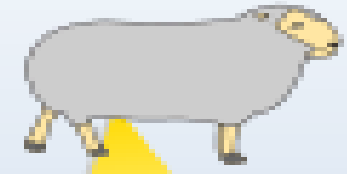
**Scottish Blackface  
(Cytoplasmic Donor)**



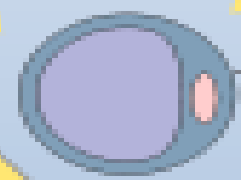
**Enucleation**



**Finn-Dorset  
(Nuclear Donor)**

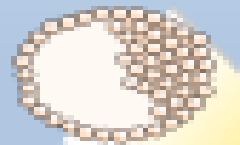


**Mammary Cells**

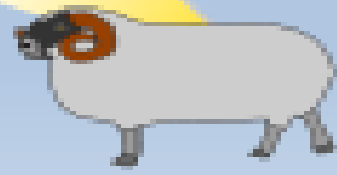


**Direct Current Pul:**

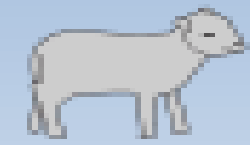
**Blastocyst**

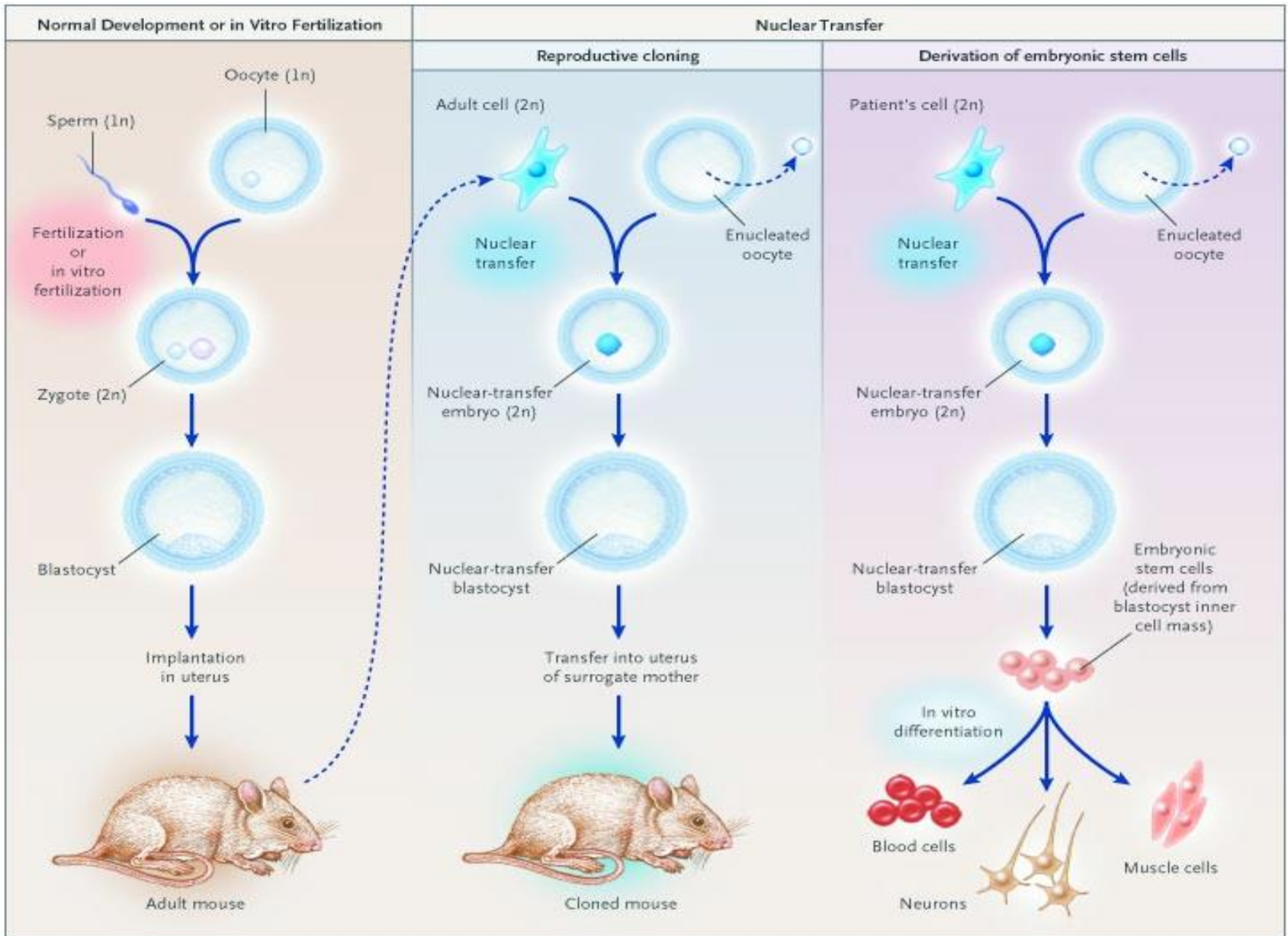


**Surrogate  
ewe**



**Dolly**

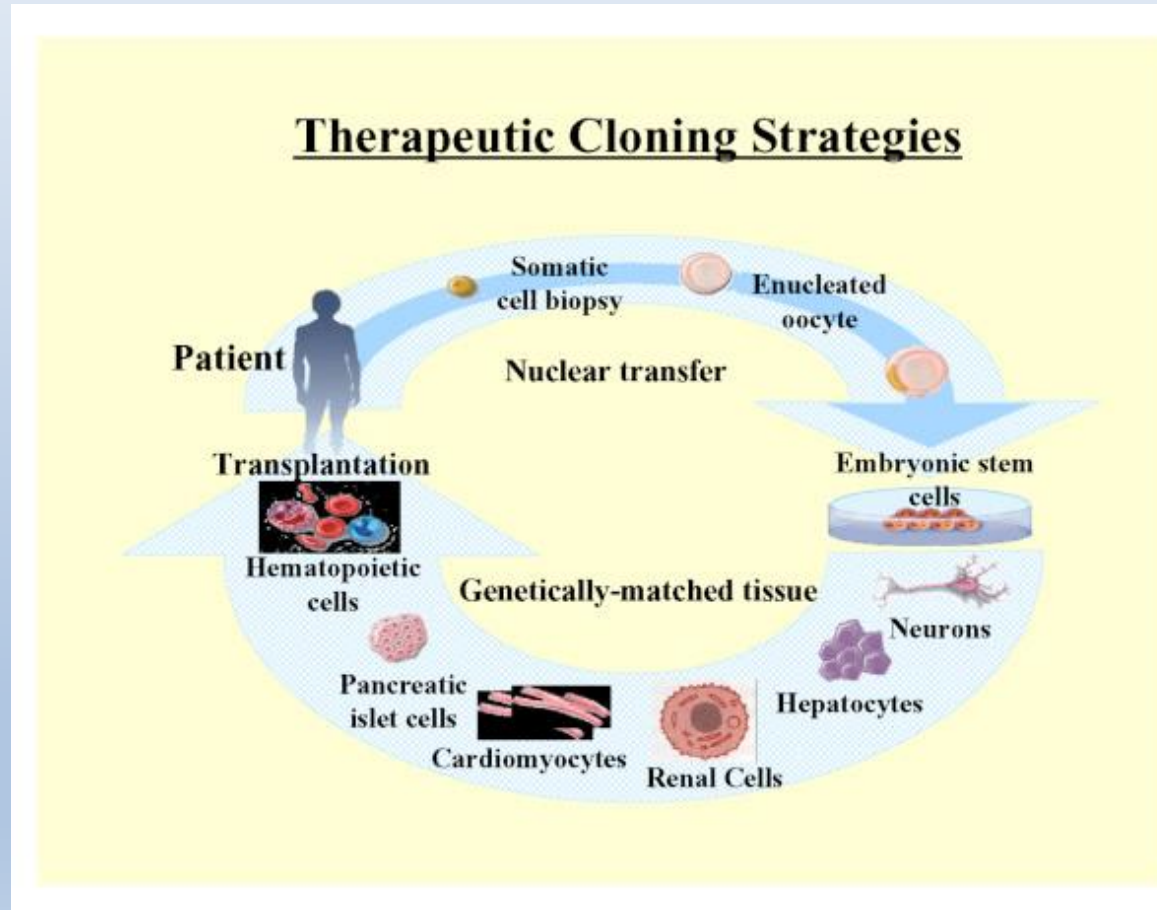






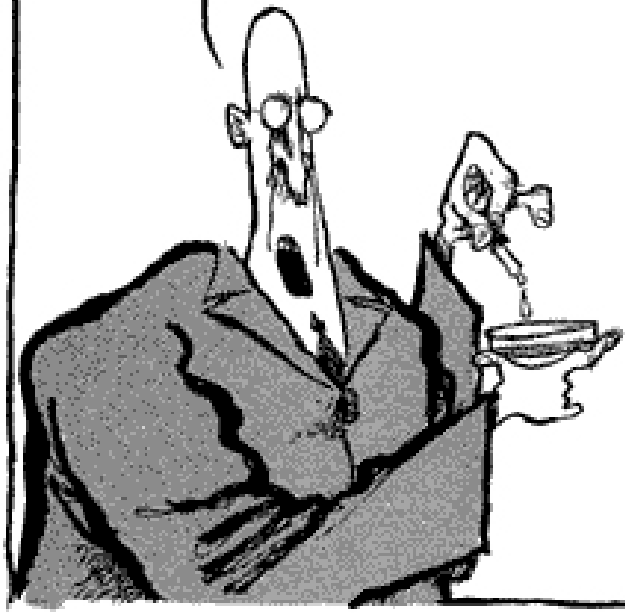
# Therapeutic Cloning

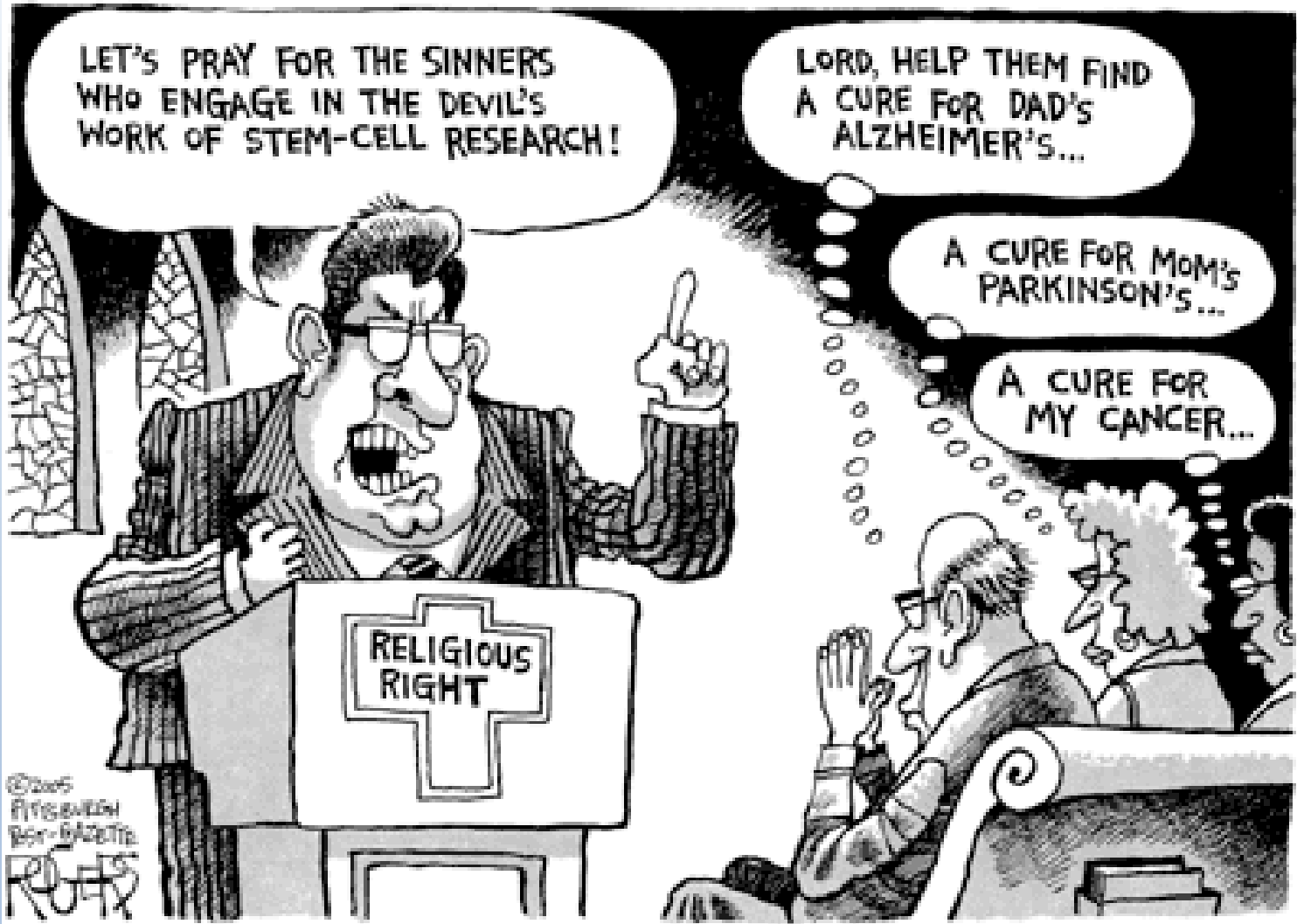
- Therapeutic cloning uses stem cells to correct diseases and other health problems that someone may encounter.
- Therapeutic cloning does not cloned to make full humans but rather is used for the stem cells of embryo



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WITH  
STEM CELLS  
WE CAN GROW  
JUST ABOUT  
ANYTHING...



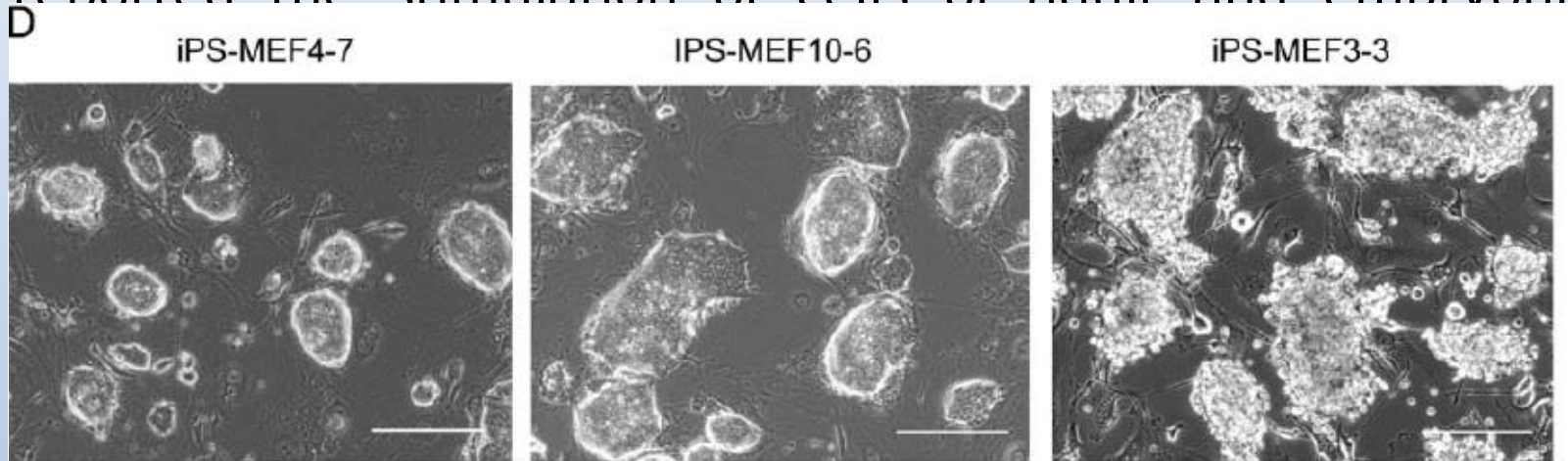


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# The first iPSCs

- In late 2006 the group of Takahashi and Yamanaka reported the stimulation of cells of adult and embryonic



\*Contact: [yamanaka@frontier.kyoto-u.ac.jp](mailto:yamanaka@frontier.kyoto-u.ac.jp)  
DOI 10.1016/j.cell.2006.07.024

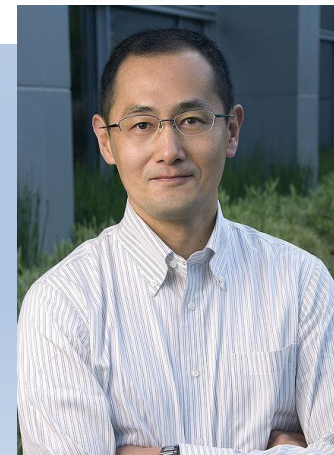




Photo: U. Munten

Sir John B. Gurdon



Photo: U. Munten

Shinya Yamanaka

The Nobel  
Prize  
in  
Physiology  
or  
Medicine  
2012

# iPS Cells



Healthy or diseased adult human or mouse



Adult cells (skin fibroblasts)



OCT4  
SOX2  
KLF4  
(Myc)



OCT4  
SOX2  
NANOG  
Lin28



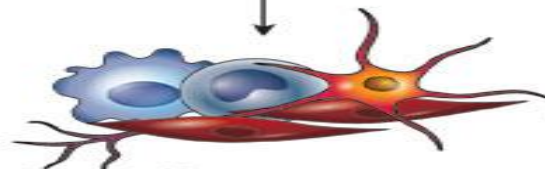
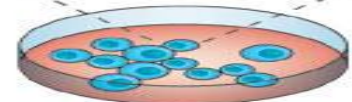
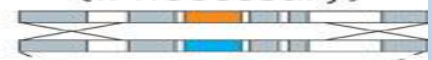
Thomson Factors

Self renewal



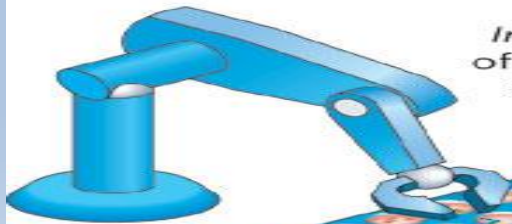
iPS cells

Genetic repair by homologous recombination (if necessary)



Differentiation

In vitro screening of drug candidates on healthy and diseased cells

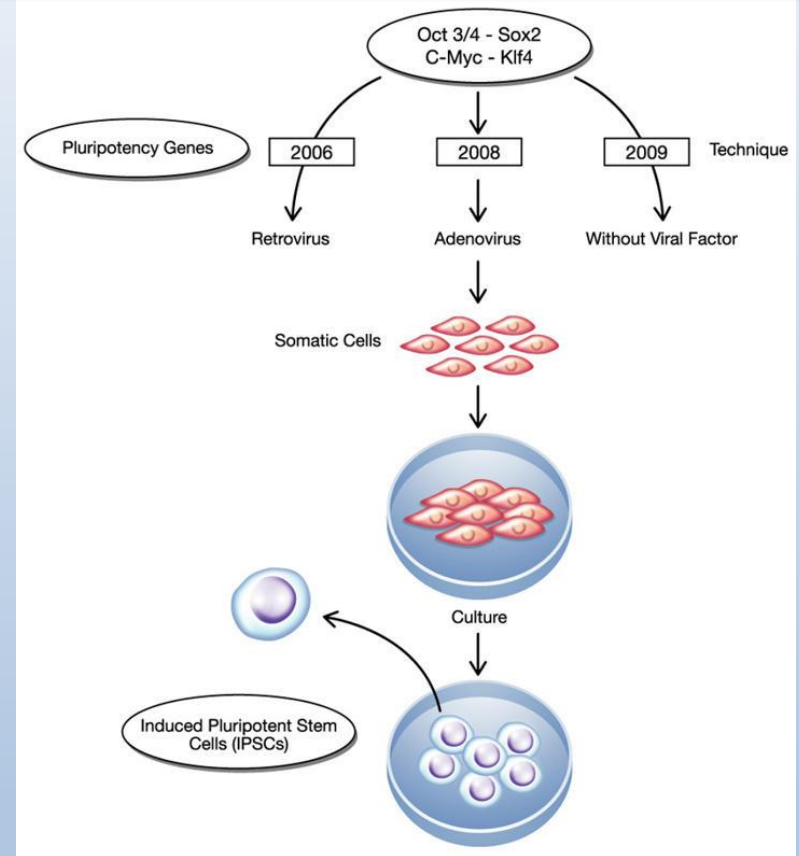


Transplantation



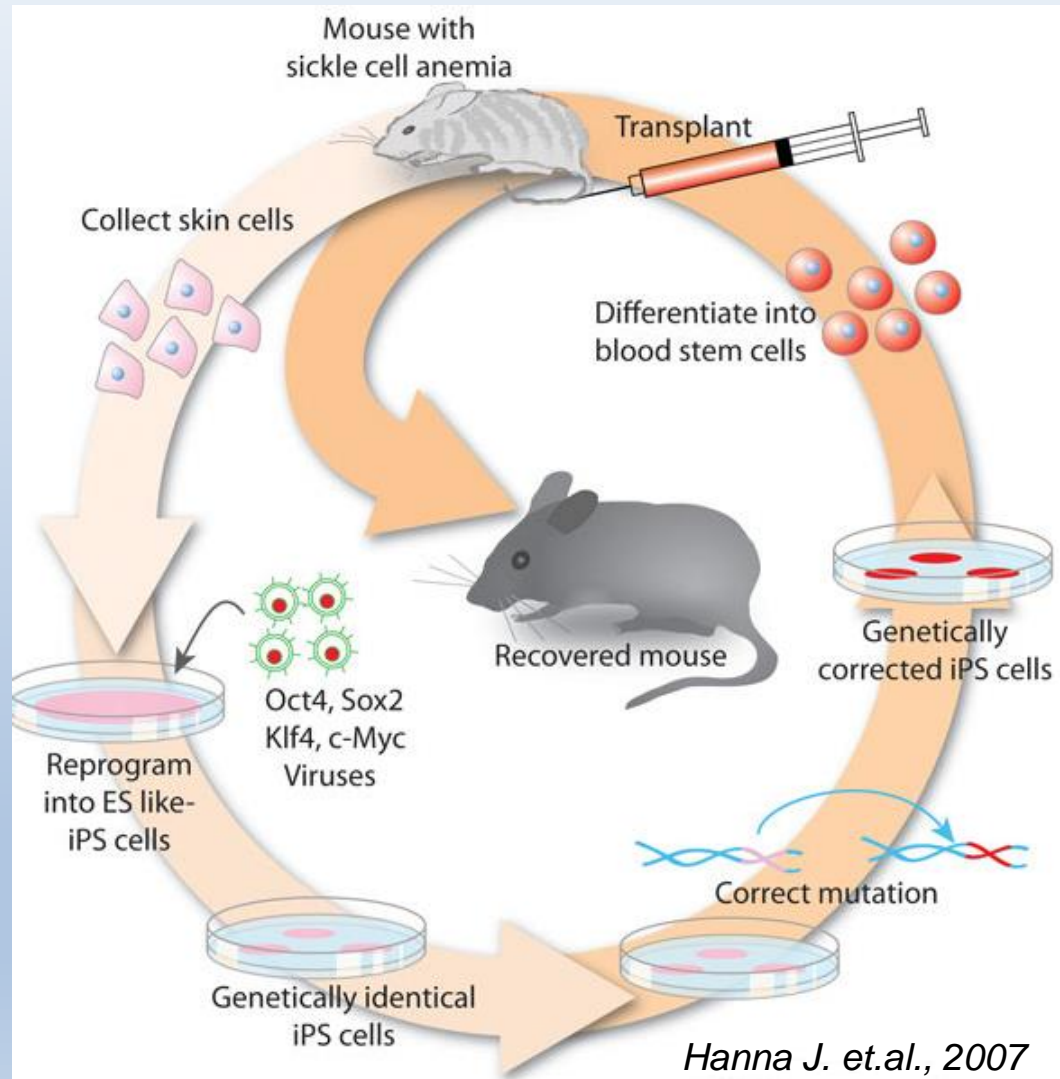
# Induced Pluripotent Stem Cell (iPS) cells

- The method was described by Yamanaka in which the skin cells of laboratory mice were genetically manipulated and returned back to their embryonic state.
- iPS are somatic cells that have been reprogrammed to a pluripotent state (embryonic stem cell like state).
- Several difficulties are to be overcome before iPS cells can be considered as a potential patient-specific cell therapy.
- It will be crucial to characterize the development potential of human iPS cell line in the future.



# Induced Pluripotent Stem Cell (iPS) cells

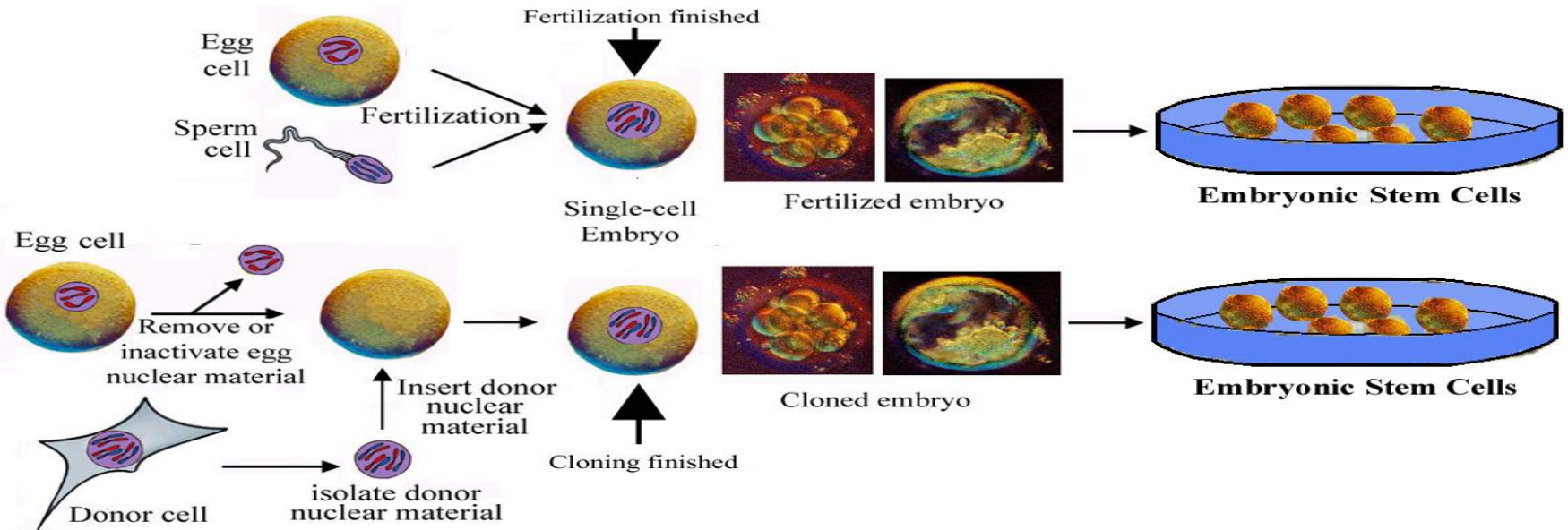
- Skin cells were taken from the tail tip of a sickle-cell model mouse.
- The cells were differentiated into hematopoietic cells.
- The produced cells were transfused back into the sick mouse





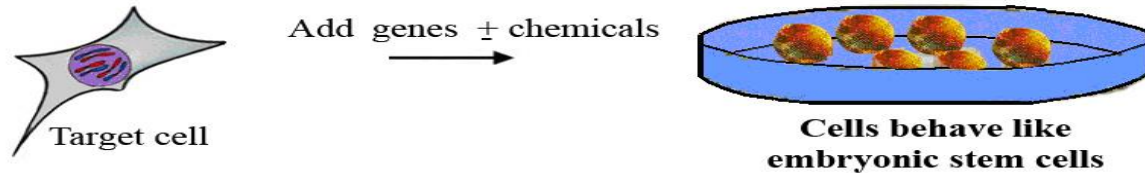
# Embryonic Stem Cells

from Embryos created by Fertilization or by Cloning (Somatic Cell Nuclear Transfer)



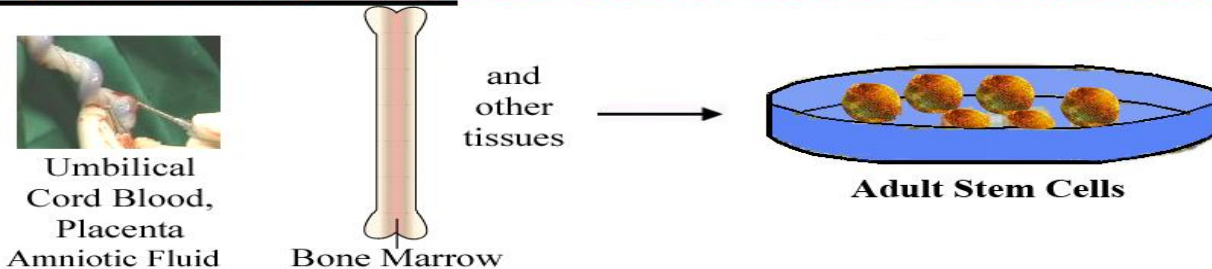
# Induced Pluripotent Stem Cells (iPS cells)

from Normal Cells that are Reprogrammed to behave like Embryonic Stem Cells

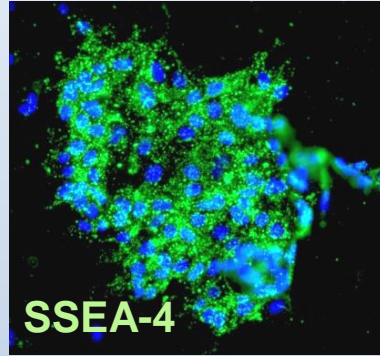
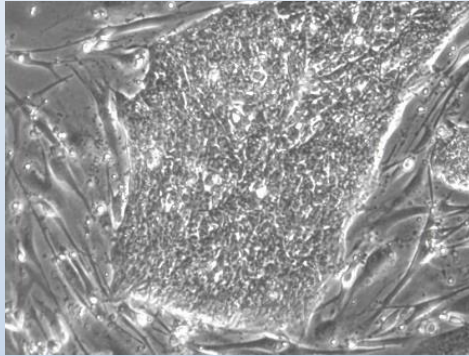


# Adult Stem Cells

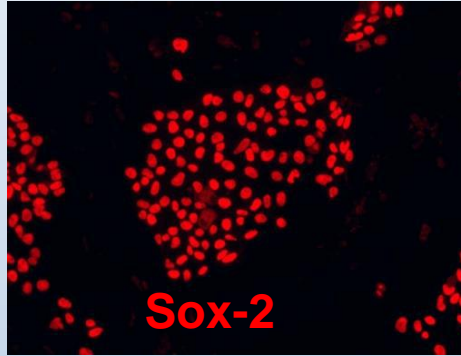
Stem Cells normally found in body tissues from birth onward, as well as umbilical cord, etc.



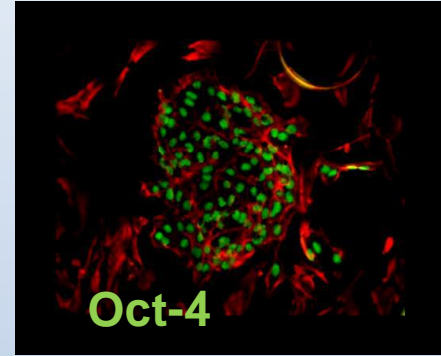
# Characterization of Human Pluripotent Stem cells (ESCs)



SSEA-4

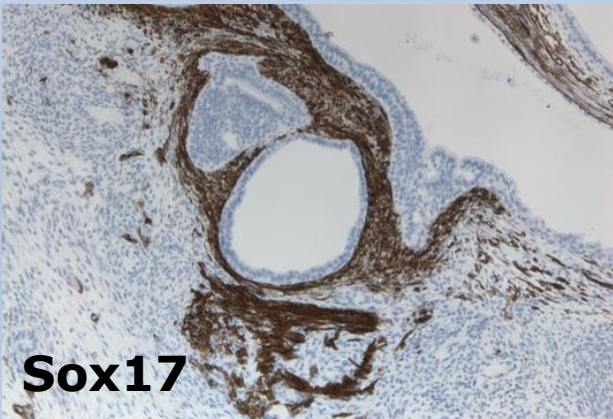


Sox-2



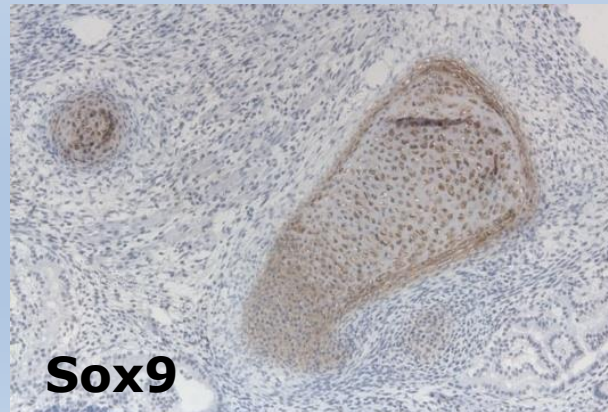
Oct-4

## ENDODERM



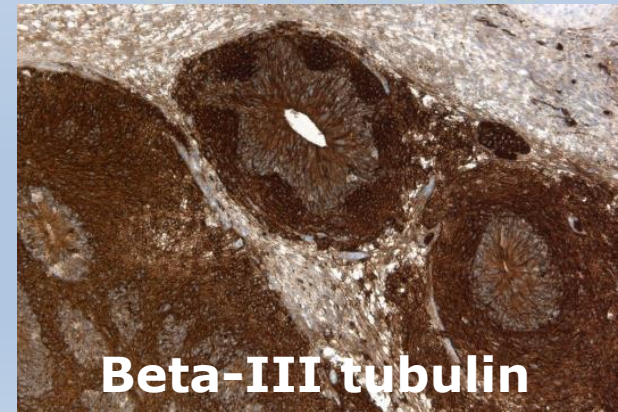
Sox17

## MESODERM

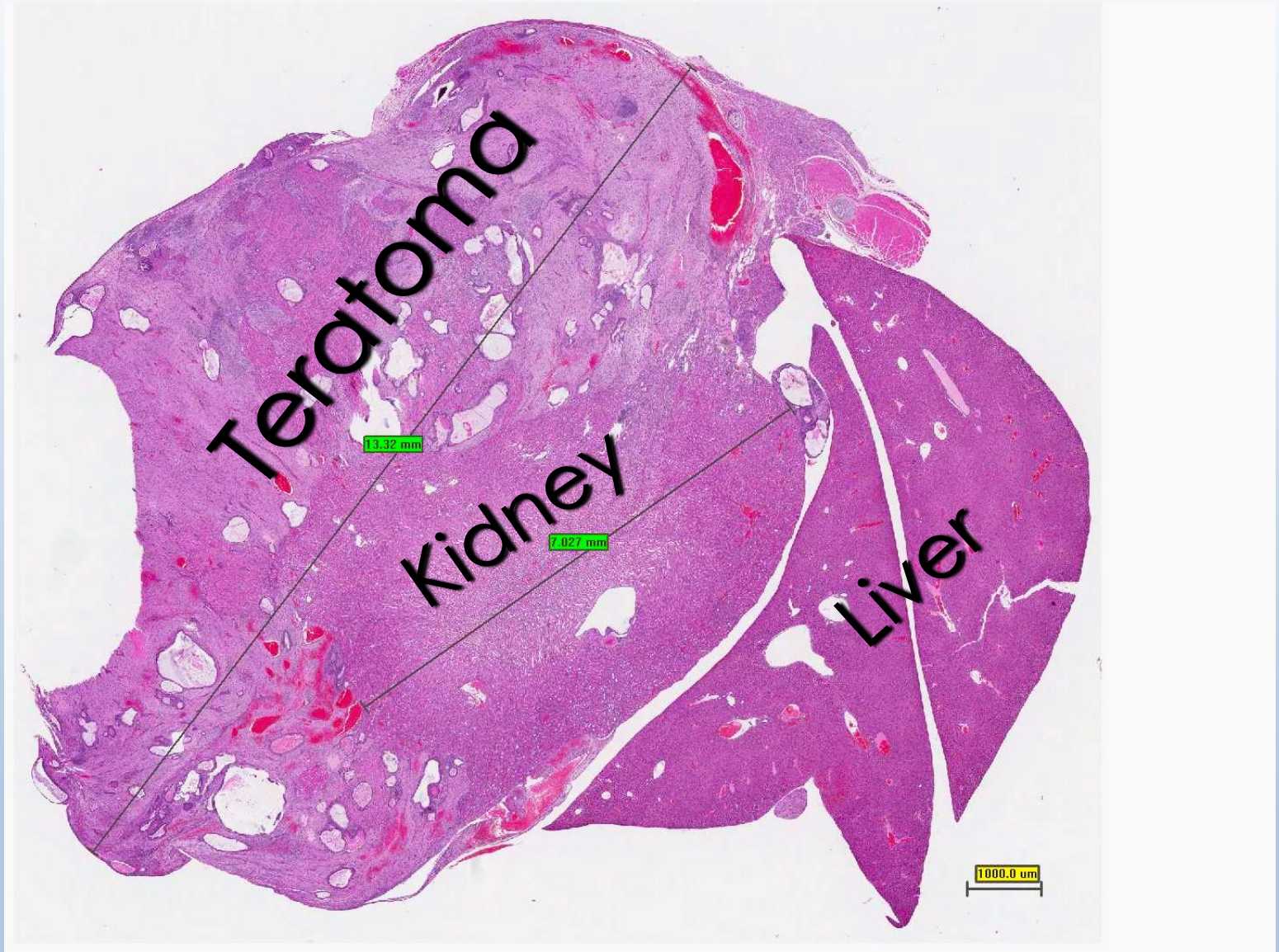


Sox9

## ECTODERM

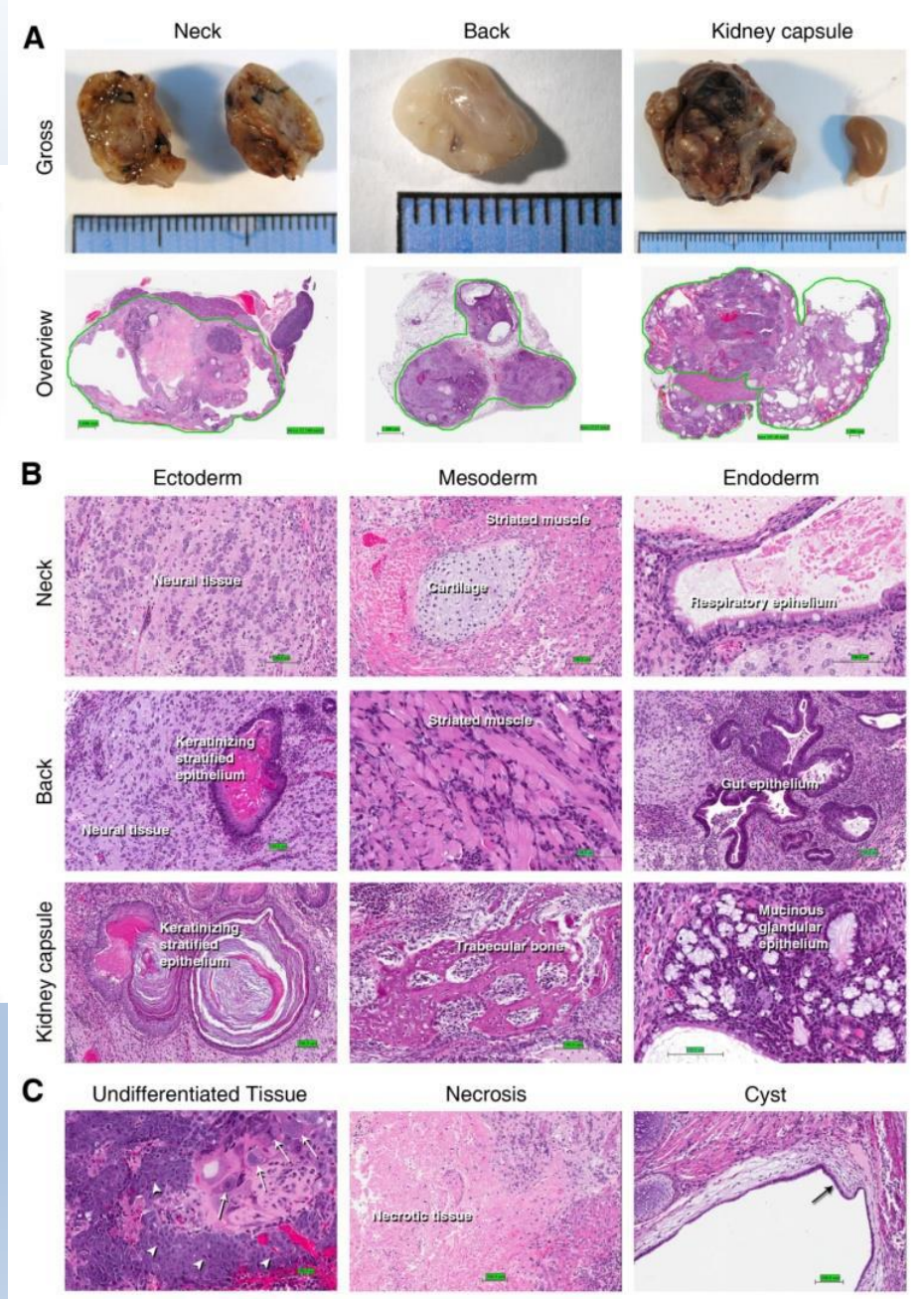
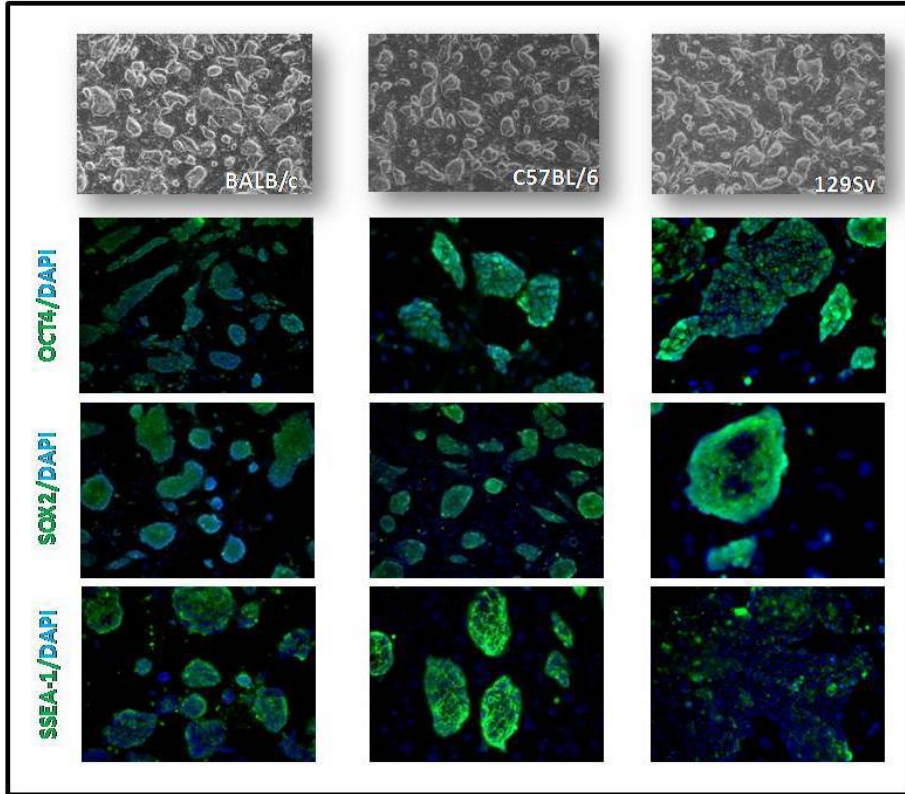


Beta-III tubulin

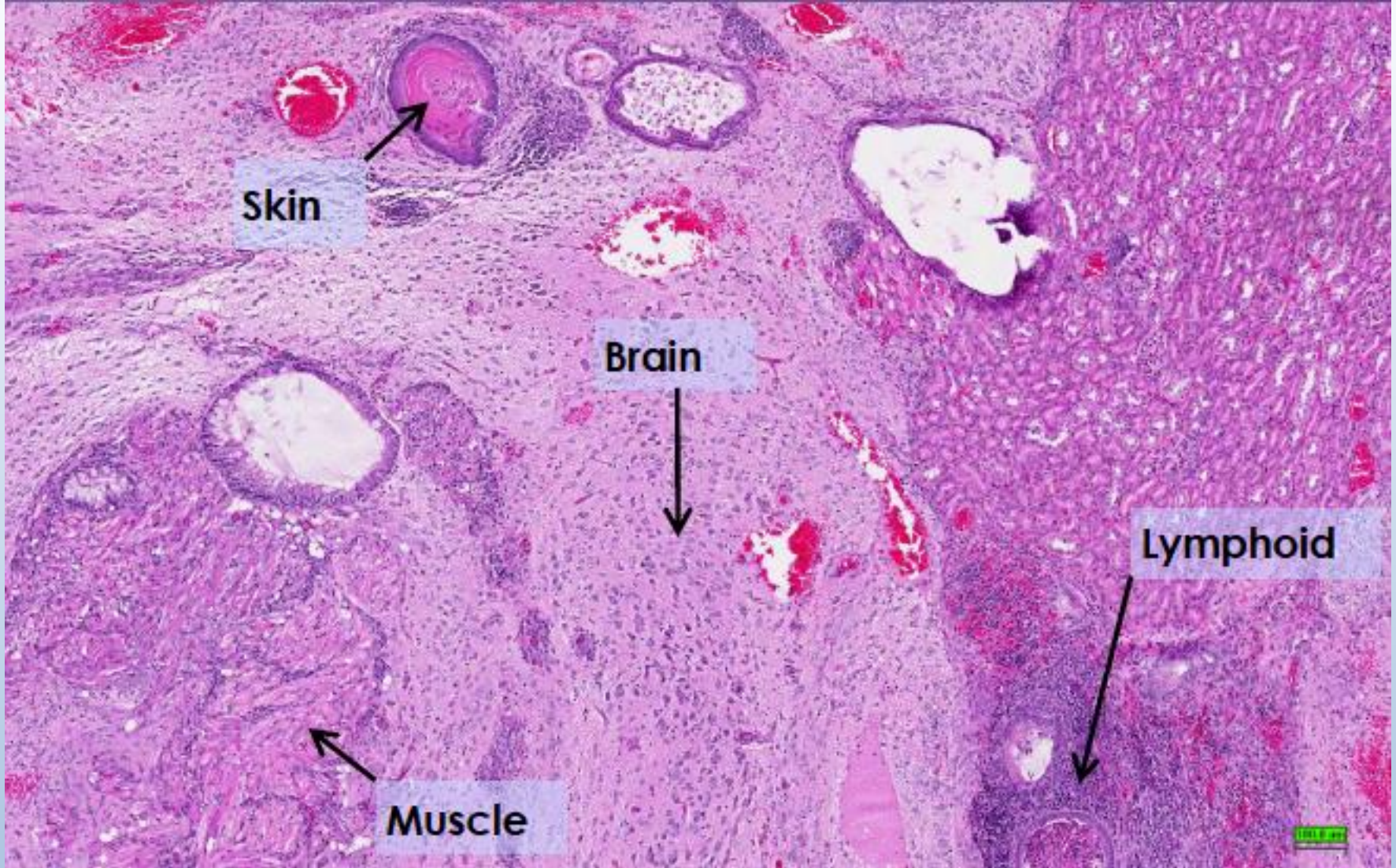


A large tumor mass measuring twice as the kidney is compressing it.

**Figure 1**



Teratoma Formation in Immunocompetent Mice After Syngeneic and Allogeneic Implantation of Germline Capable Mouse Embryonic Stem Cells, 2013



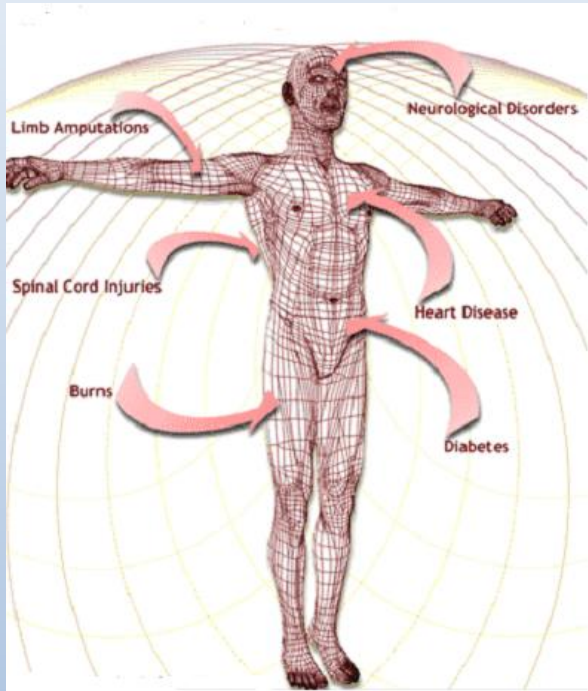
The teratoma was composed of mixed tissue patterns: skin with keratin, brain tissue, striated and smooth muscle, lymphoid tissue,....

# Goal of Stem Cell Therapies

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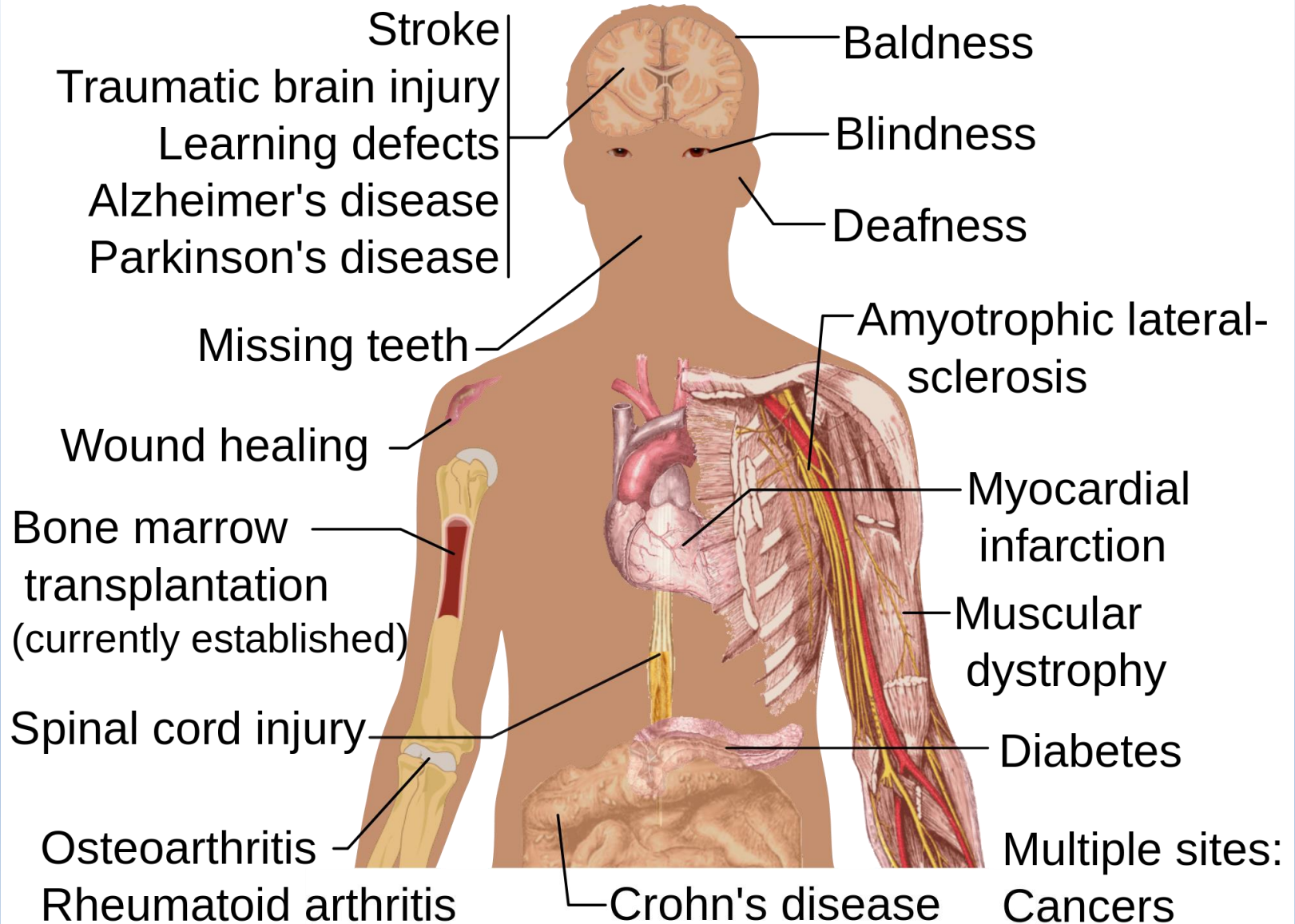
**The goal of stem cell therapies is to promote cell replacement in organs that are damaged and do not have the ability for self repair**

# The Promise of Stem Cell Technology



- Replacement of tissues/organs
- Repair of defective cell types
- Study cell differentiation
- Toxicity testing.
- Understanding prevention and treatment of birth defects.
- Study of development and gene control.
- Study of drugs therapeutic potential.

# Potential uses of **Stem cells**





# Obstacles of Stem Cell Research

---

- How to find the right type of stem cells?
- How to completely differentiate Stem Cells to desired cell type?
- How to put the stem cells into the right place?
- Will the stem cells perform the desired function in the body?
- Differentiation protocols for many cell types have not been developed.

# Question 1

- Which of the following are pluripotent stem cells?
  - a. Cells has the potential to differentiate into any adult cell type forming an entire organism
  - b. Cells that has limited potential to form only multiple adult cell types
  - c. Cells that don't have the ability for self renewal
  - d. Cells has the Potential to form all differentiated cell types except placenta

# Question 2

- **Important limitation of using cloned ESCs (SCNT-ESCs) clinically:**
  - a. Immune rejection
  - b. Produce limited number of cell types
  - c. Destruction of human embryos
  - d. Difficult to grow and culture in the laboratory

# Question 3

- **What are Yamanaka factors?**
  - a. OCT3/4, SOX2, KLF4, c-Myc
  - b. Growth factors
  - c. Cytokines
  - d. OCT3/4, SOX2, Nanog

# Question 4

- **Mesenchymal stem cells are examples of:**
  - a. Pluripotent stem cells
  - b. Multipotent stem cells
  - c. Totipotent stem cells
  - d. Induced pluripotent stem cells (iPS cells)



Thank You