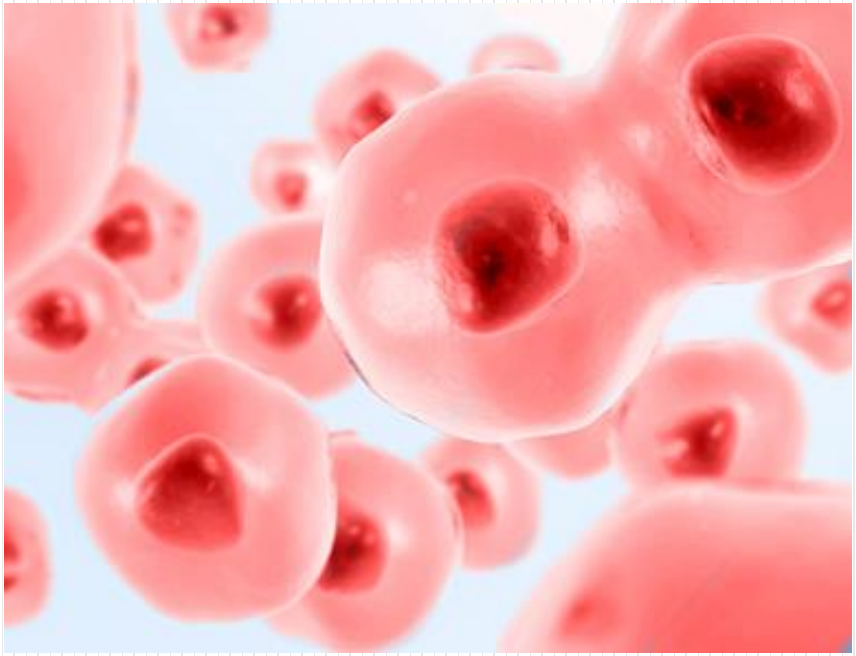
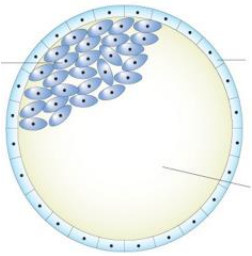
STEM CELLS AND THEIR USE IN MEDICINE



created by Morenko A.
Full PhD
Lesya Ukrainka Eastern European National University

What are Stem Sells, Why are They Important?

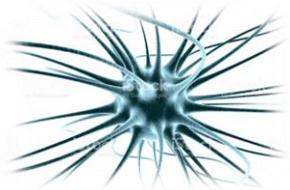


Stem cells are the cells that have the remarkable potential to develop into many different cell types in the body during early life and growth.

Internal repair system

Potential in further differenti ation

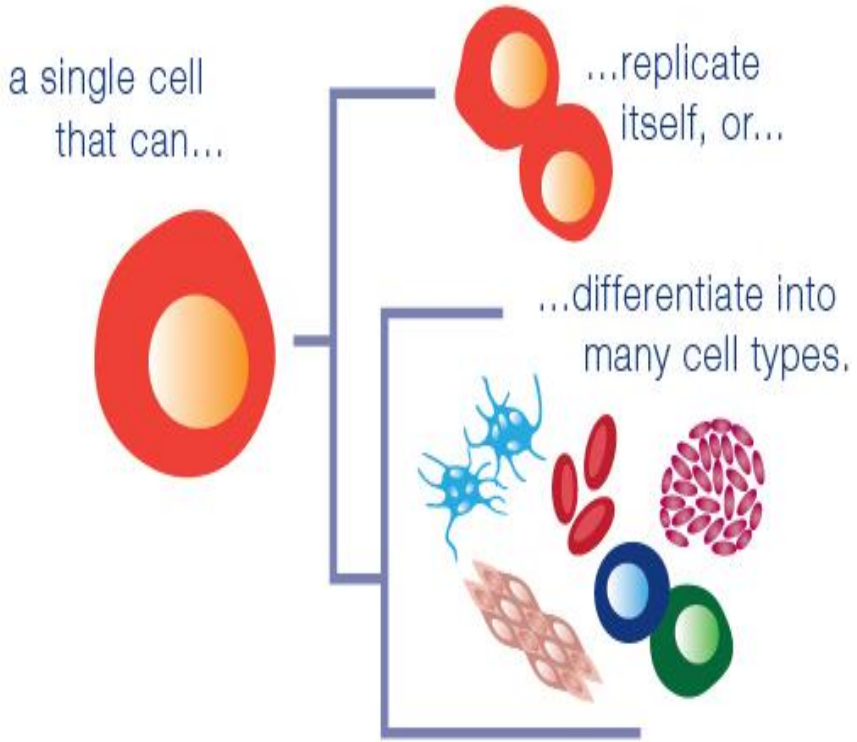
Dividing without a limit they replenish other cells from person's or animal's birth to death.



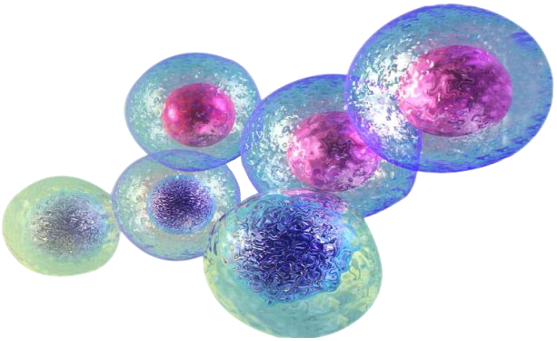
Stem Cells are Distinguished from Other Cell Types by Two Important Characteristics

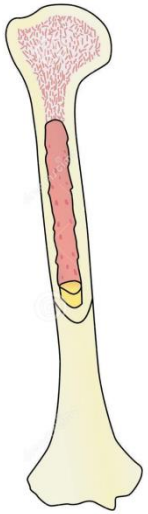
1. They are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity.

2. Under certain physiologic or experimental conditions, they can be induced to become tissue or organ specific cells with special functions.

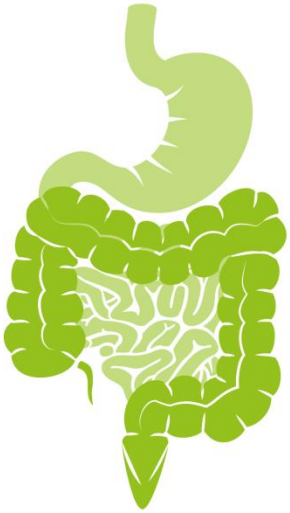


Muscle Tissue

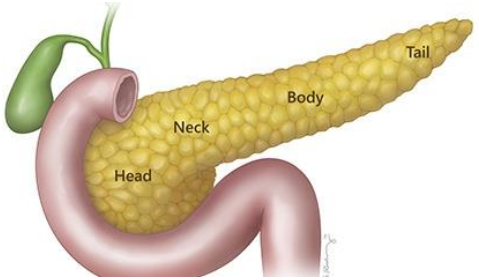




Bone marrow



Gut



Pancreas

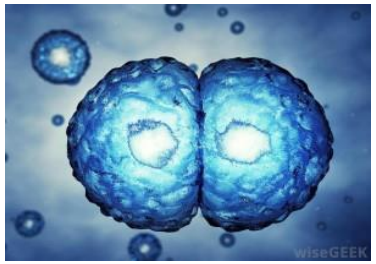
Heart

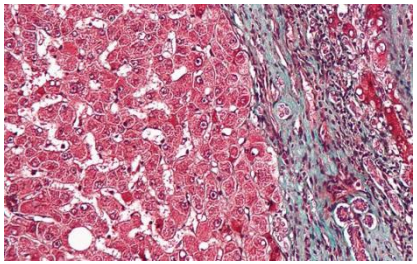
Stem cells regularly divide to repair and replace worn out or damaged tissues.

Stem cells only divide under special conditions.

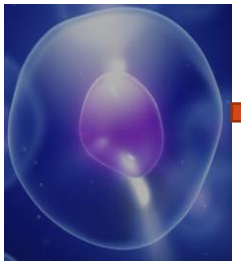
Short History of Stem Cell Research

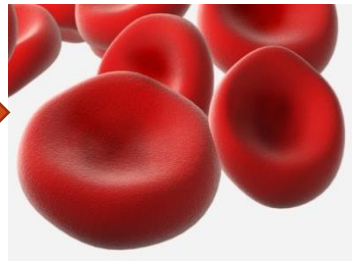
Stem cells have an interesting history that has had many debate and controversy.





In the mid 1800s it was discovered that cells were basically the building blocks of life and that some cells had the ability to produce other cells.





Attempts were made to fertilise mammalian eggs outside of the human body.
In the early **1900s**, it was discovered that some cells had the ability to generate blood cells.

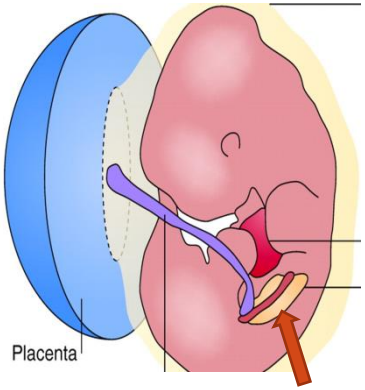
Minnesota, pediatric immunologist Robert Good.

In 1968, the first bone marrow transplant was performed to successfully treat two siblings with severe combined immunodeficiency.

Other Key Events in Stem Cell Research Include:

- 1978: Stem cells were discovered in human cord blood,
- 1981: First in vitro stem cell line was developed from mice,
- 1988: Embryonic stem cell lines were created from a hamster,
- 1995: First embryonic stem cell line was derived from a primate,
- 1997: Cloned lamb Dolly was created and grown from stem cells,
- 1997: Leukaemia origin was found as haematopoietic stem cell, indicating possible proof of cancer stem cells.

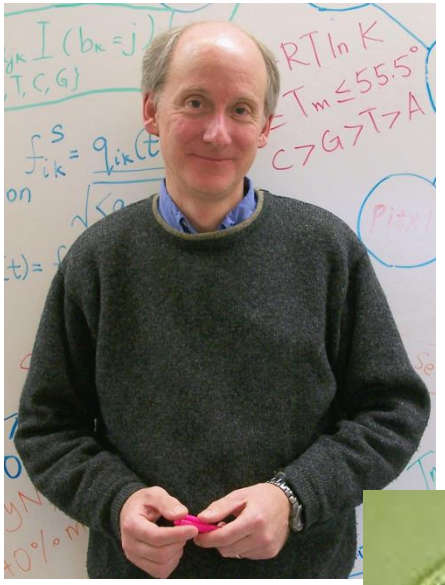
John Gearhart, cell biologist from Johns Hopkins University.



Fetal gonad tissue

He first identified and isolated human pluripotent stem cells from human primordial germ cells.

Pluripotent stem cell lines were developed from both sources



James Thompson, cell biologist from the University of Wisconsin,

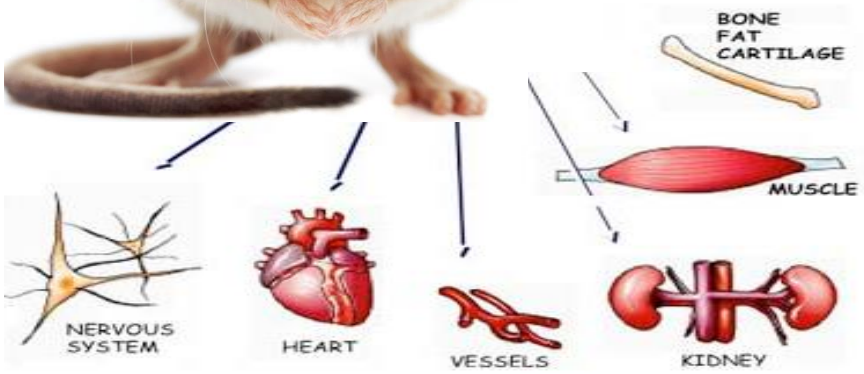
In 1998, He isolated cells from the human blastocysts inner cell mass of early embryos.

He developed the first embryonic stem cell lines in lab.

Fabricating studies and findings

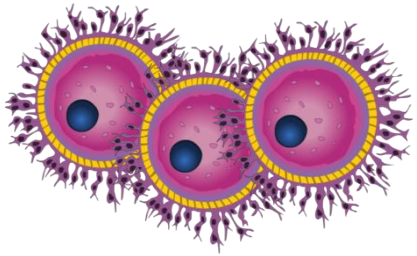
In1999 and 2000, scientists discovered that manipulating stem cells of adult mouse tissues could produce different cell types.

Adult mouse tissues

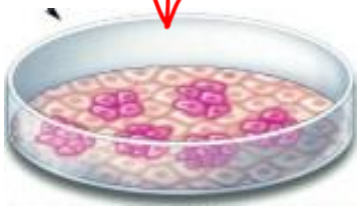


It promised of greater scientific control over stem cell differentiation and proliferation.

In 2004 to 2005, when Hwang Woo-Suk claimed…



Unfertilised human eggs

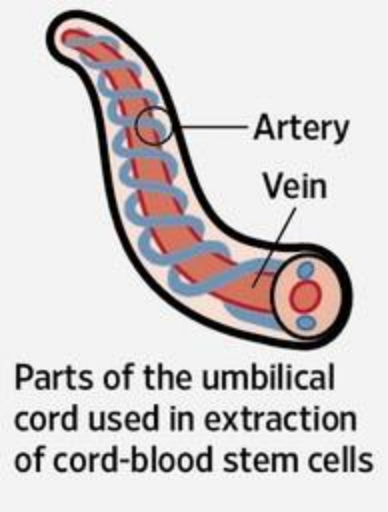


Embryonic stem cell lines

The lines were eventually shown to be completely false and therefore fabricated.

In 2005, scientists at Kingston University in England found out another category of stem cells.

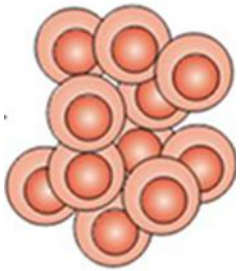
The ability to differentiate into more cell types than adult stem cells

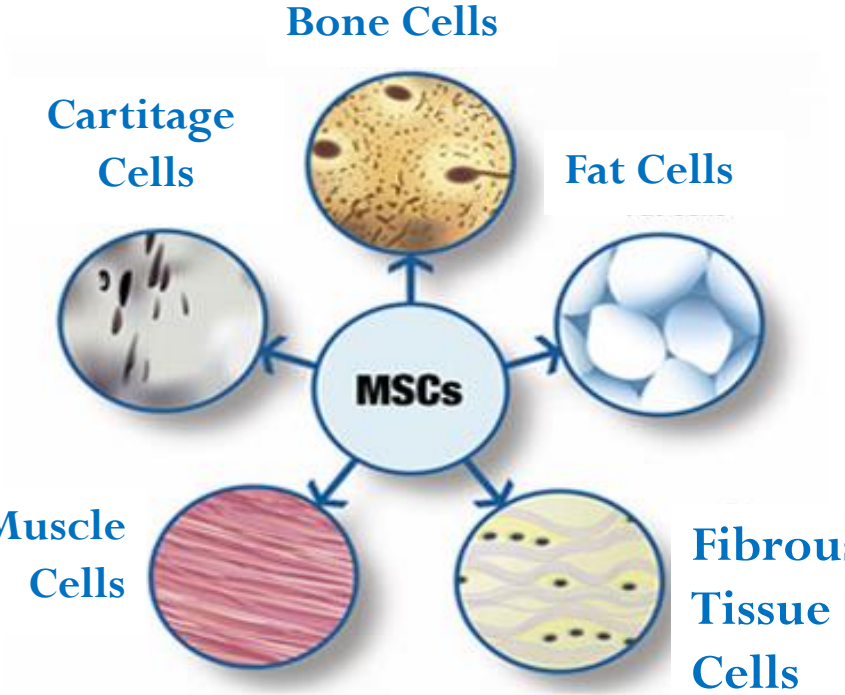


Parts of the umbilical cord used in extraction of cord-blood stem cells

Umbilical Blood Cord

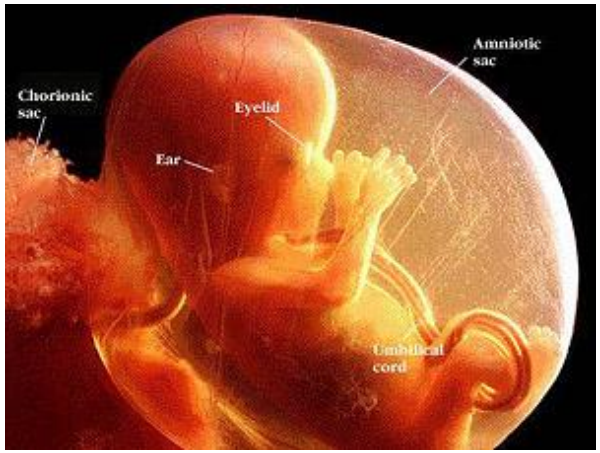
Cord blood embryonic stem cells

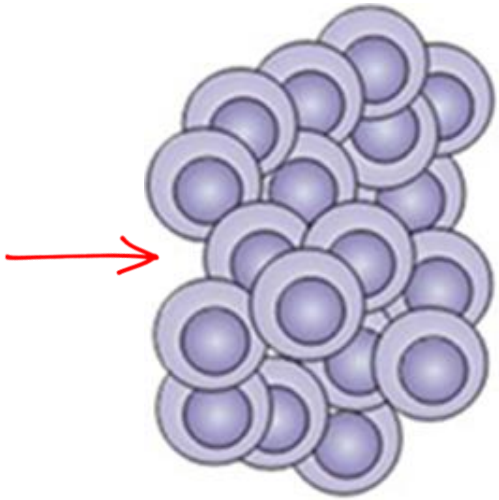




It opens up greater possibilities for cell-based therapies..

In 2007, researchers lead by ***Dr. Anthony Atala*** claimed that a new type of stem cell had been isolated in amniotic fluid.

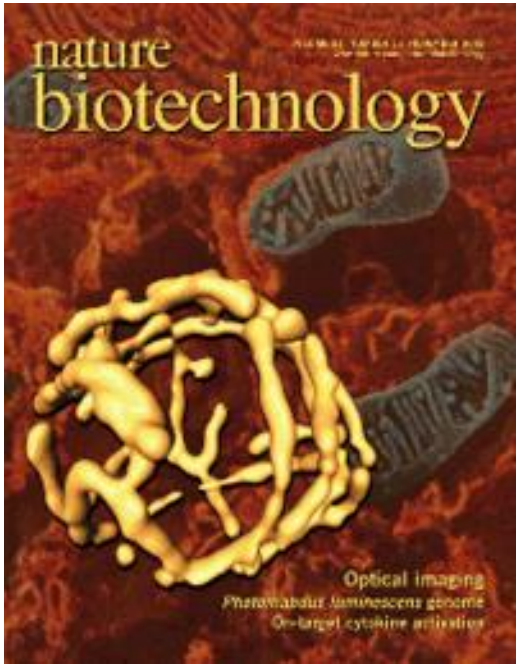




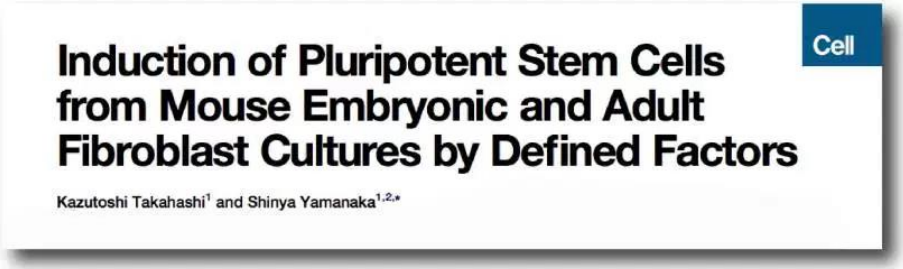
These stem cells could prove to be a viable alternative to the controversial use of embryonic stem cells

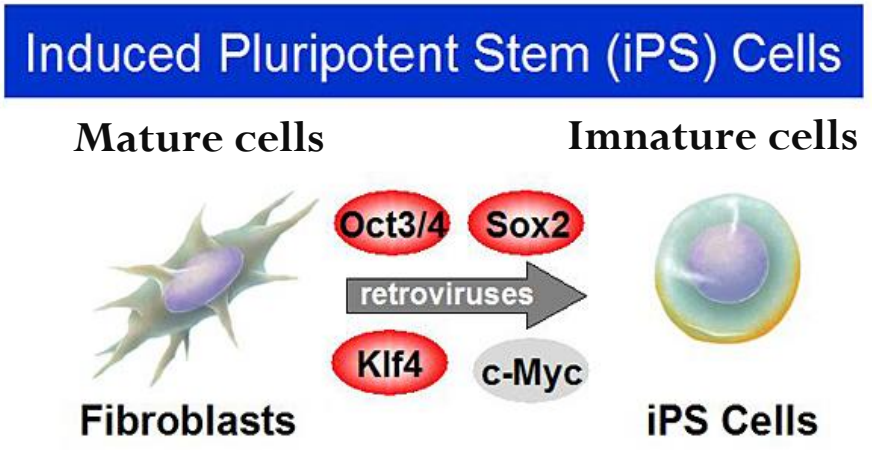
Dr. Anthony Atala is a practicing surgeon and a researcher in the area of regenerative medicine and one of the world's most influential people in biotechnology.

In 2016 scientists directed by Dr. Atala at Wake Forest Baptist Medical Center have proved that it is feasible to 3D-print living tissue structures to replace injured or diseased tissue in patients.



Reporting in ”Nature Biotechnology”, the scientists said they printed ear, bone and muscle structures.



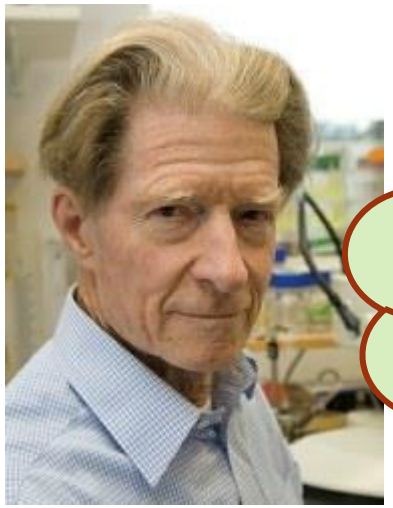


His 2006 publication in the journal *Cell* was hailed a breakthrough, and recent research has confirmed that iPS cells can give rise to all the different cell types of the body.

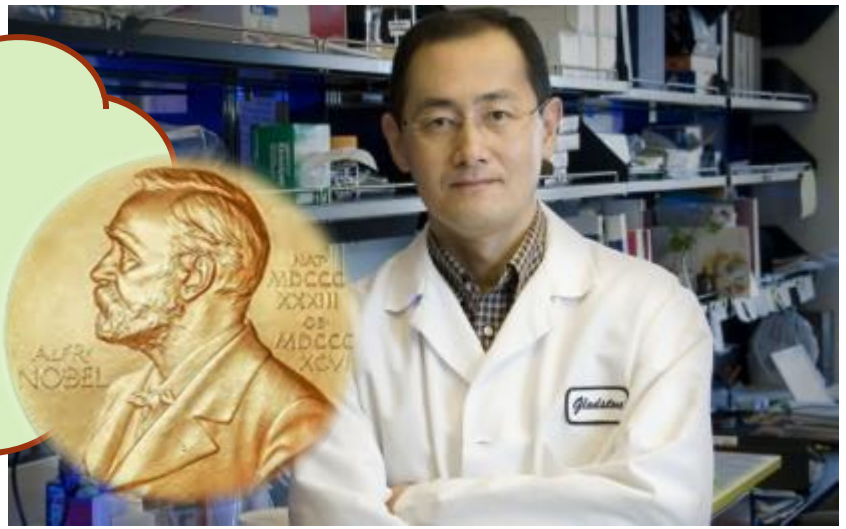
Mouse iPS cells reported in 2006

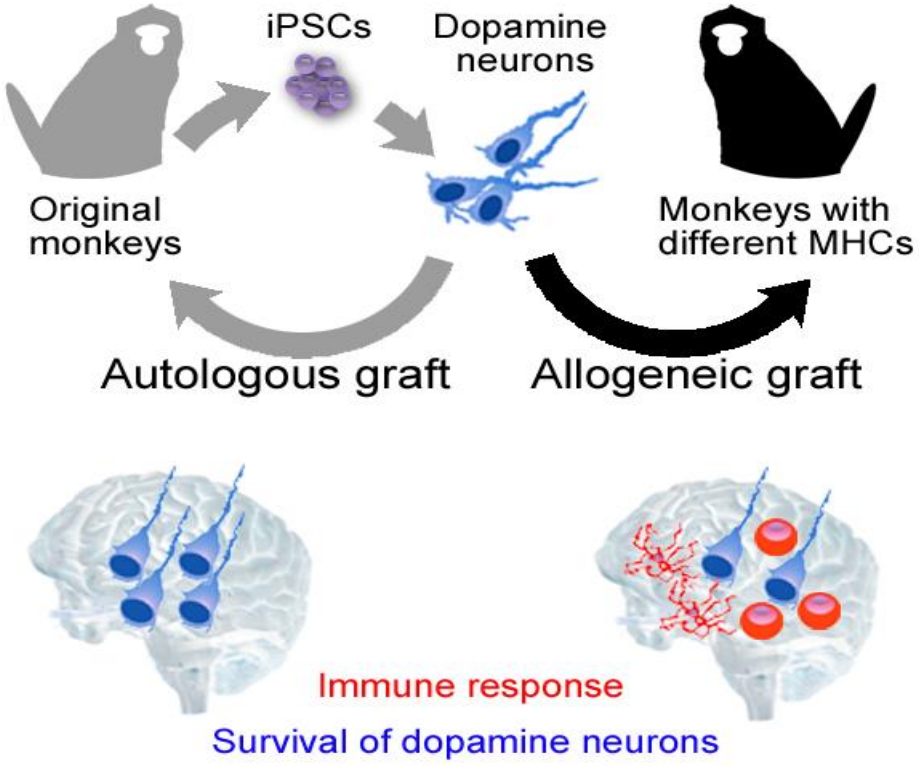
Human iPS cells reported in 2007

The Content of Yamanaka's Discovery

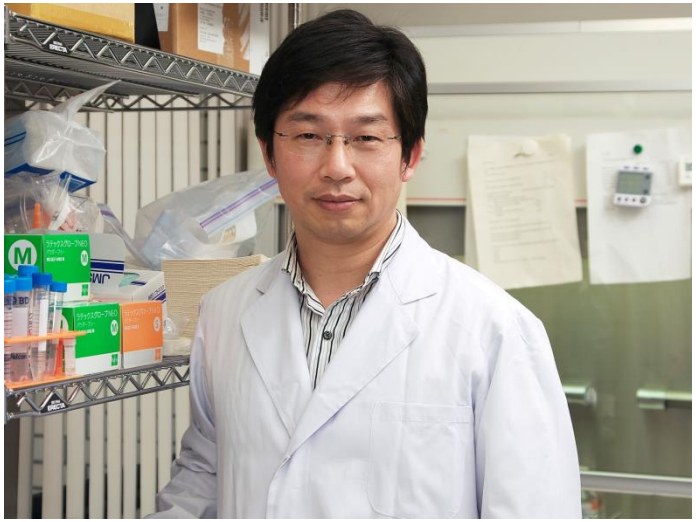


Prof. Shinya Yamanaka and Prof. Sir John B. Gurdon of the UK have jointly won the Nobel Prize in Physiology or Medicine 2012.



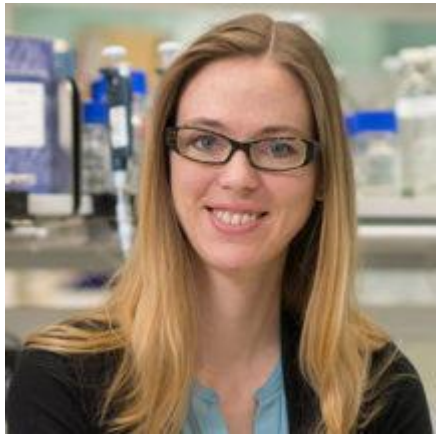


In 2013 Researchers led by ***Professor Jun Takahashi*** at Kyoto University, Japan, have carried out a study to compare the impact of immune response in autologous transplantation and allogeneic transplantation



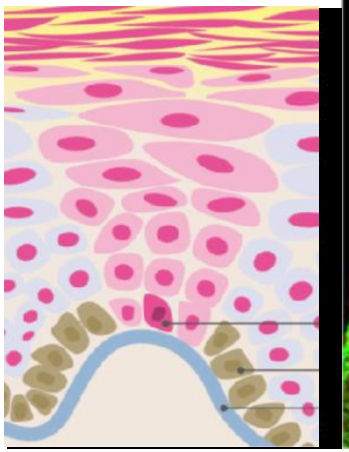
Transplantation of cells from the subject's own body

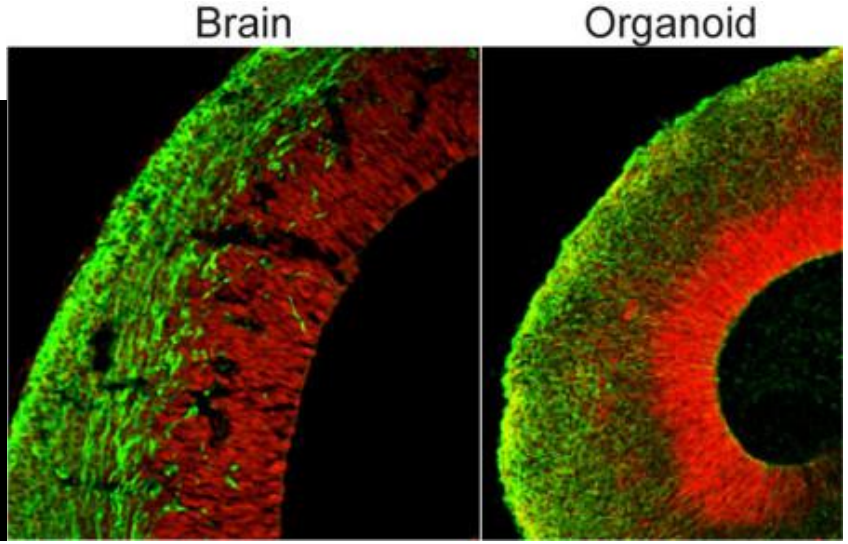
Transplantation of cells from a different individual of the same species



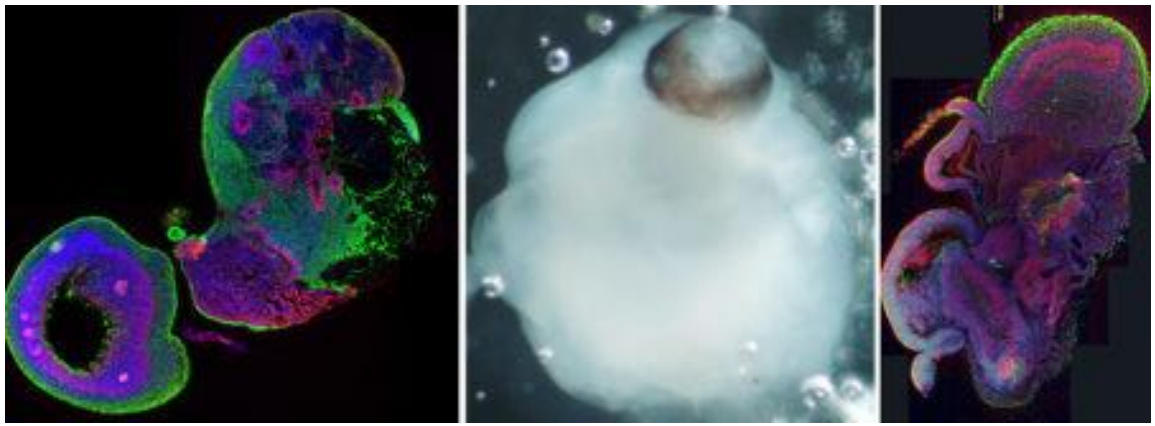
In 2016 - neuroscientist ***Madeline Lancaster*** from Cambridge in vitro bred human brain from skin stem cells, treating them with special nutrients.

Skin stem cells

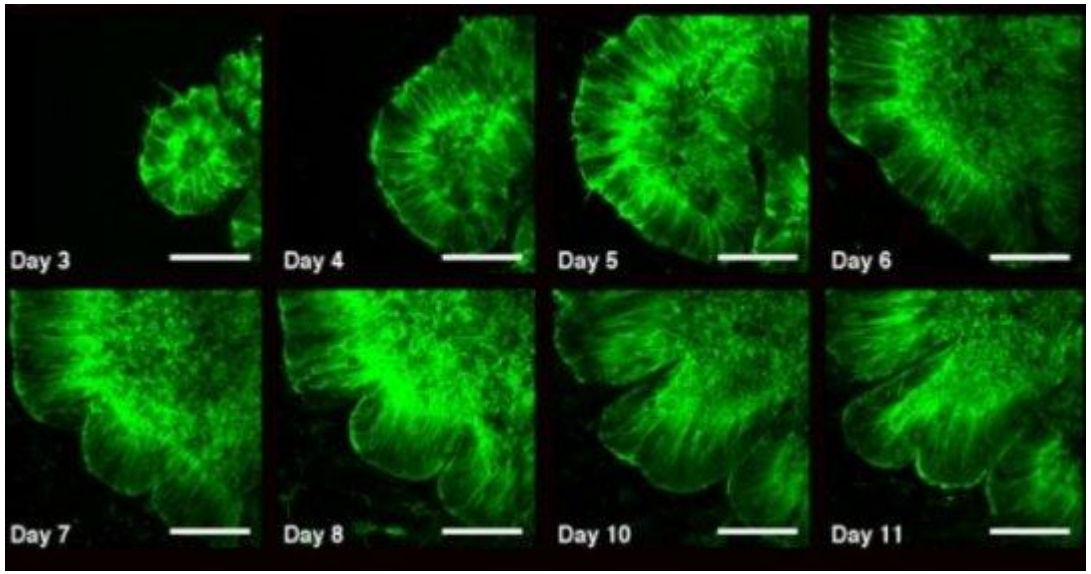




Madeline's group is studying brain development and disease using in vitro methods to model human disease.

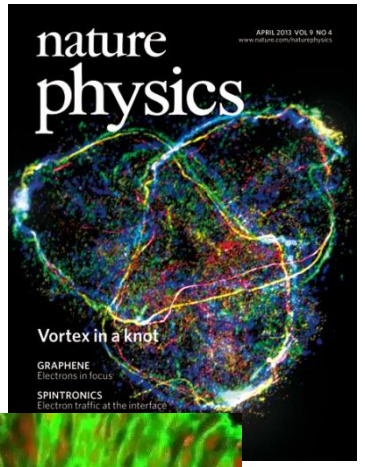


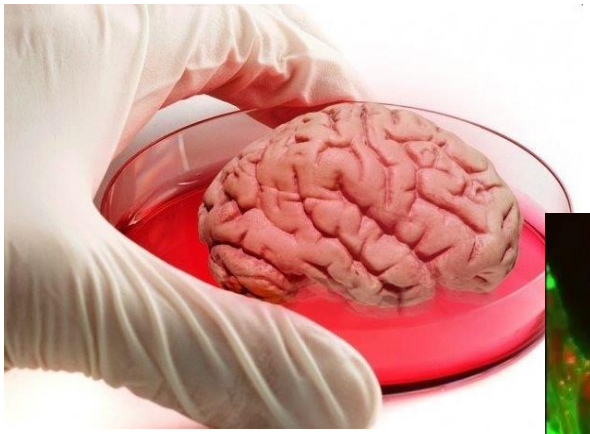
The stages of human brain growing as were presented in **"The EMBO Journal"** by M.Lancaster

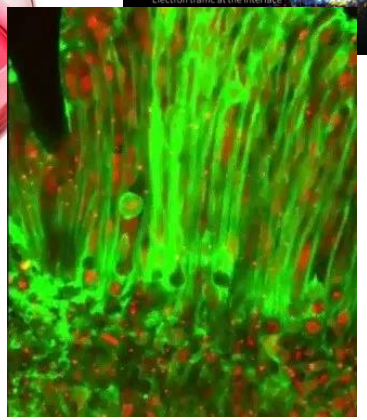


That method enables them to track the physical and biological mechanisms underlying the wrinkling process. They managed to identify groups of genes responsible for the violation of the normal development of brain wrinkles

Fluorescence images show the development of an organoid over days 3-11, in which the emergence of wrinkles is clearly seen

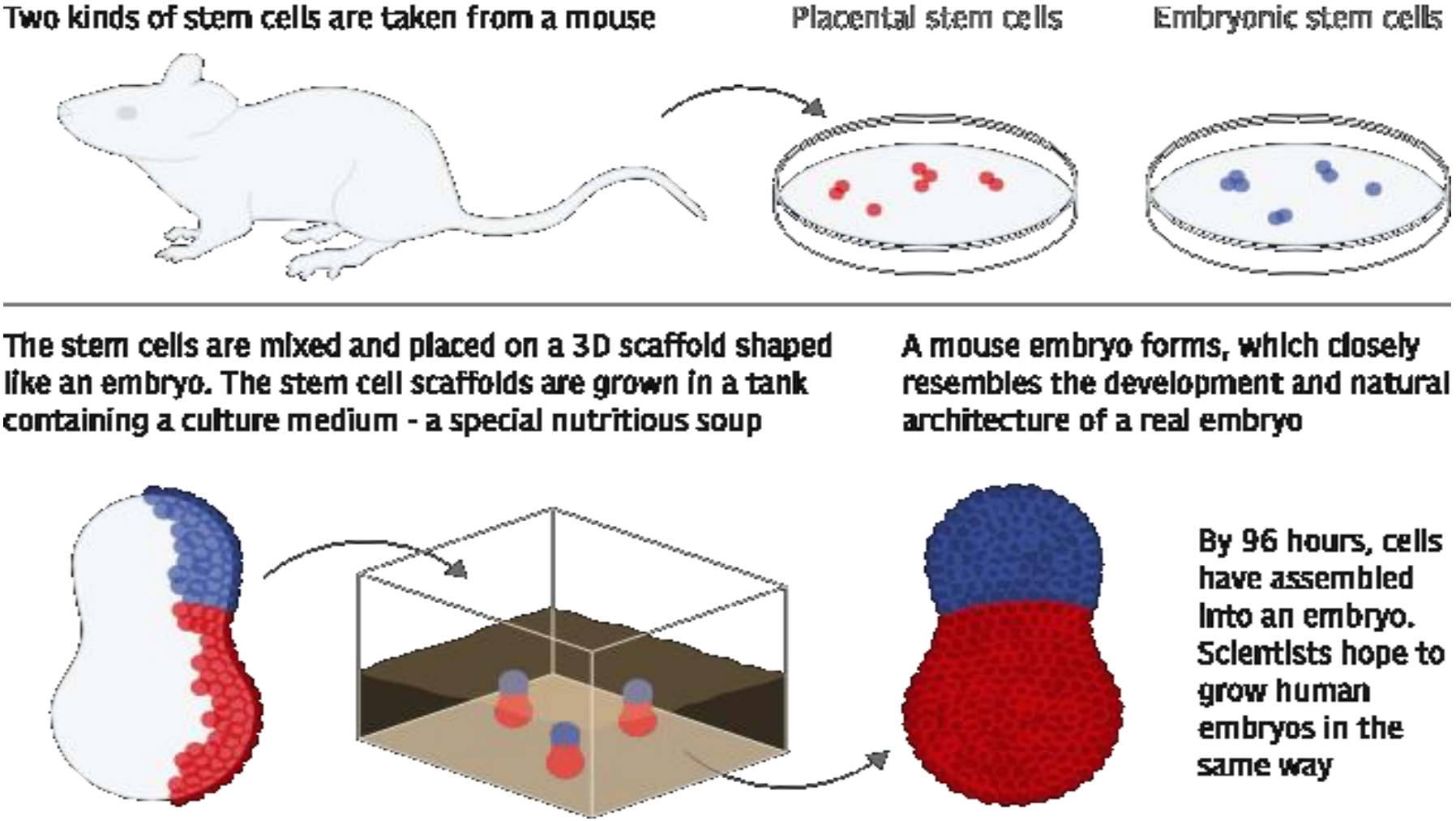




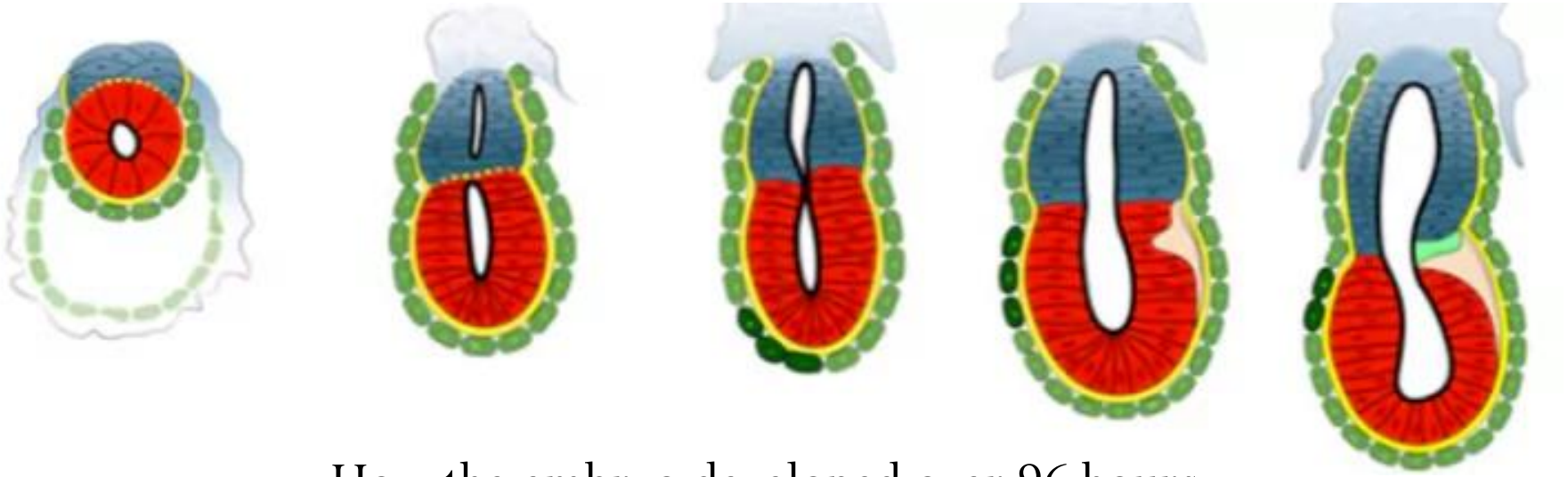


In 2018 Professor Orly Rainer with scientists from the Weizmann Institute of Science (Israel) has developed a mini brain in vitro using embryonic stem cells.

Artificial human life could soon be grown in lab



Magdalena Zernicka-Goetz from Cambridge, 2017



How the embryo developed over 96 hours
(added from Cambridge University site)

Eventually an embryonic structure was formed. It had two distinct clusters of cells at each end and a cavity in the middle in which the embryo would continue to develop.

The embryo would not grow into a mouse because it lacked the stem cells which made a yolk sack.

Research on stem cells continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms.

Stem cell research raises scientific questions as rapidly as it generates new discoveries.

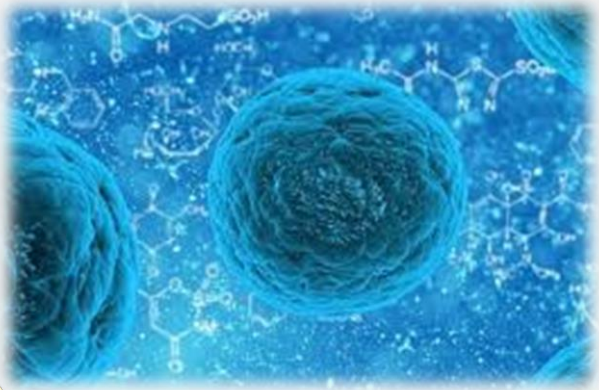
What are The Unique Properties of All Stem Cells?

Dividing and renewing themselves for long periods

Unspecificity

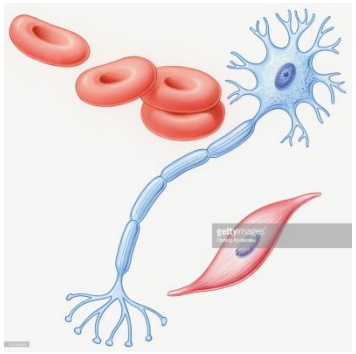
Capability to specialize into cell types.

All stem cells have three general properties

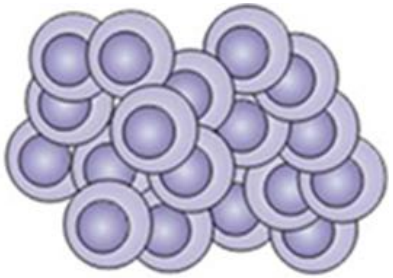


Stem cells differ from other kinds of cells in the body.

Stem Cells are Capable of Dividing and Renewing Themselves for Long Periods



Do not normally replicate themselves

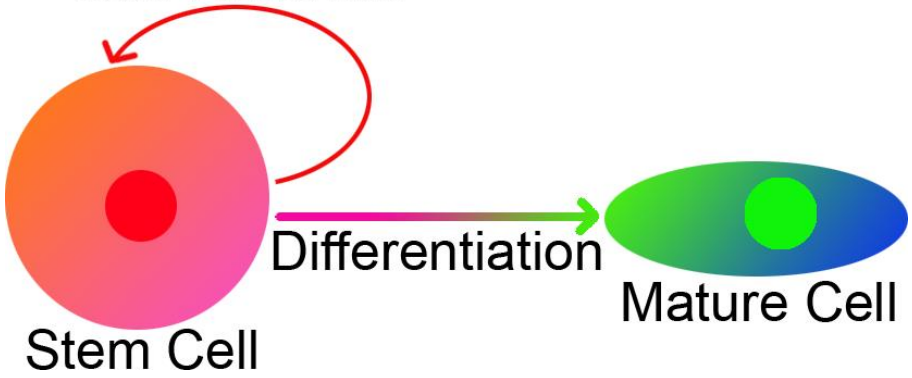


A starting population of stem cells…

…may replicate many times, or proliferate

… to yield millions of cells for many months in the lab.

If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are capable of long-term self-renewal.

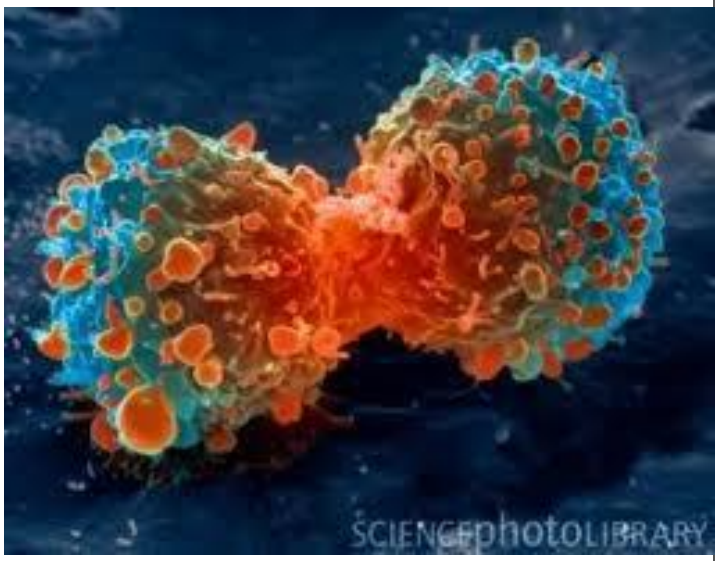


Scientists are trying to understand two fundamental properties of stem cells that relate to their long-term self-renewal:

- Why can embryonic stem cells proliferate for a year or more in the laboratory without differentiating, but most adult stem cells cannot;
- What are the factors in living organisms that normally regulate stem cell proliferation and self-renewal?

How cell proliferation is regulated during normal embryonic development or during the abnormal cell division that leads to cancer.

Such information would also enable scientists to grow embryonic and non-embryonic stem cells more efficiently in the laboratory

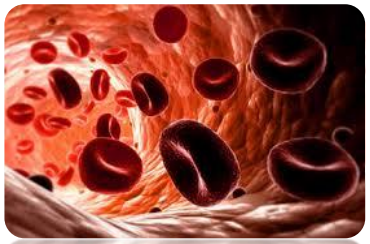


Dividing Cancer Cell in Microscope

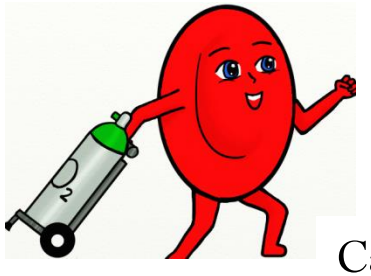
Stem Cells are Unspecialized

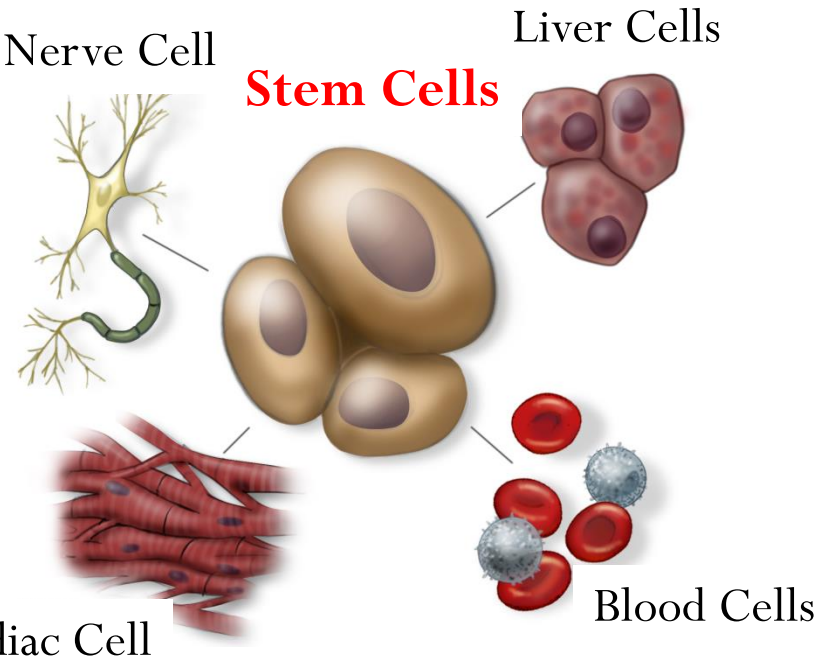
One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions.

A stem cell cannot work with its neighbors to pump blood through the body.



It cannot carry oxygen molecules through the bloodstream.

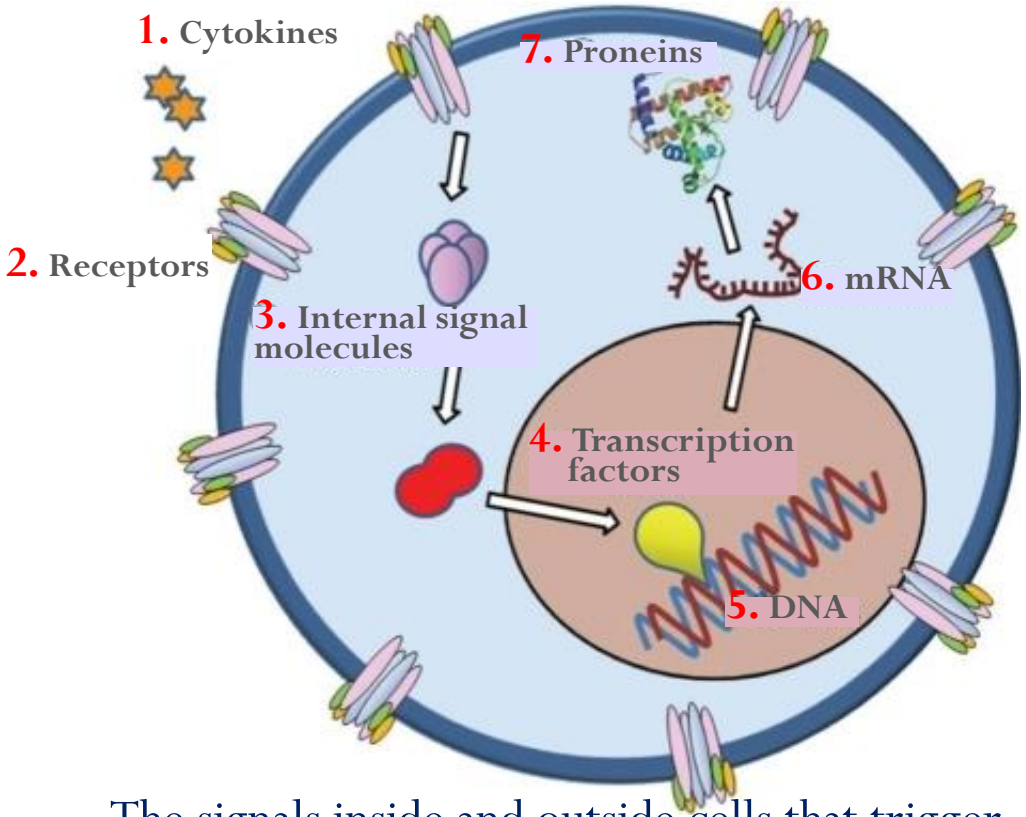




Stem cells can give rise to specialized cells

Stem Cells Can Give Rise to Specialized Cells

When unspecialized stem cells give rise to specialized cells, the process is called **differentiation**.
While differentiating, the cell usually goes through several stages, becoming more specialized at each step.

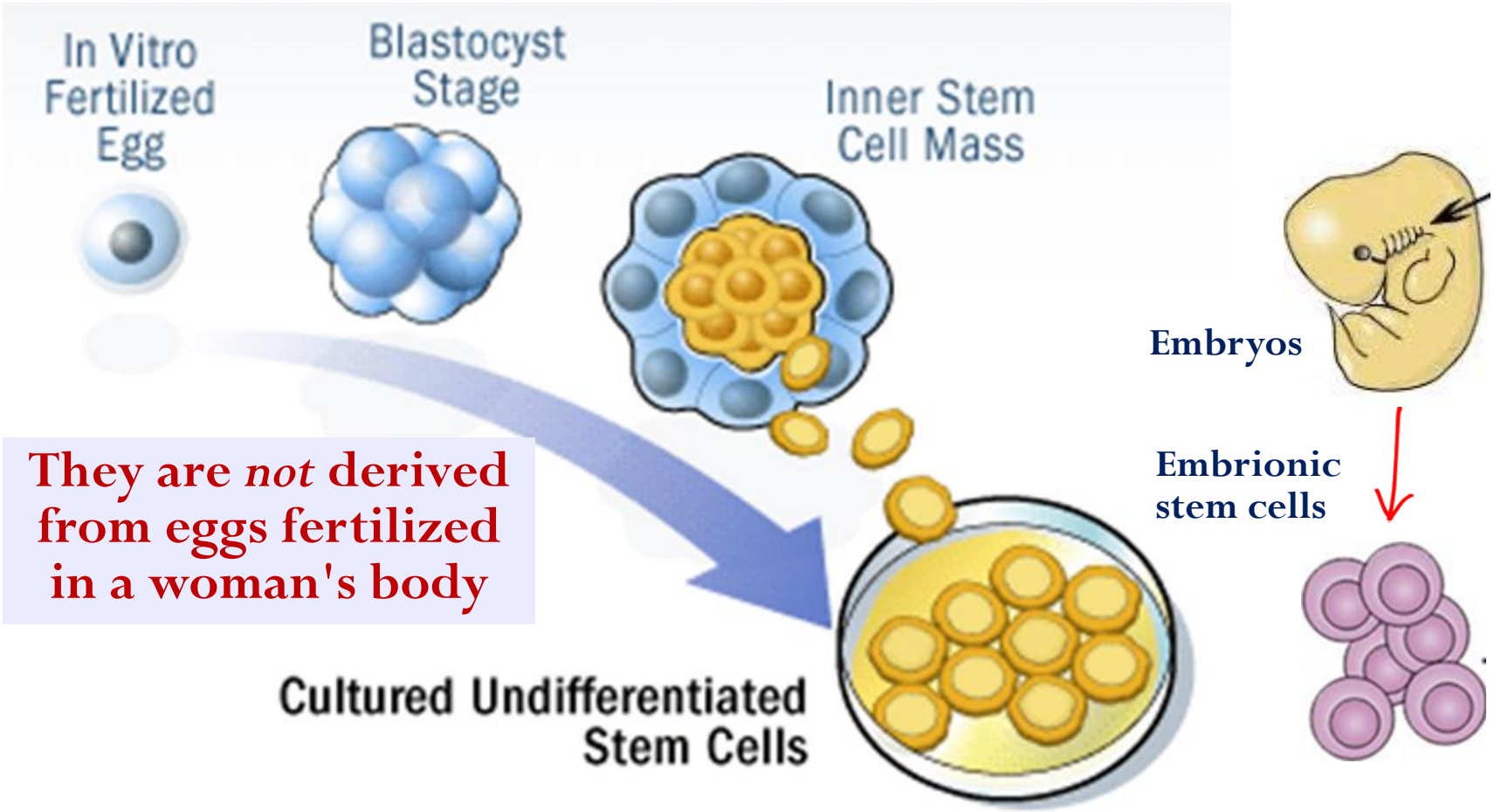


The signals inside and outside cells that trigger each step of the differentiation process.

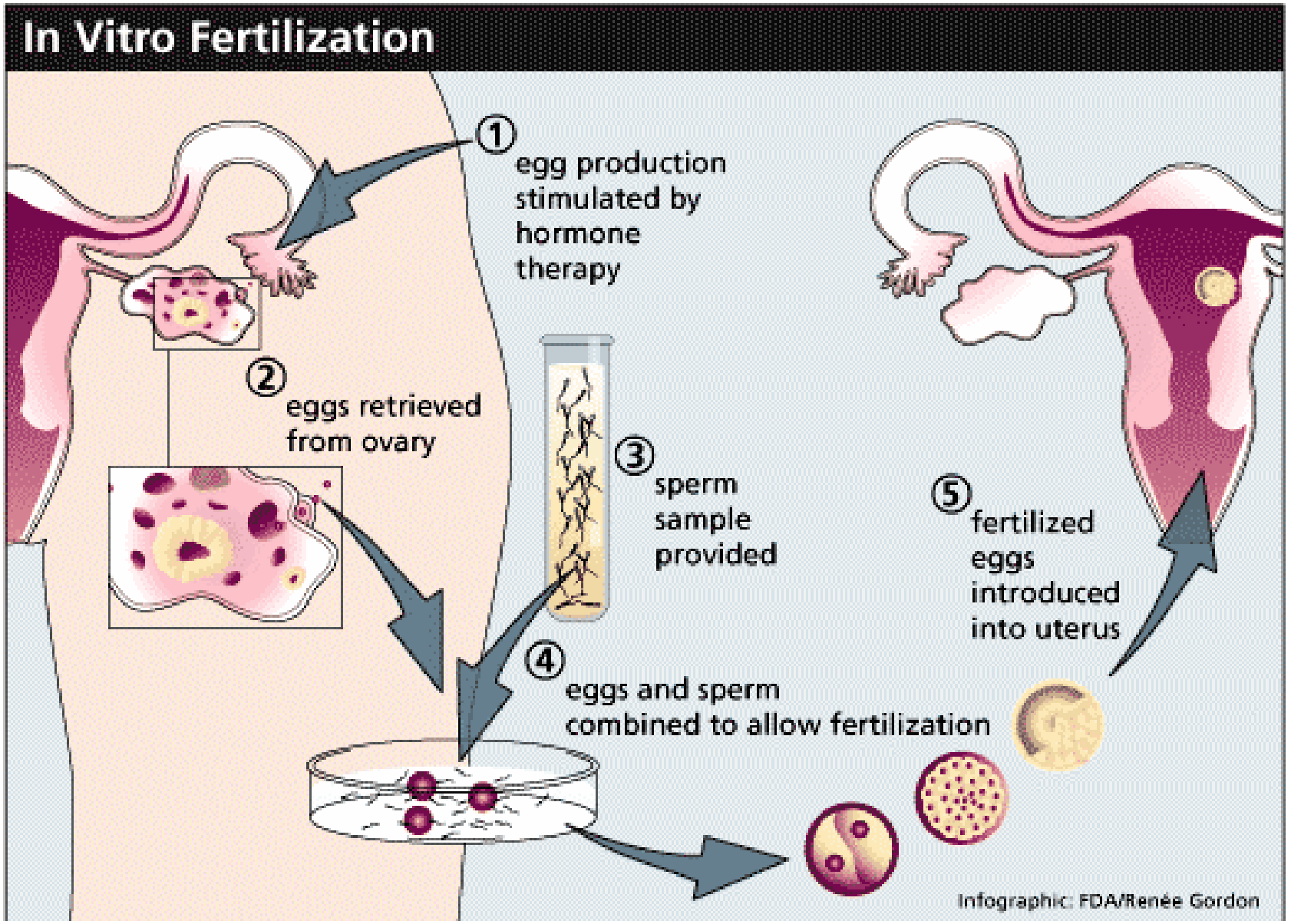
The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the microenvironment.

The internal signals are controlled by cells' genes, which are interspersed across strands of DNA and carry coded instructions for all cellular structures and functions.

What are Embryonic Stem Cells?



Cultured in-vitro Embryonic Stem Cells may be donated for research purposes with informed consent of the donors.



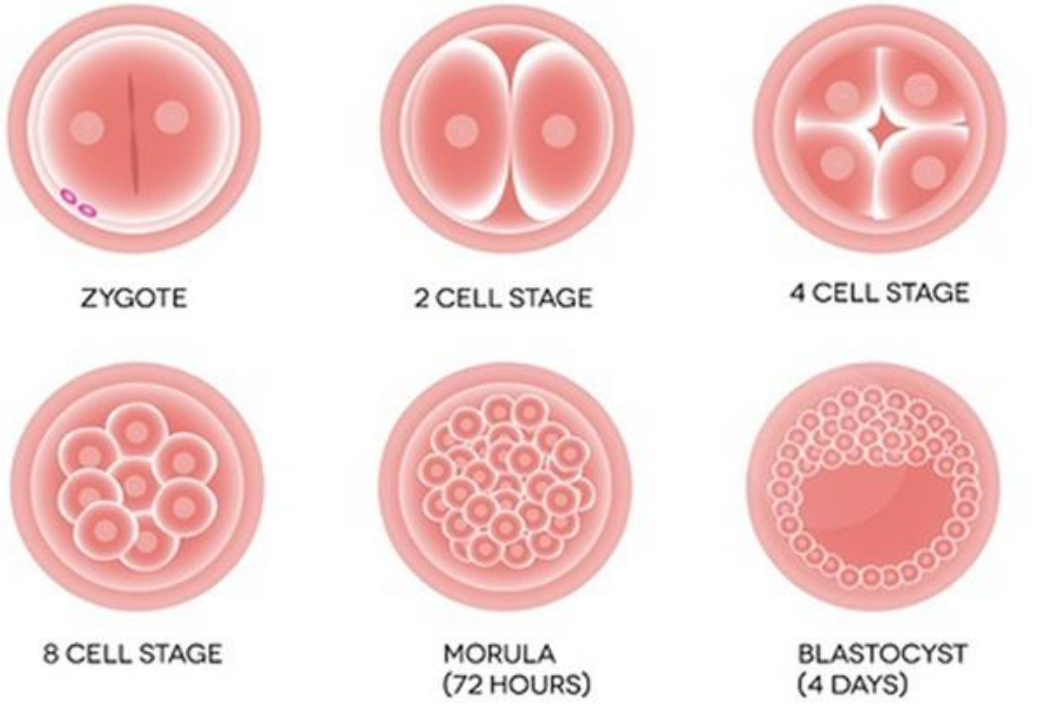
Among several eggs that fertilized in a test tube in vitro, only one is implanted into a woman.

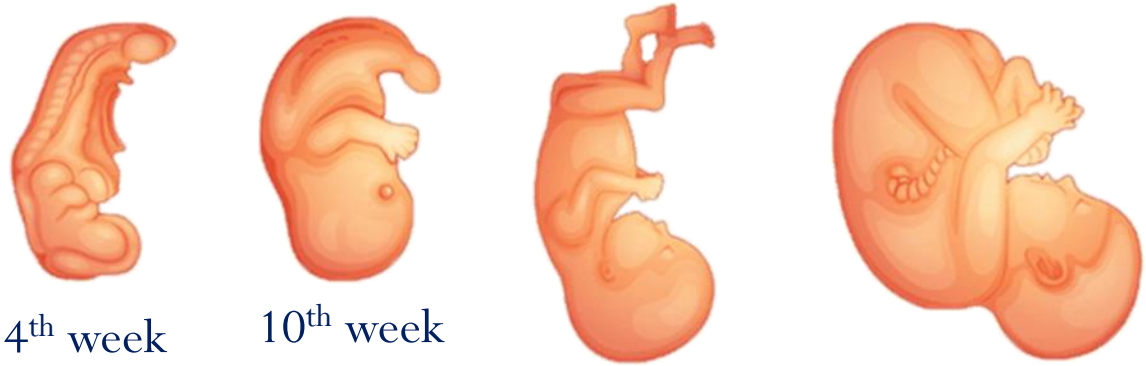
Embrio Development

The single zygote cell then begins a series of divisions

The blastocyst stage continues until implantation of the embryo in the uterus, at which point the embryo is referred to as a fetus.

Embryo becomes a Fetus usually by the end of the 10th week of gestation after all major organs of the body have been created.





4th week

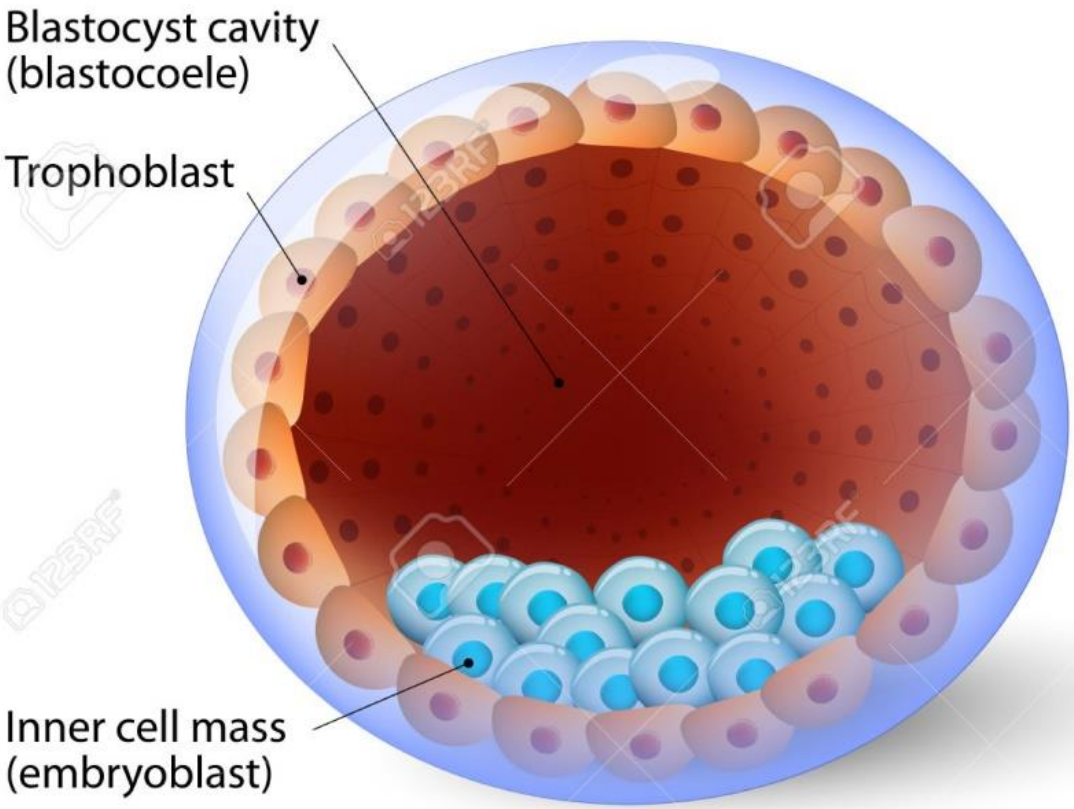
10th week

16th week

20th week

BLASTOCYST

consists of…



Embryonic stem cells are the source of totipotent cells.

Totipotent cells are the cells with total potential to develop into any cell in the body)

the group of cells that will differentiate to become all the structures of an adult organism.

Outer Cell Mass

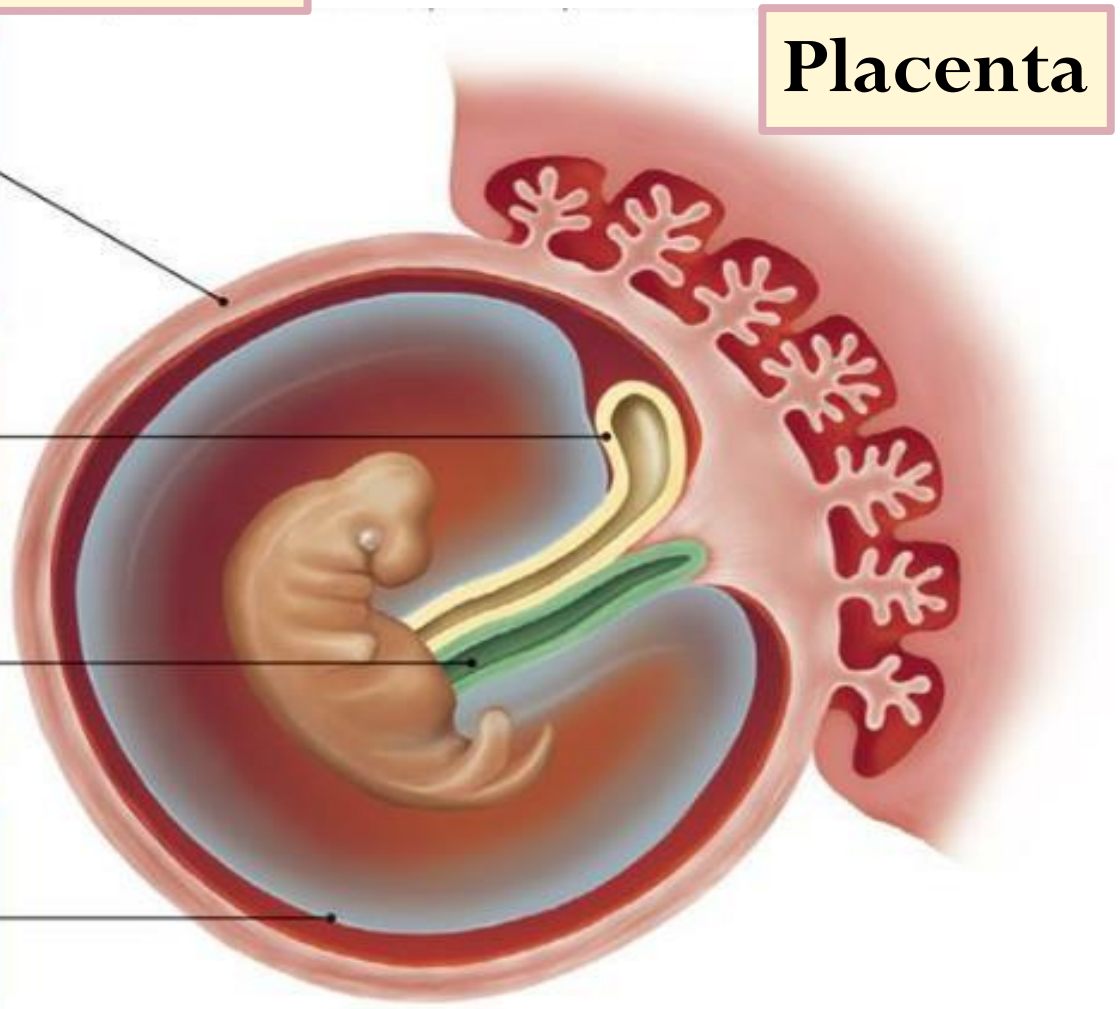
Extra embryonic Tissues

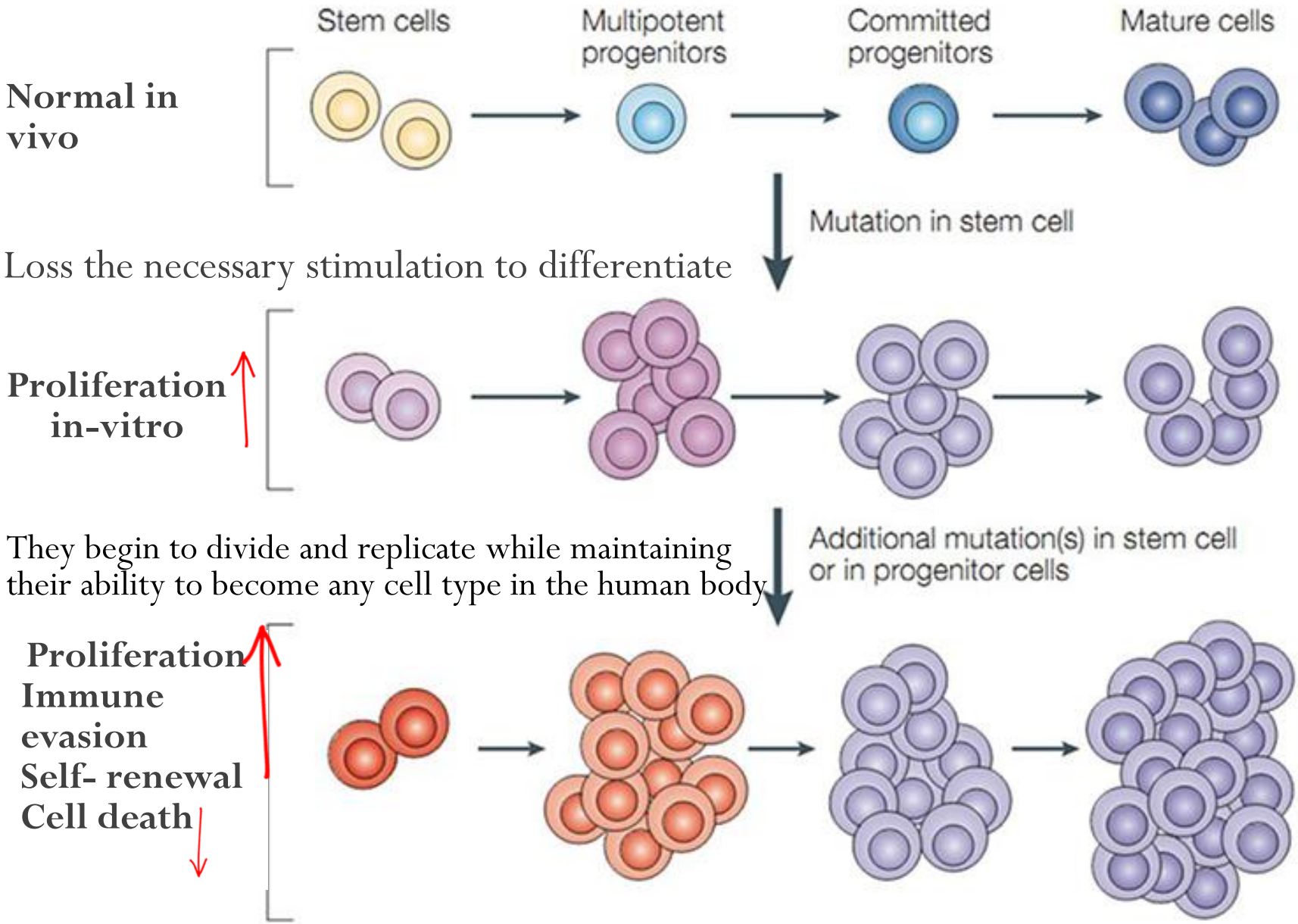
chorion
becomes part of the placenta where the embryo/ fetus receives oxygen and nutrient molecules and rids itself of waste molecules

yolk sac
first site of blood cell formation

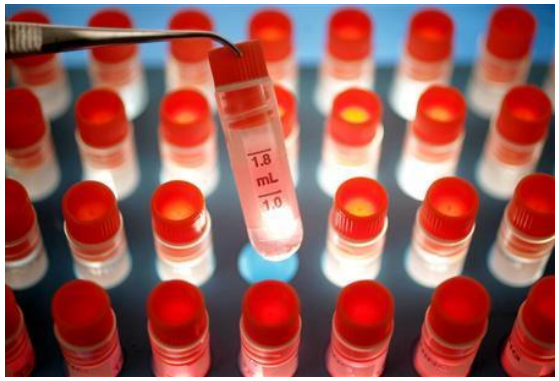
allantois
its blood vessels become the blood vessels of the umbilical cord

amnion
contains the amniotic fluid, which cushions and protects the embryo



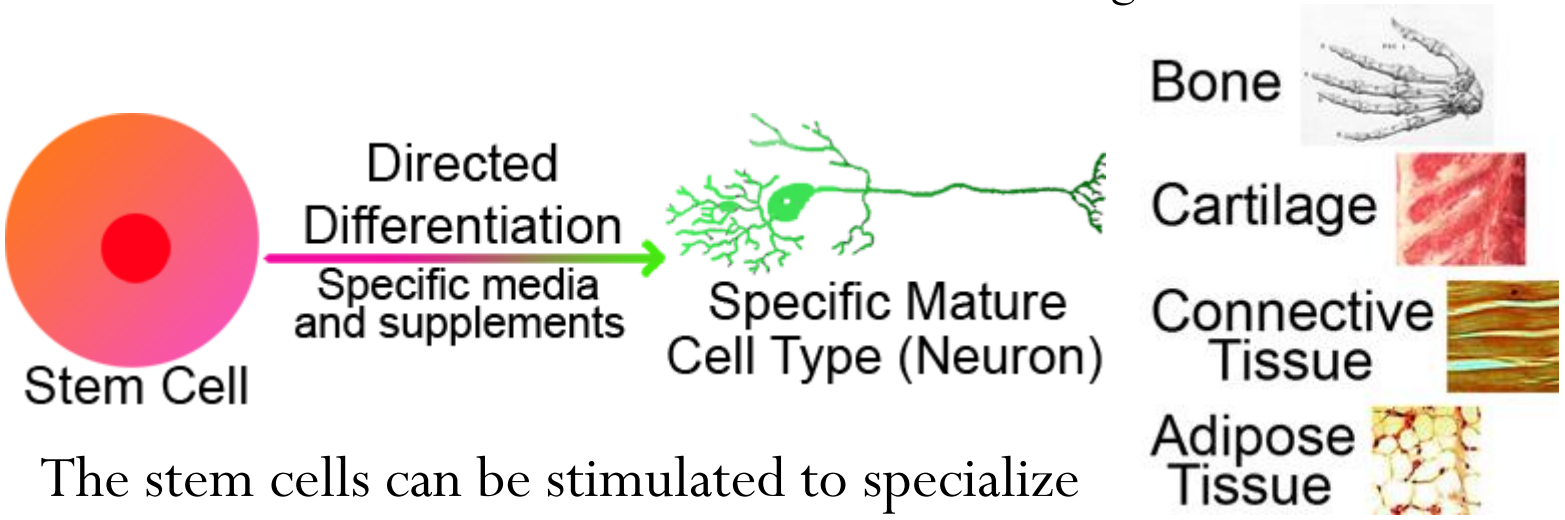

Stem cells
Multipotent progenitors
Committed progenitors
Mature cells
Normal in vivo
Mutation in stem cell
Loss the necessary stimulation to differentiate
Proliferation in-vitro
They begin to divide and replicate while maintaining their ability to become any cell type in the human body
Additional mutation(s) in stem cell or in progenitor cells
Proliferation
Immune evasion
Self- renewal
Cell death
These undifferentiated cells can be stimulated to create specialized cells

Stem Cell Lines



A stem cell line - a controlled culture of healthy, dividing, and undifferentiated cells.

These stem cell lines are subsequently managed and shared among researchers



The stem cells can be stimulated to specialize

Potency

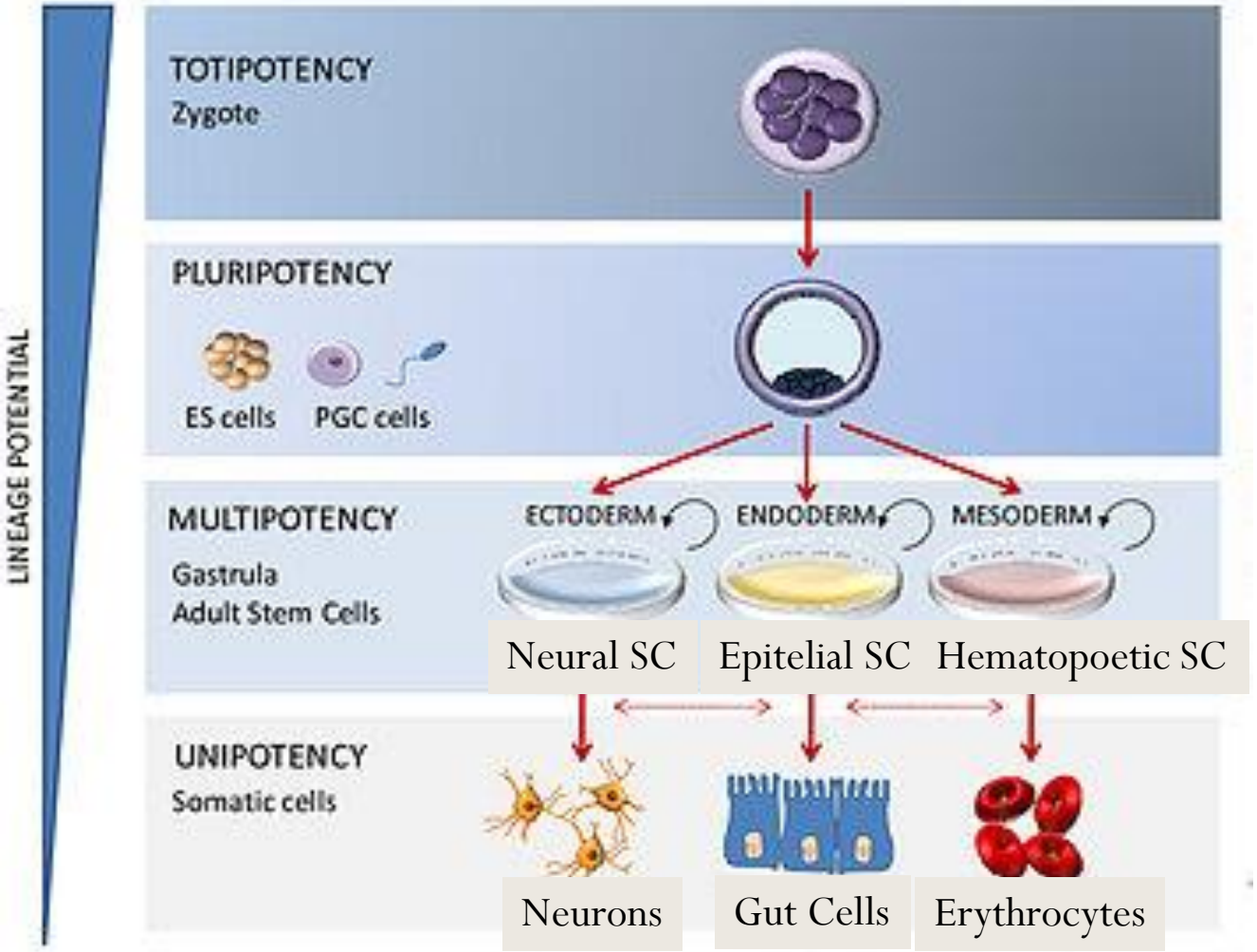
Stem cells are categorized by their potential to differentiate into other types of cells.

Differentiating into all possible cell types

Differentiating into almost cell types

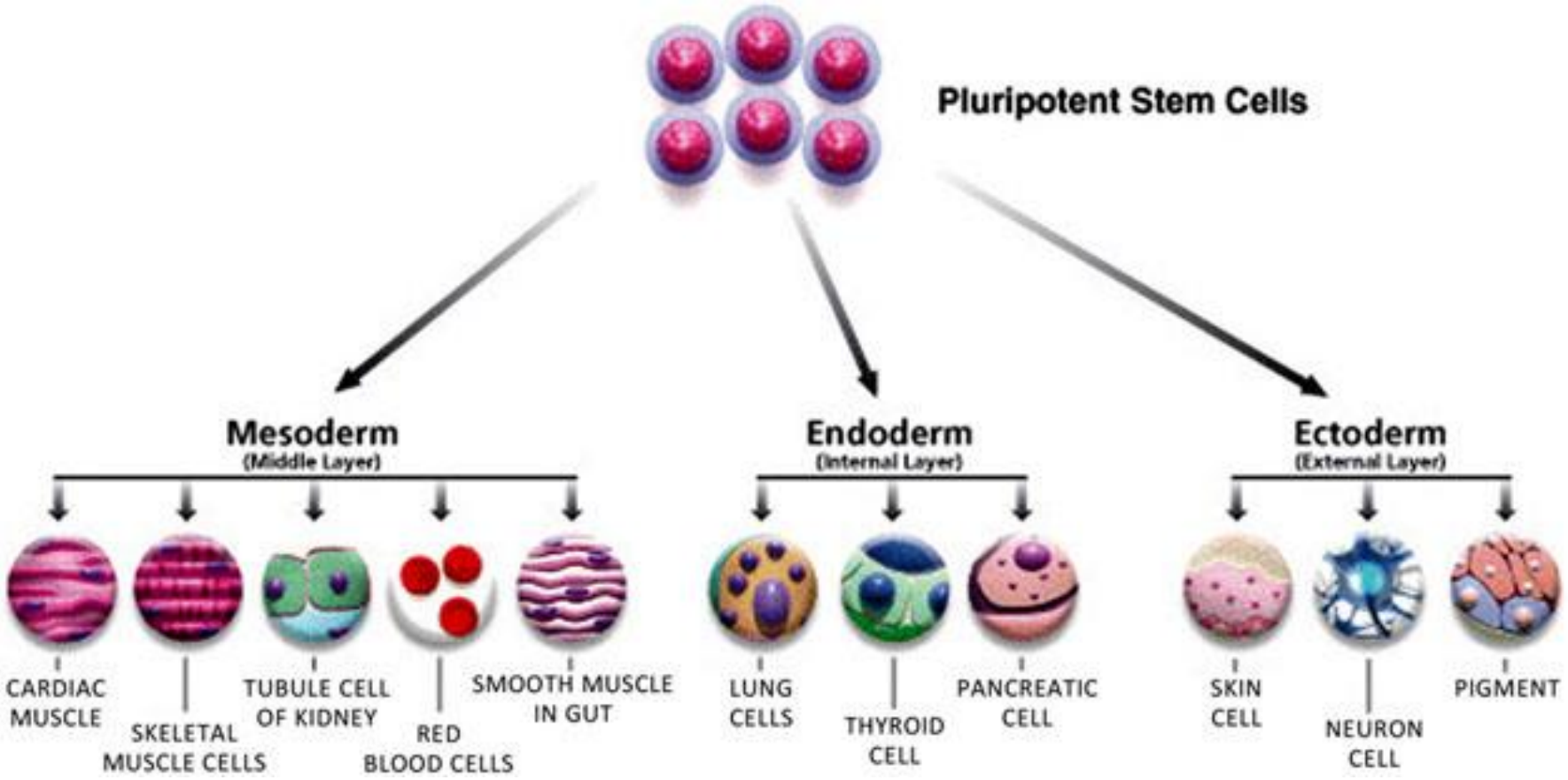
Differentiating into a closely related family of cells.

Only produce cells of their own type.



They have the property of self-renewal required to be labeled a stem cell. Examples include (adult) muscle stem cells.

Normal Differentiation Pathways of Adult Stem Cells



Embryonic stem cells are considered pluripotent instead of totipotent because they do not have the ability to become part of the extra-embryonic membranes or the placenta.

Normal Differentiation Pathways of Adult Stem Cells

That gives hope for the treatment of various diseases.

Signal of any "problem"

Self-Renewing

Hematopoietic Stem Cell

RBC

WBC

Platelet

Self-Renewing

Mesenchymal Stem Cell

Chondrocyte

Osteoblast

Fibroblast

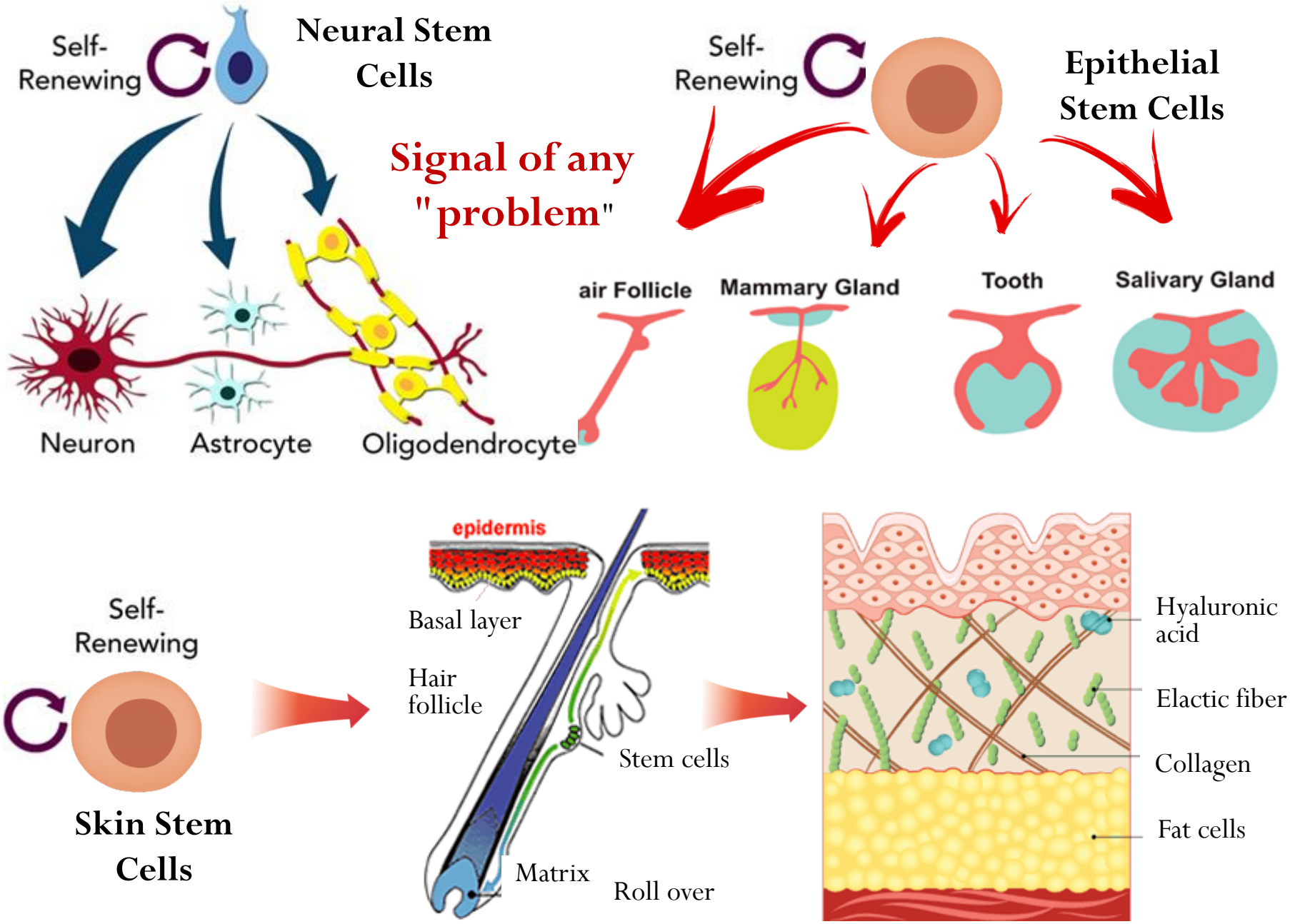
Adipocyte

Epithelial cell

Skeletal muscle

Neuron

Glial cell


Self-
Renewing
Neural Stem
Cells
Signal of any
"problem"
Neuron
Astrocyte
Oligodendrocyte
Self-
Renewing
Epithelial
Stem Cells
air Follicle
Mammary Gland
Tooth
Salivary Gland
Self-
Renewing
Skin Stem
Cells
epidermis
Basal layer
Hair
follicle
Stem cells
Matrix
Roll over
Hyaluronic
acid
Elactic fiber
Collagen
Fat cells

Renewable Function of Stem Cells

The correlation of stem cells and advanced cells in different age

In the moment of birth the correlation of stem cells and advanced cells is 1:10 000

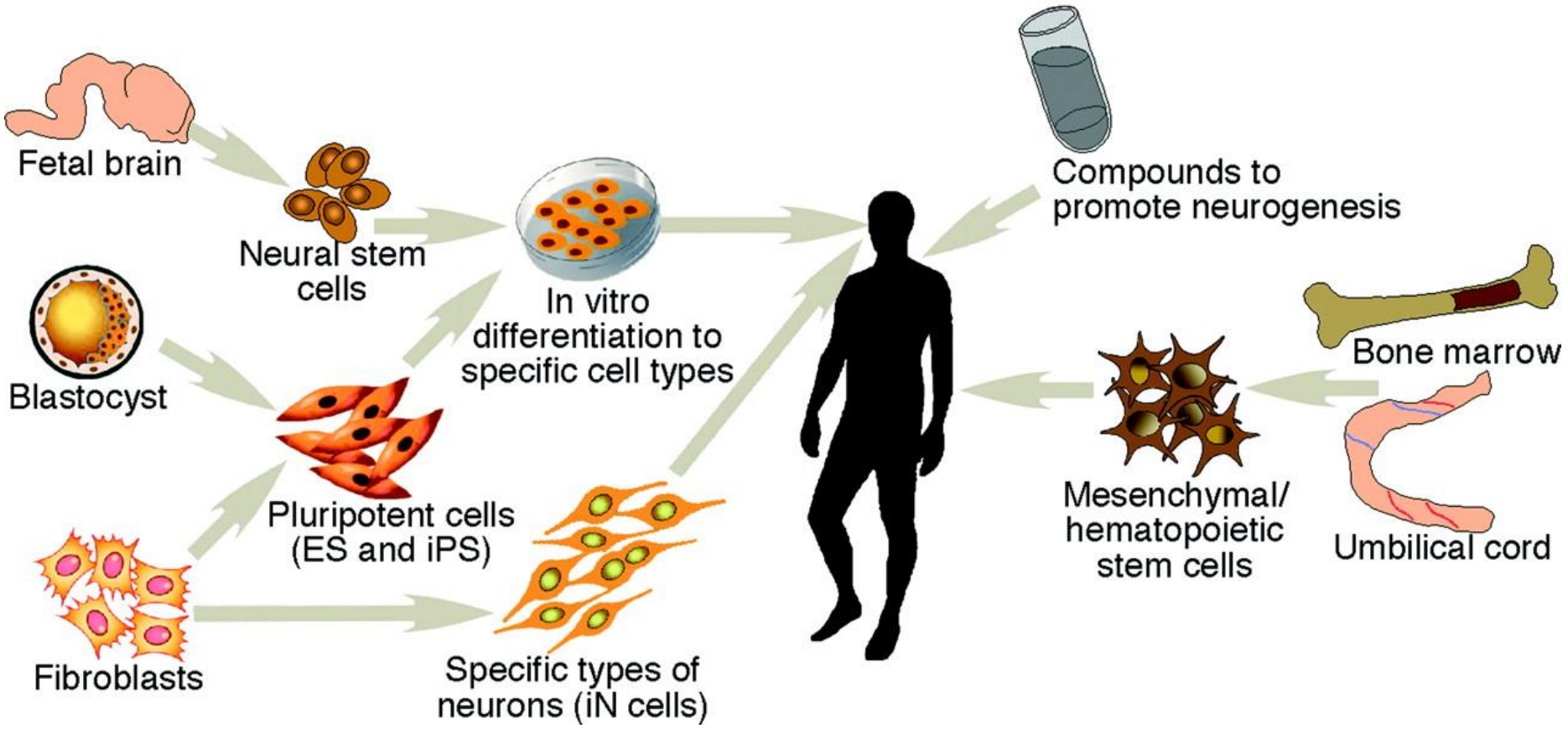
In the moment of 50 years old - 1: 500 000.

In the moment of 70 years old - 1: 1 000 000.

It happens that organism is not able to restore lost body cells itself - or cell destruction is very large, or the organism is weakened, or age is not the same.

Therefore the most convenient donor stem cells obtained directly at birth from the umbilical cord and placenta, where they are also sufficient.

STEM CELL SOURCES FOR STROKE THERAPY



Potential Sources for Stroke Therapy

- Umbilical Cord
- Bone Marrow
- Peripheral blood
- Fat
- Small quantities in almost every organ

Cord Blood Bankings

The reject phenomena on their own stem cells are eliminated completely

Since the use of stem cells is very effective in almost any diseases, associated with aging, we can expect that their maintenance from birth to senility will postpone the senile changes and radically lengthen life.

Cord banking at temperature -196 ° C (in liquid nitrogen) can be carried over decades (or even hundreds) of years. This allows you to store children's donor stem cell in case of the disease or vital senility.

Steps in cord blood banking

1

After birth the umbilical cord is clamped and cut

2

Cord blood is collected from umbilical cord vein by experts

3

Collected cord blood is safely packed in proprietary transfer kits and within 24 hours reach our labs

6

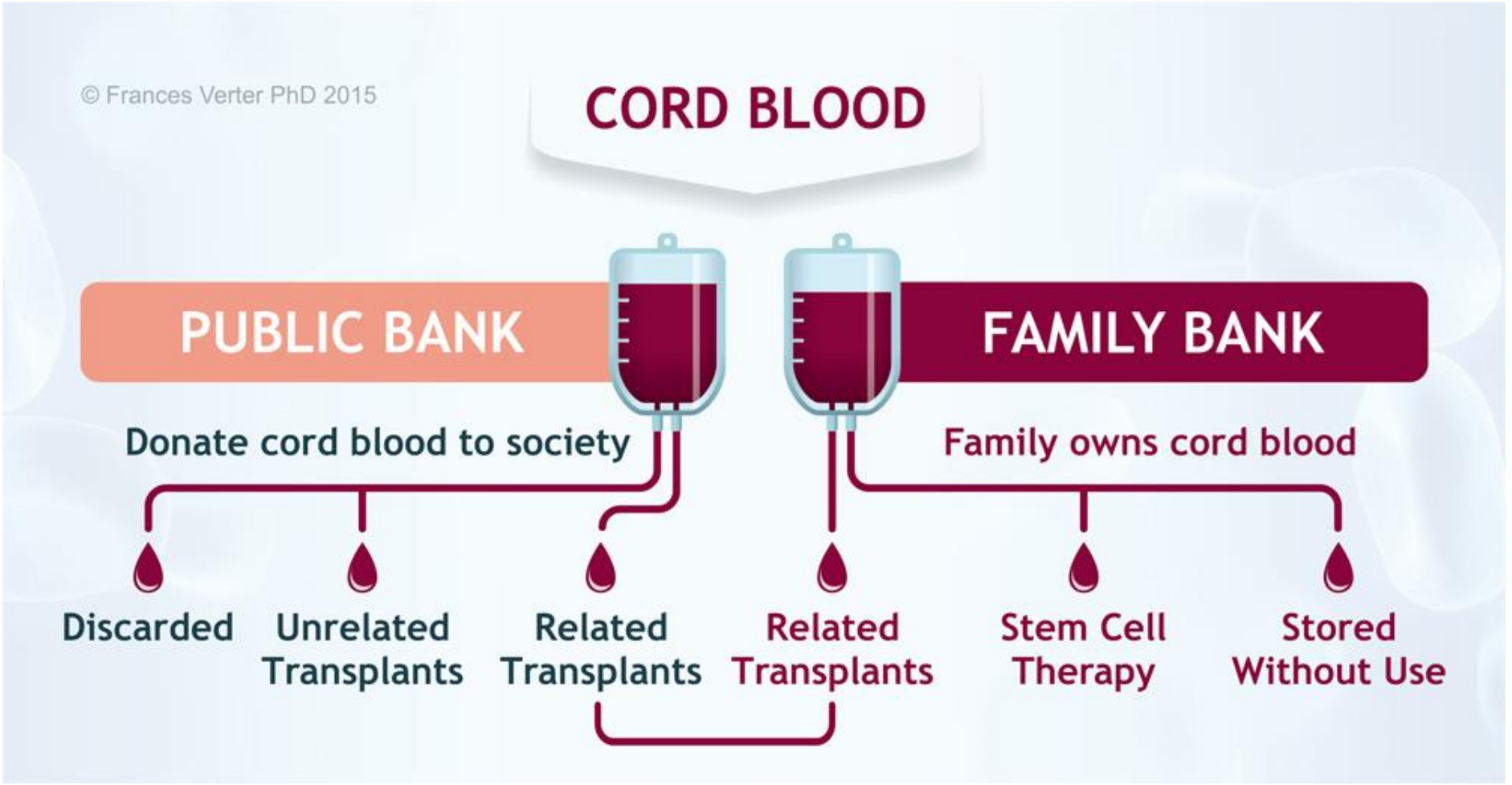
The end product is stored at -196 deg. C for 21 years

5

Cord blood is processed by patented technologies to yield maximum number of stem cells

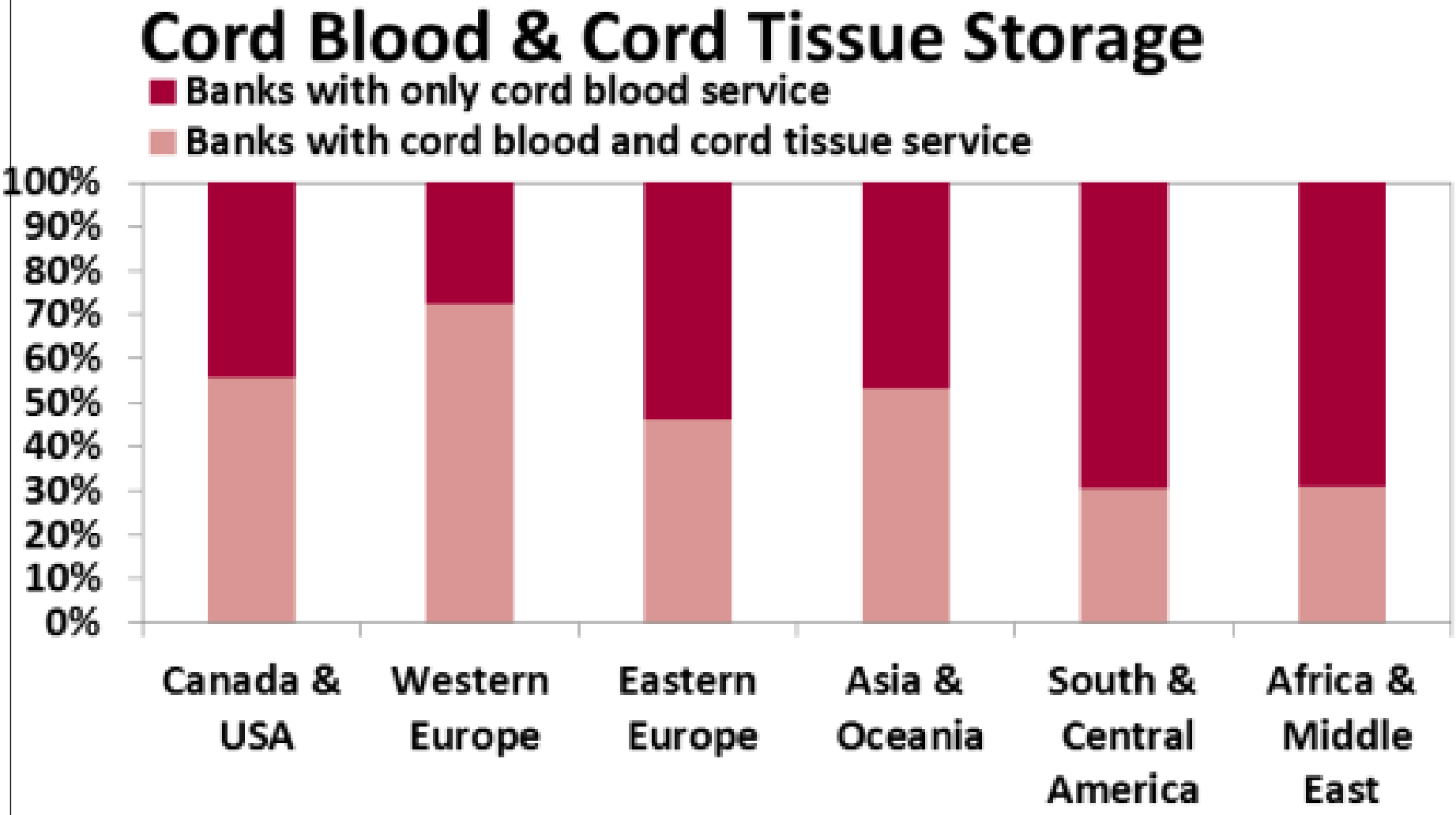
4

The sample quality is evaluated and all the required tests are conducted for maximum safety

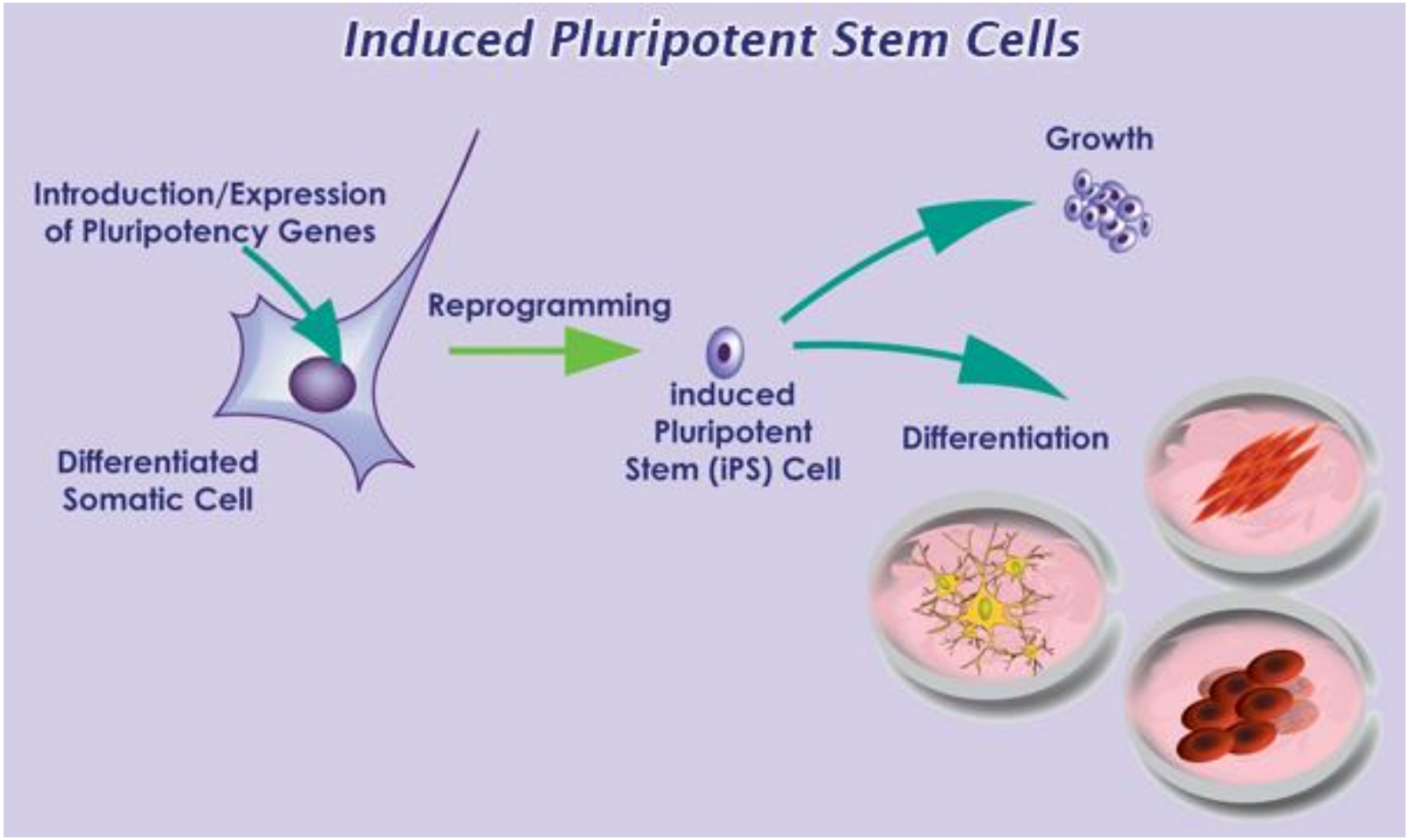


In many countries there are cord blood bankings.

Cord Blood & Cord Tissue Storage



Induced Pluripotent Stem Cells



IPS cells are critical tools to help scientists learn more about normal development and disease onset and progression, and they are also useful for developing and testing new drugs and therapies.

Applications of iPS cell

Cell reprogramming

Do not use of human embryos

Patient

iPS cells

Absence of the risk of immune rejection.

Neural cells

Cardiac cells

Hepatocytes

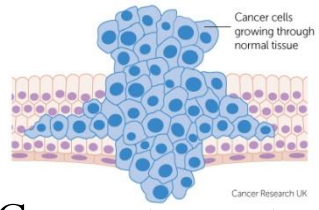
Beta cells

If it is the only chance for salvation

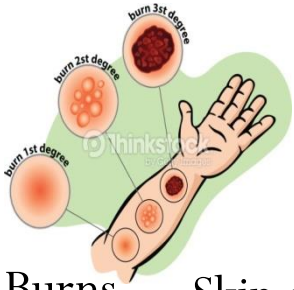
Heart

Regenerative medicine (*in vivo* use)

Toxicology disease model, drug screening (*in vitro* use)



Cancer tumort



Burns

Skin grat

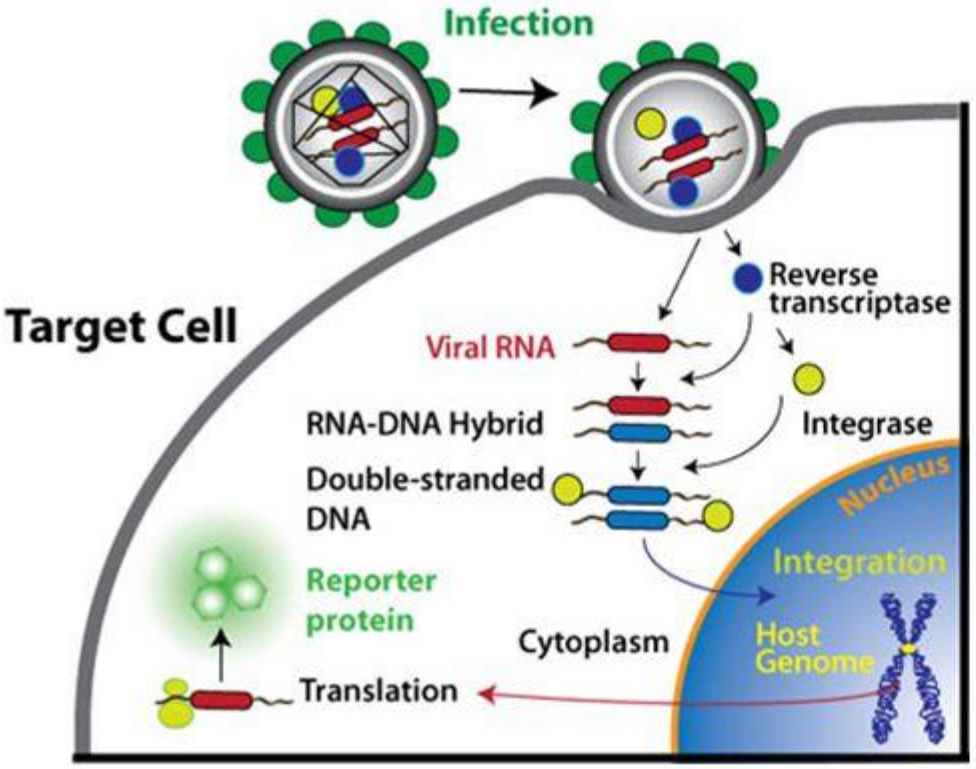
A model system to study the causes of diseases, development of treatment methods and new drugs.

Toxicological testing of the drugs' efficiency.

iPS cells – using retrovirus/lentivirus

Uses oncological retroviruses and transgenes

chemicals or viruses instead of…



Advantages:

- easy to use
- reproducible
- good efficiency
- controlled expression (inducible)

Disadvantages:

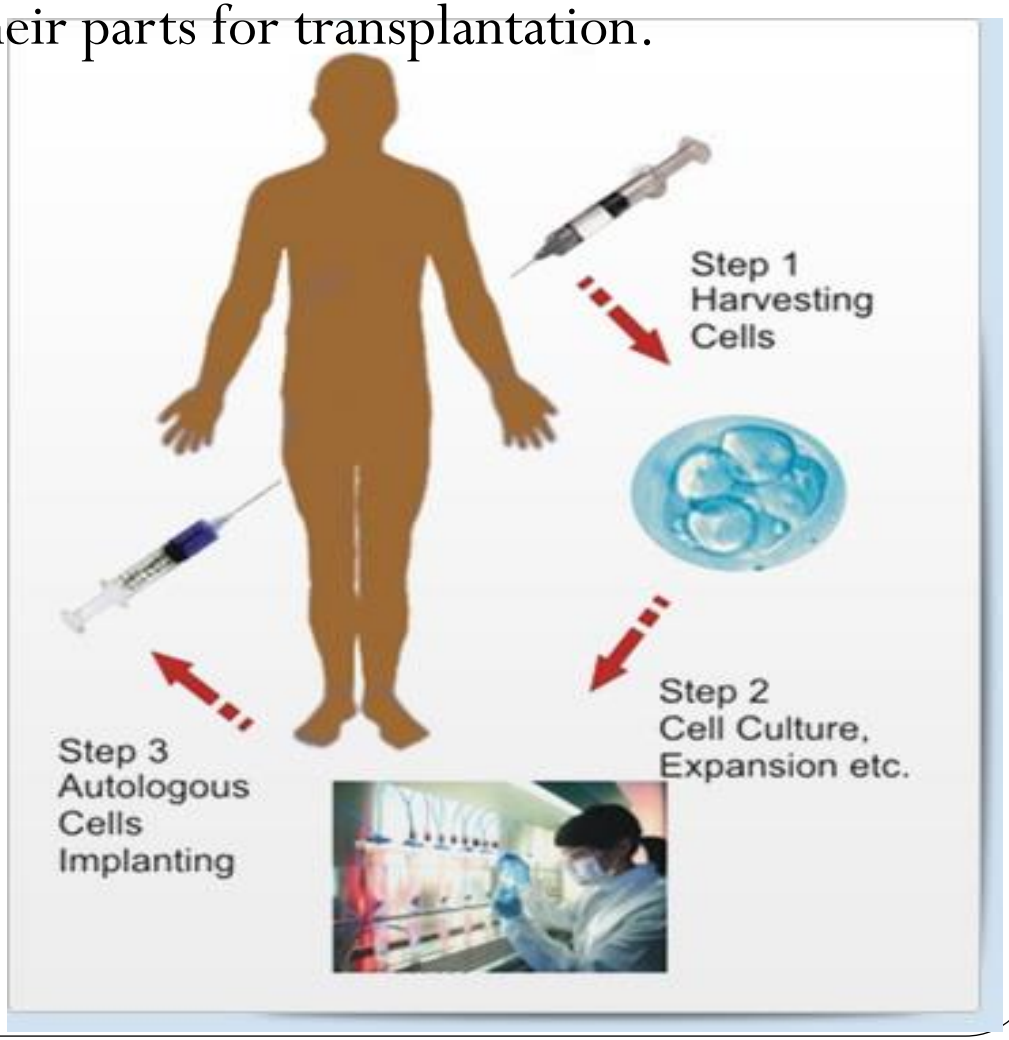
- increased risk of insertional mutagenesis
- possibility of transgene reactivation
- incomplete silencing
- clone to clone variation
- can cause mutations and other genomic disorders.

Research with Stem Cells

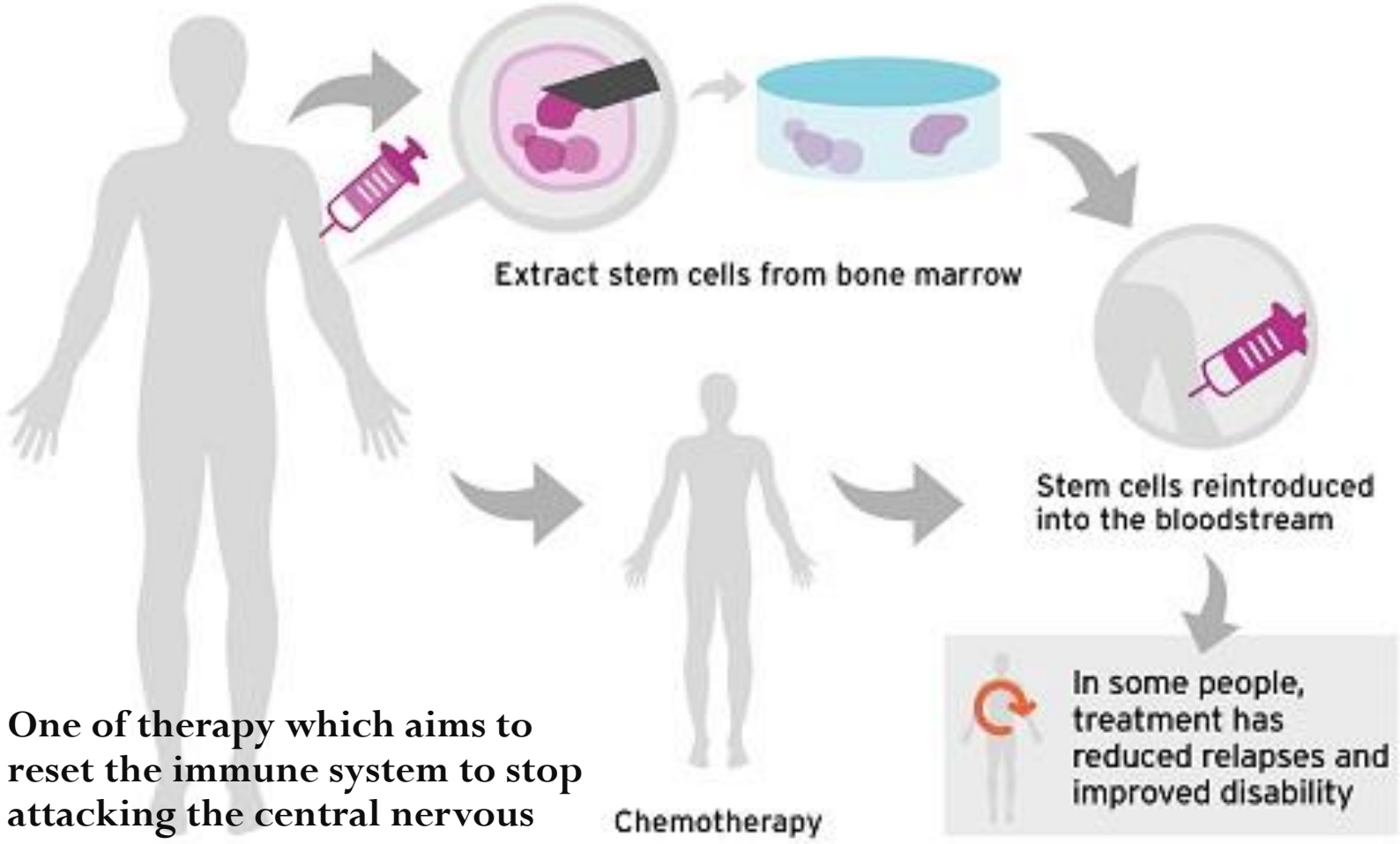
Today we can distinguish two uses of stem cells in the clinic:

- Cell therapy,
- Cultivation of organs or their parts for transplantation.

Based on the use of the regenerative potential of stem cells for number of serious diseases' treatment, rehabilitation of patients after trauma, struggle with premature signs of aging.

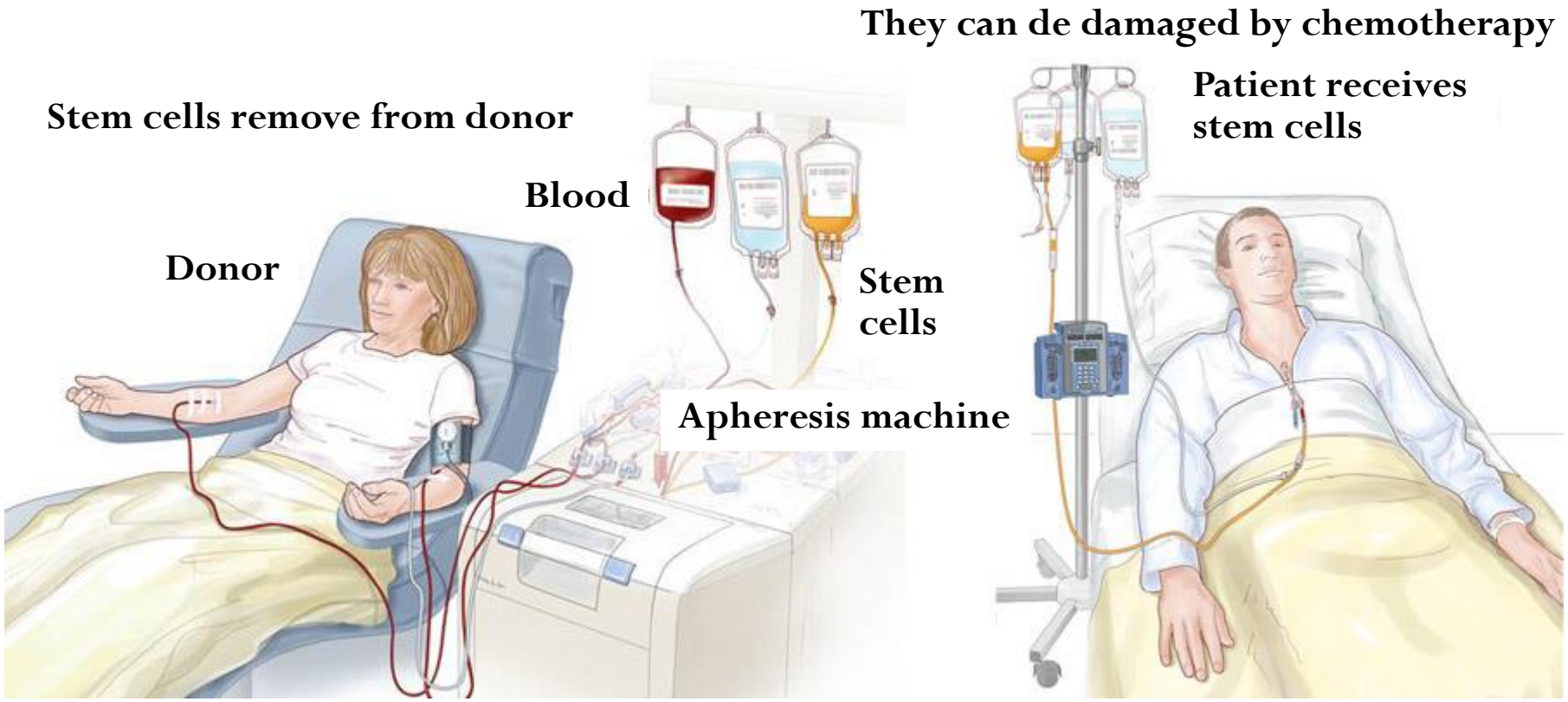


Autologous bone marrow stem cells transplantation



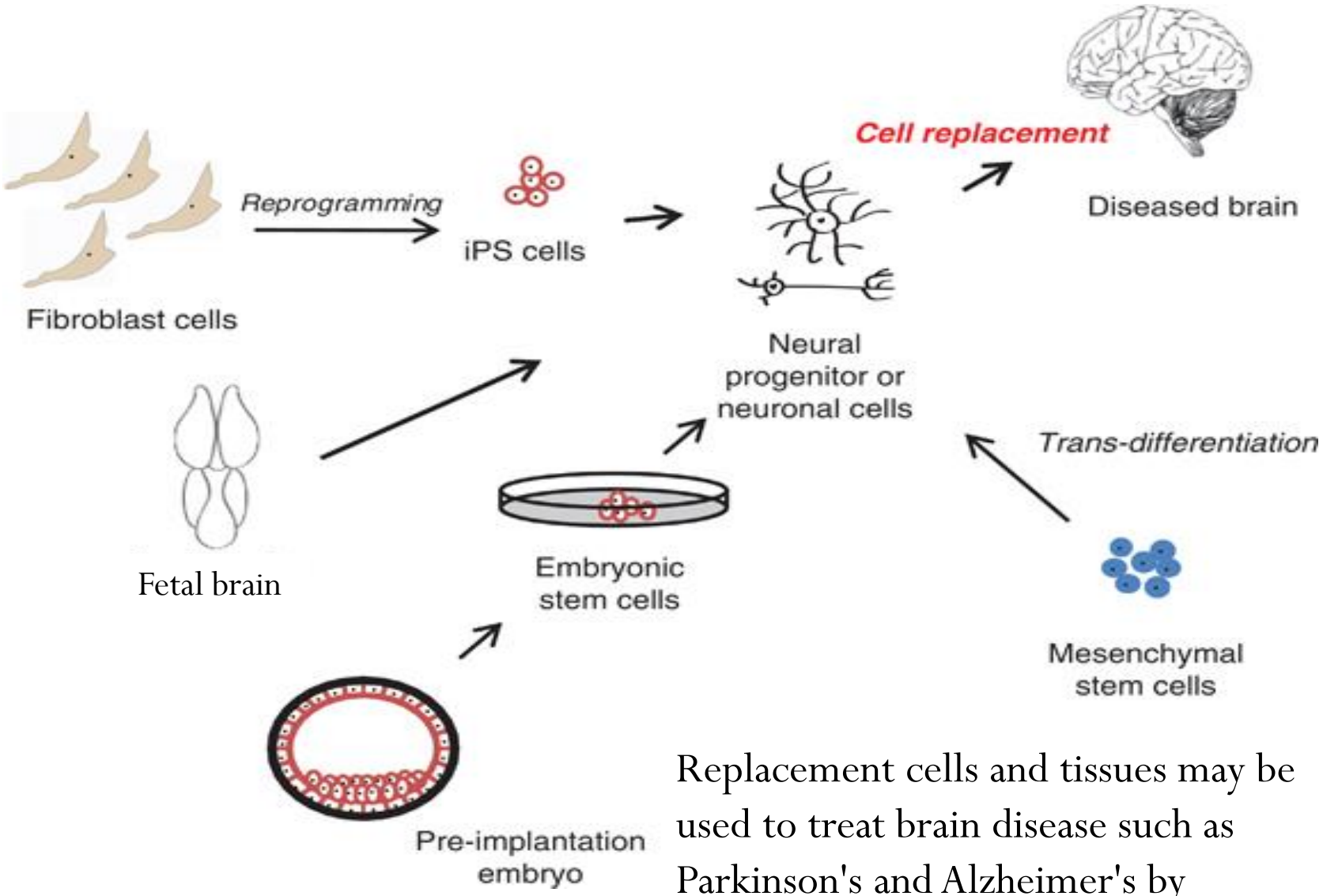
One of therapy which aims to reset the immune system to stop attacking the central nervous system,

Allogenic bone marrow stem cells transplantation



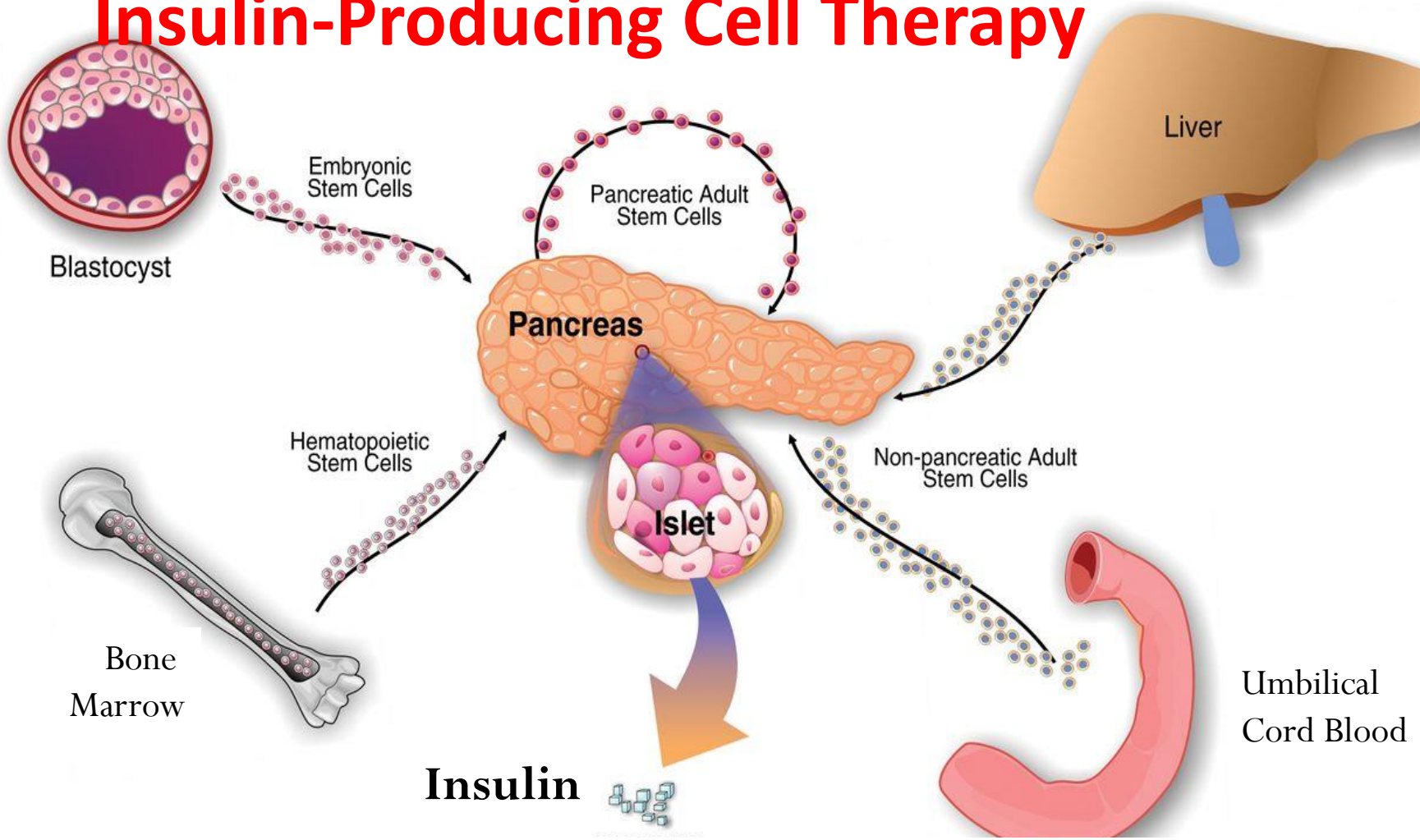
The donor's immune system can fight some types of cancer and blood-related diseases, such as leukemia.

Brain Disease Treatment



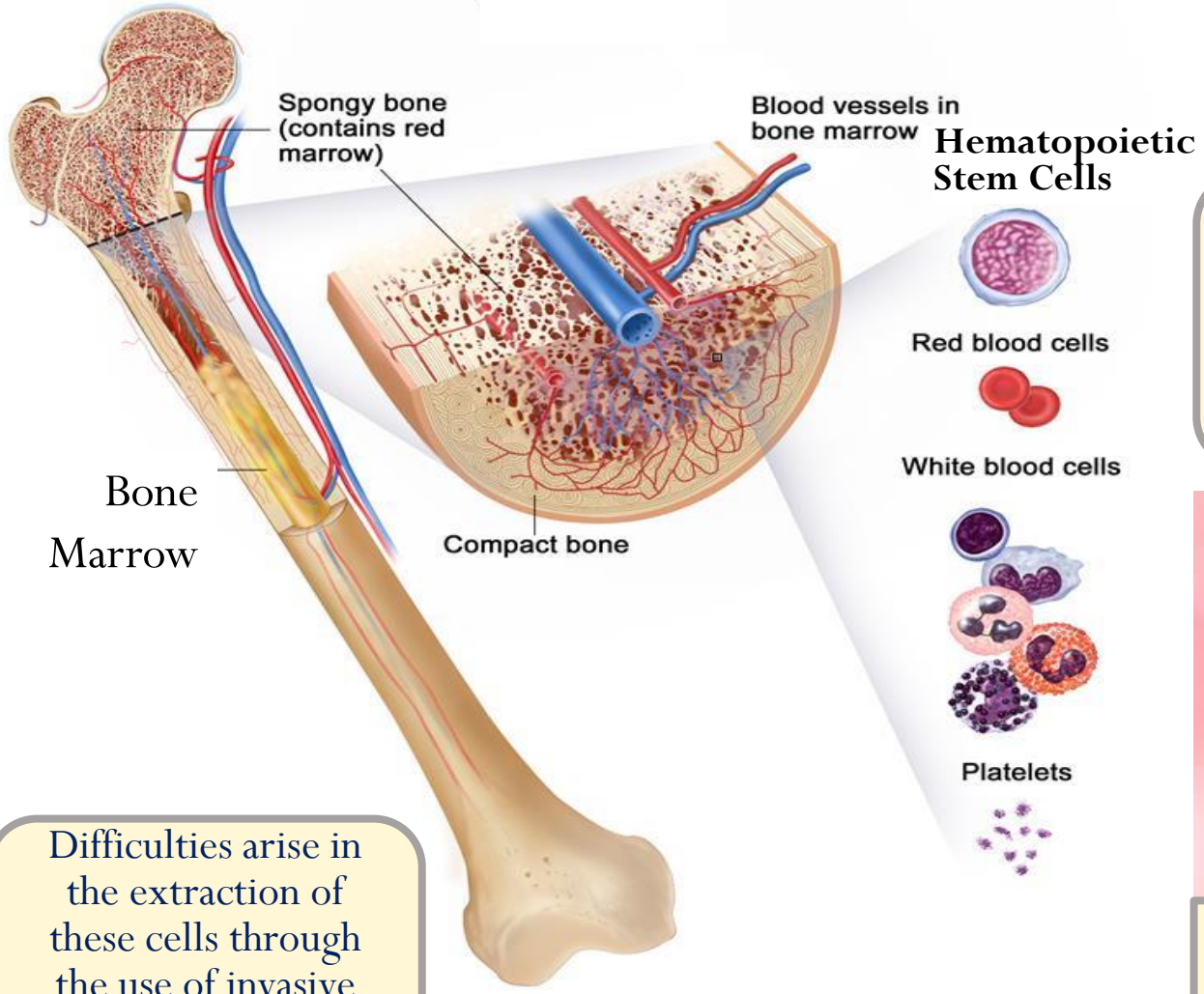
Replacement cells and tissues may be used to treat brain disease such as Parkinson's and Alzheimer's by replenishing damaged tissue

Insulin-Producing Cell Therapy



People with type I diabetes may receive pancreatic cells to replace the insulin-producing cells that have been lost or destroyed by the patient's own immune system.

Blood Disease Treatments



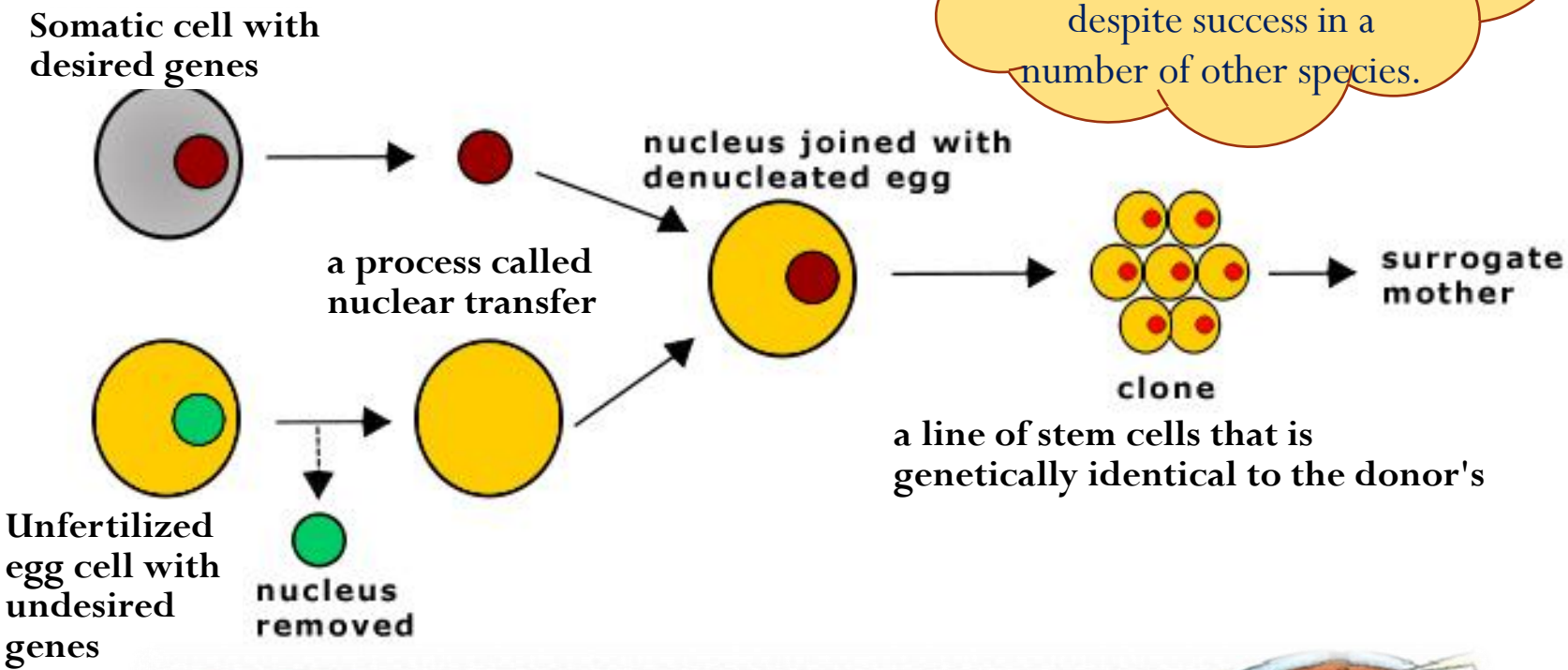
Treatment diseases such as leukemia, sickle cell anemia, and other immunodeficiencies.

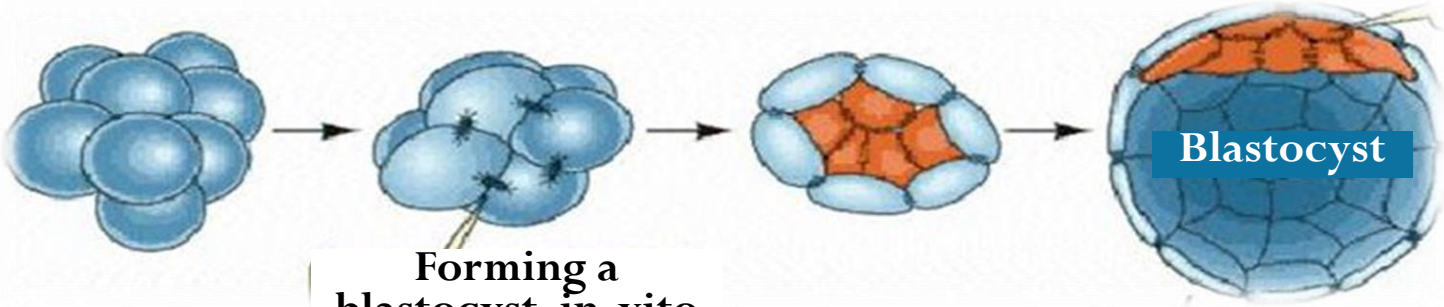
Umbilical Cord Blood and Placenta may be additional sources of hematopoietic stem cells

Difficulties arise in the extraction of these cells through the use of invasive bone marrow transplants..

Therapeutic Cloning

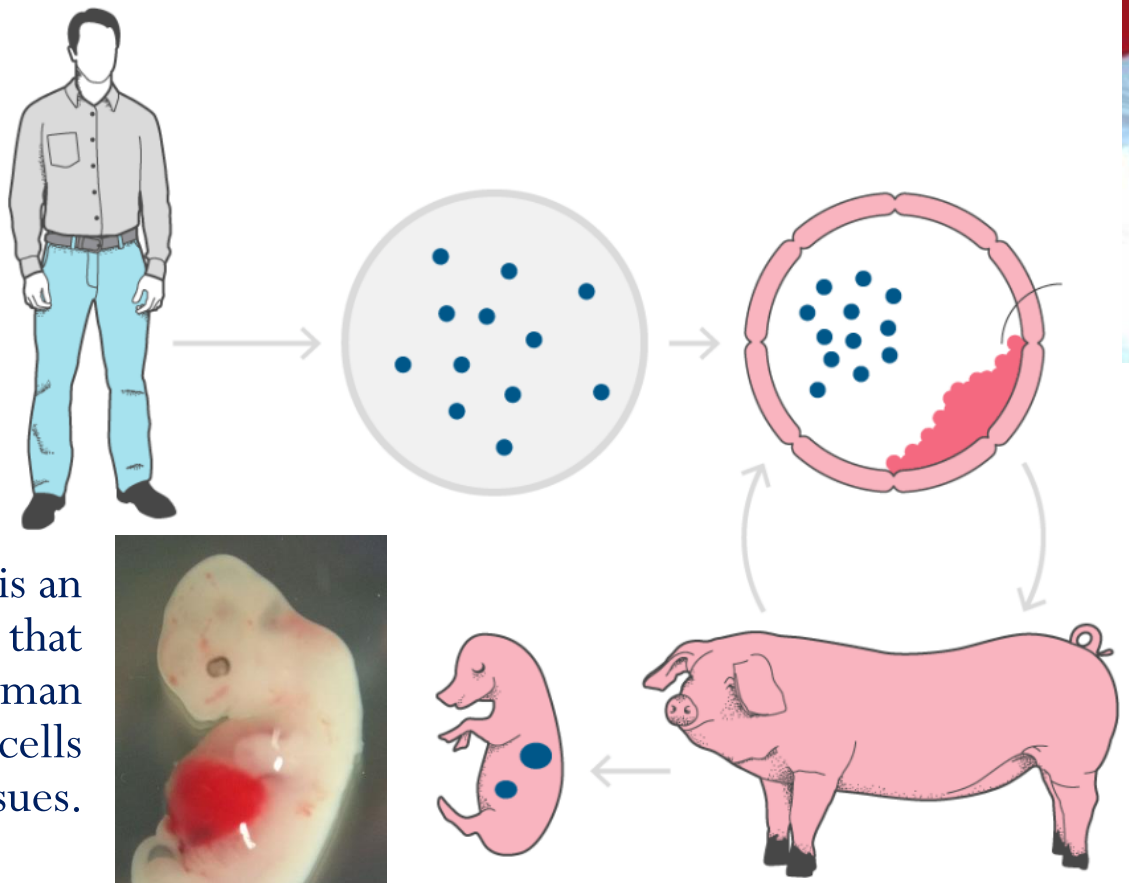
But. Researchers haven't been able to successfully perform therapeutic cloning with humans despite success in a number of other species.

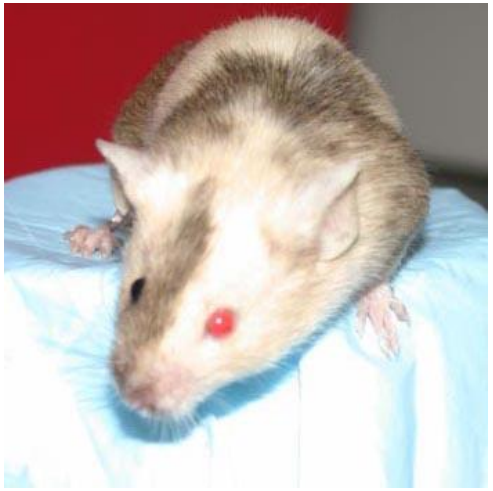




Forming a blastocyst in-vito

Chimeras





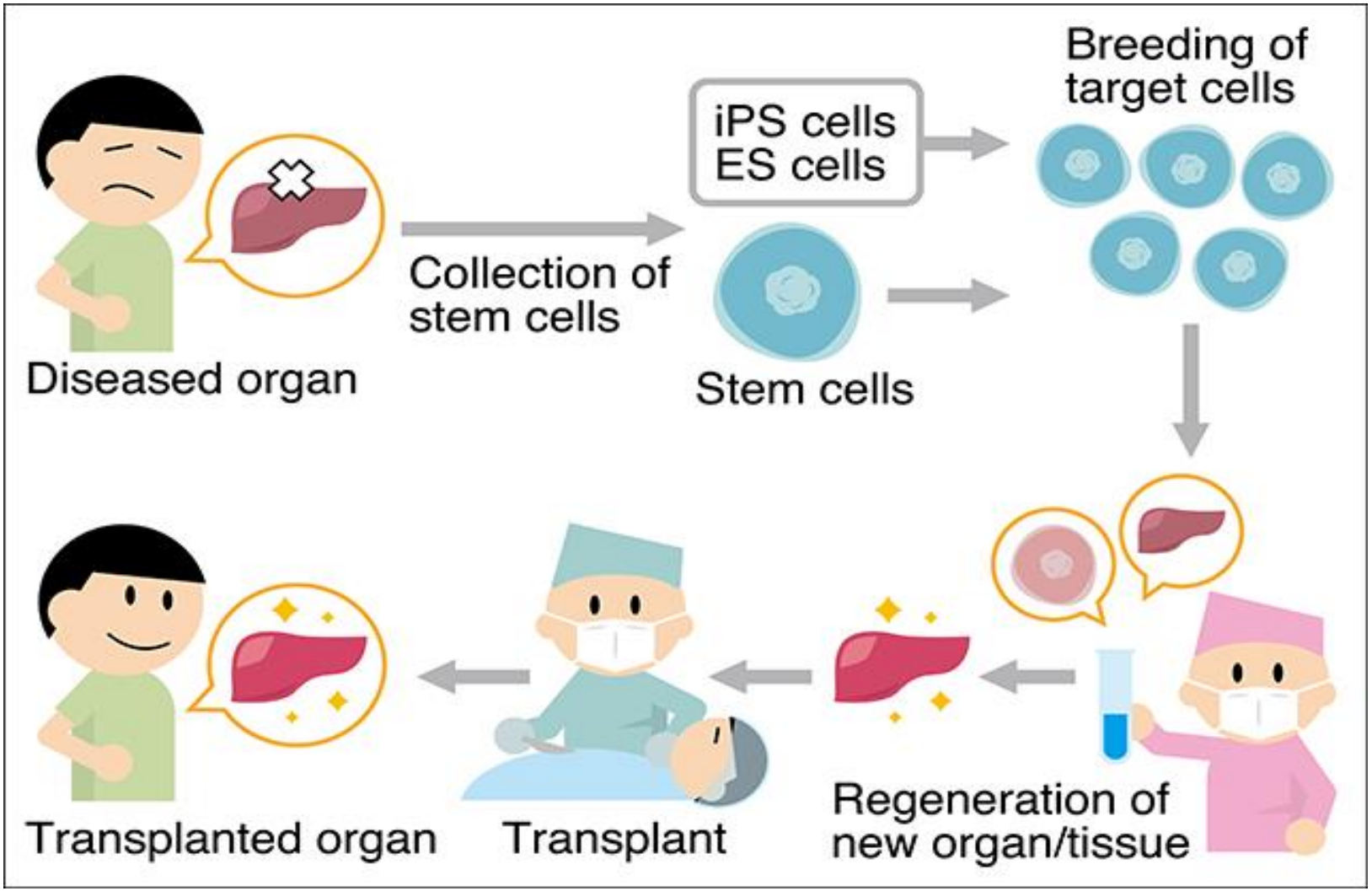
Chimeric Mouse

A chimera is an organism that has both human and animal cells or tissues.

Human-Pig Hybrid Created in the Lab in 2017

Nowadays this creates the opportunity for researchers to see what happens when stem cells are implanted.

Stem cells' Transplantation is the Symbol of the New Century

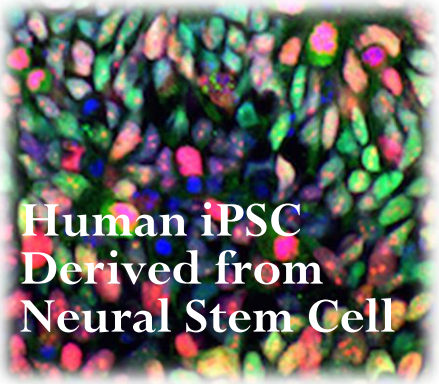


What are the Key Questions About Adult Stem Cells?

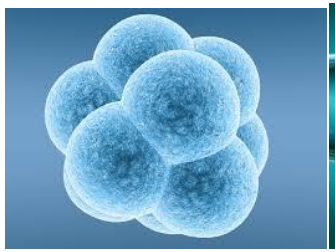
Adult Stem Cells

- How many kinds of SCs exist?
- In which tissues do SCs exist?
- How do adult SCs evolve and maintaine
- Why do SCs remain in an undifferentiated state and by what time?
- What are the mechanisms of SCs transplantation?
- What are the factors that control adult stem cell proliferation and differentiation?

- Is it possible to control this process to improve its reliability and efficiency?
- Do adult stem cells have the capacity to transdifferentiate?

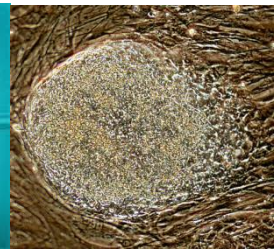


The Ethics of Embryonic Stem Cell Research



Human Embryos

In-vitro fertilization



ESCs colony in lab

The National Institutes of Health created guidelines for human stem cell research in 2009.

Several questions and issues have been raised about the ethics of embryonic stem cell research.

Defining embryonic stem cells

how they may be used in research and donation .

ESCs may only be used from embryos created by in vitro fertilization when the embryo is no longer needed.

The aims of National Policy

To safeguard the public from unethical stem cell research and use

Scientists

They debate about various laws and procedures regarding stem cell harvesting, development and treatment for research or disease purposes.

Religious groups

Support new advancements in the field of stem cells

Government officials

Those nations with a strong religious presence, particularly Roman Catholic, tend to be less supportive of stem cell research.

The most important international organizations that monitor procurement and clinical application of stem cells

Germen Stem Cell Network

International Society for Cellular Therapy

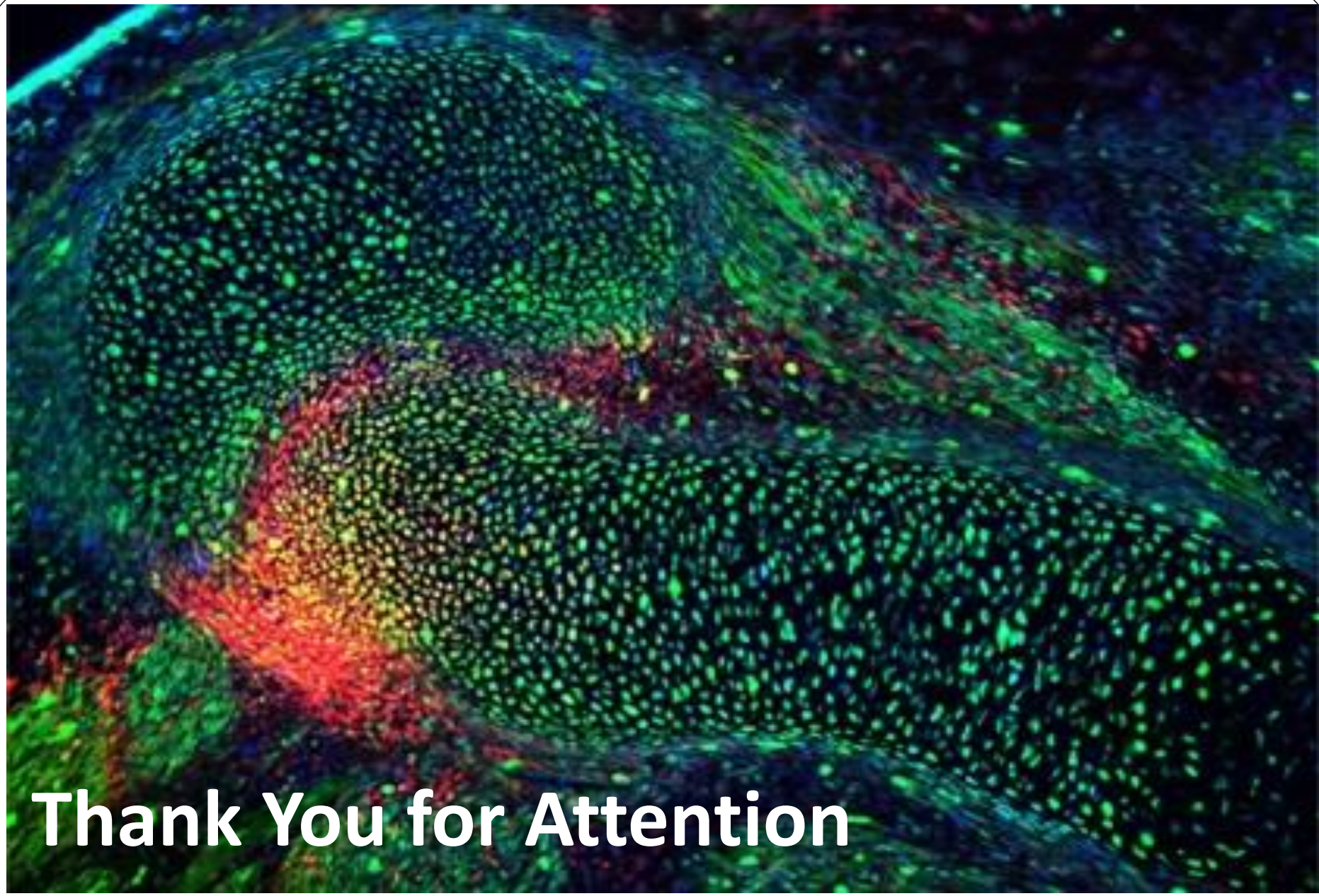
World Marrow Donor Association

Swiss Stem Cell Foundation®

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

PERINATAL STEM CELL SOCIETY

European Society for Blood and Marrow Transplantation



Stem Cells were labeled with fluorescent dyes