# Stem Cells: Hope, Hype and Progress

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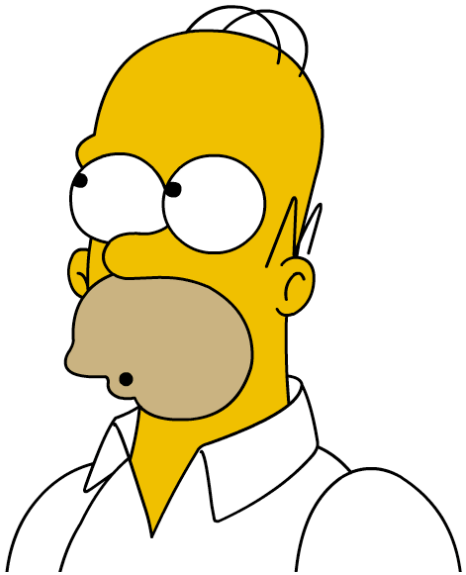
# Humans come in all different types…

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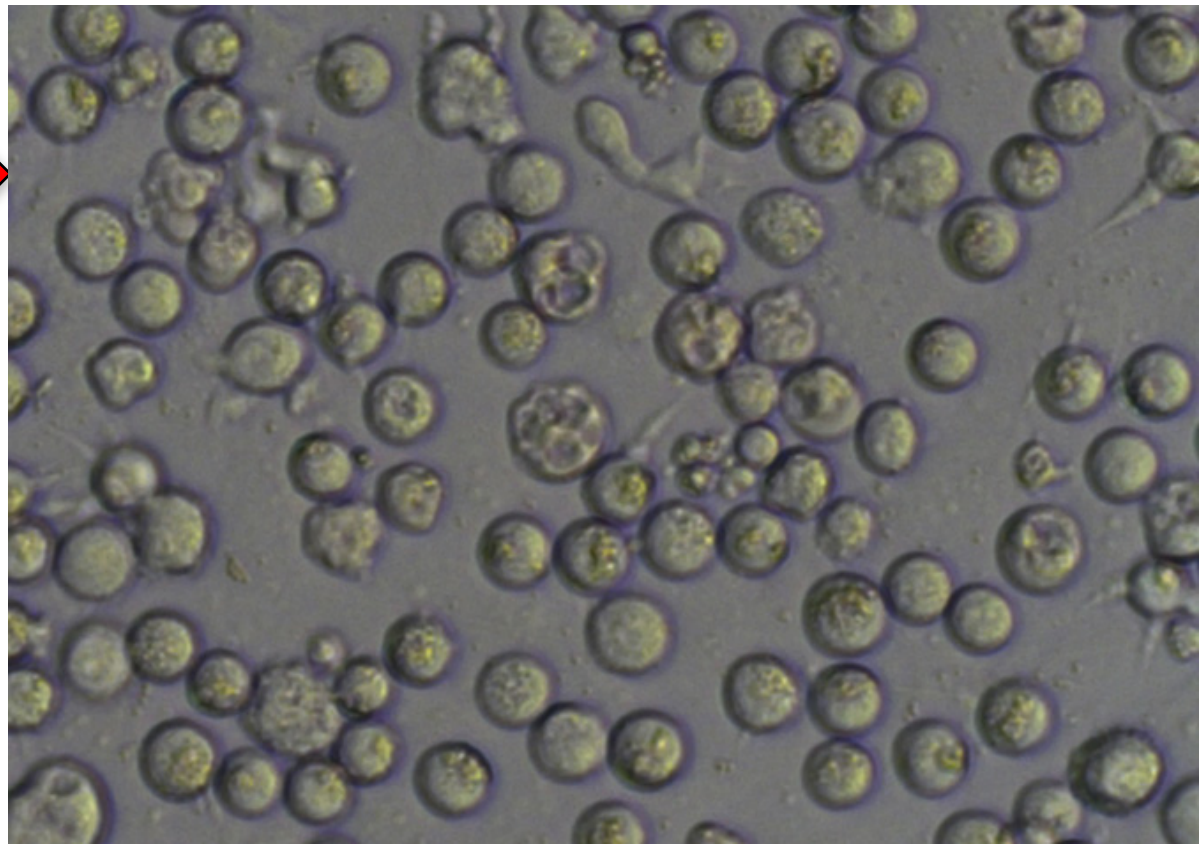
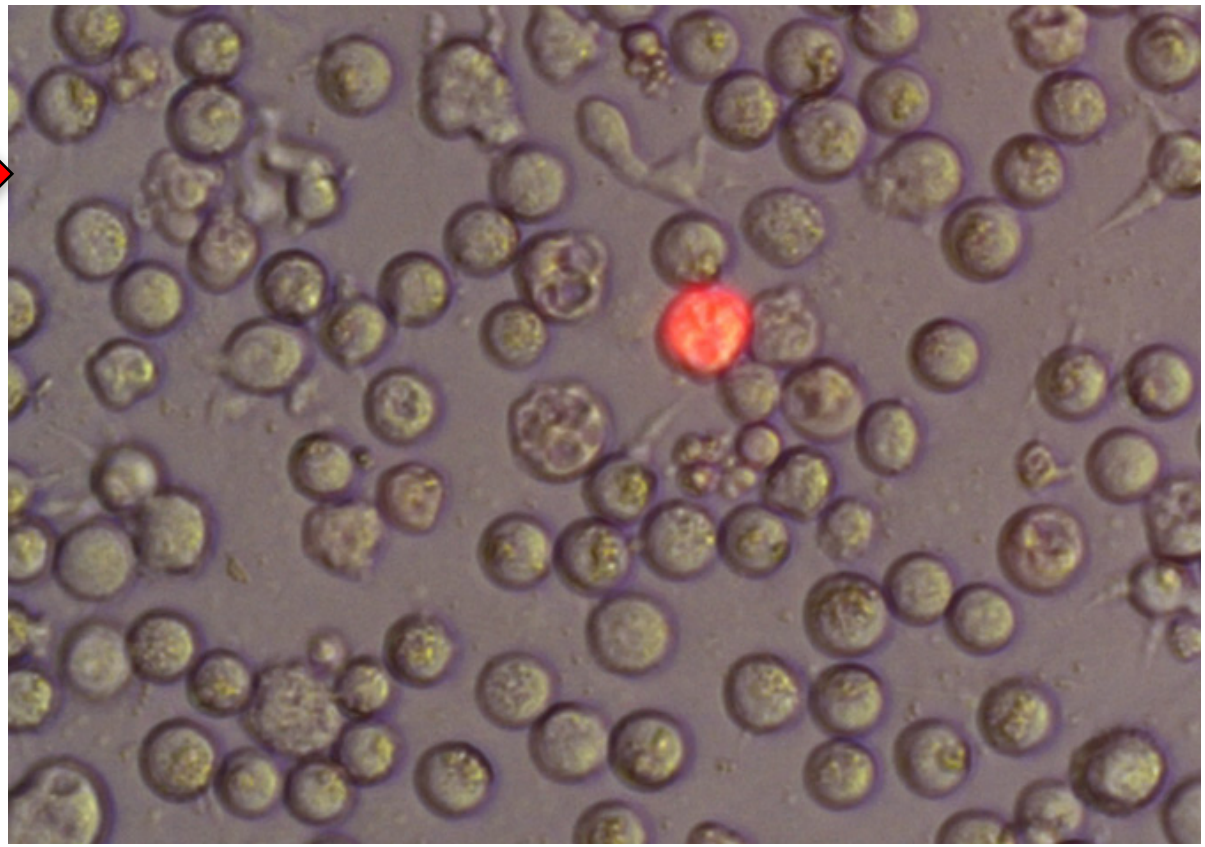
# …but we are all very similar deep down! All our organs contain billions of cells of many different types…



# …within which there are incredibly rare cells called **STEM CELLS**

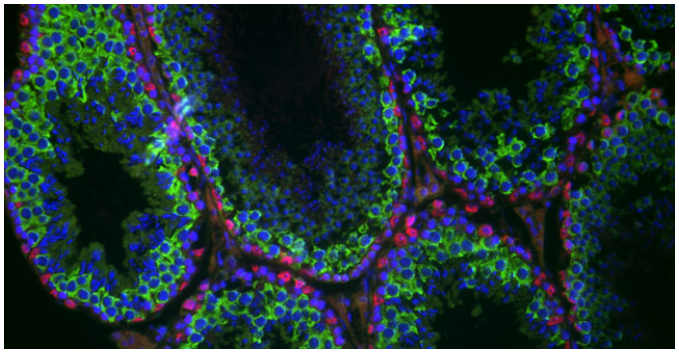


# What are stem cells?

- the body is made up of about 200 different kinds of specialised cells such as muscle cells, nerve cells, fat cells and skin cells
- all cells in the body come from **stem cells**
- a stem cell is a cell that is not yet **specialised**
- the process of **specialisation** is called **differentiation**
- once the differentiation pathway of a stem cell has been decided, it can **no longer** become another type of cell on its own

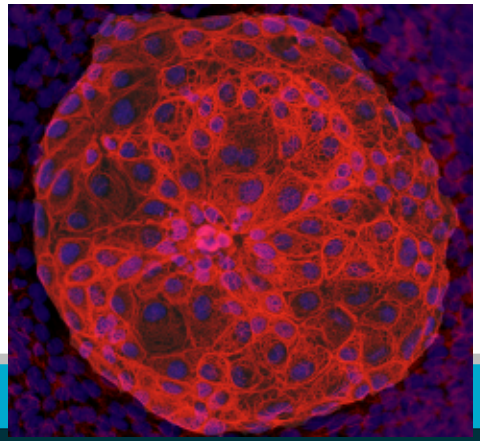
# Stem cells are not one cell type.

Stem cells that can become every type of cell in the body are termed **totipotent**



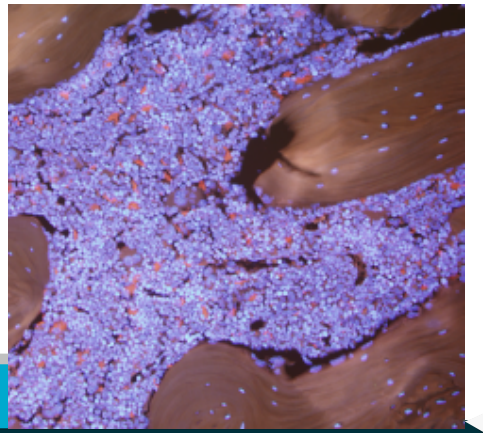
Germ stem cells (totipotent)

Stem cells that can become many types of cells in the body are termed **pluripotent**



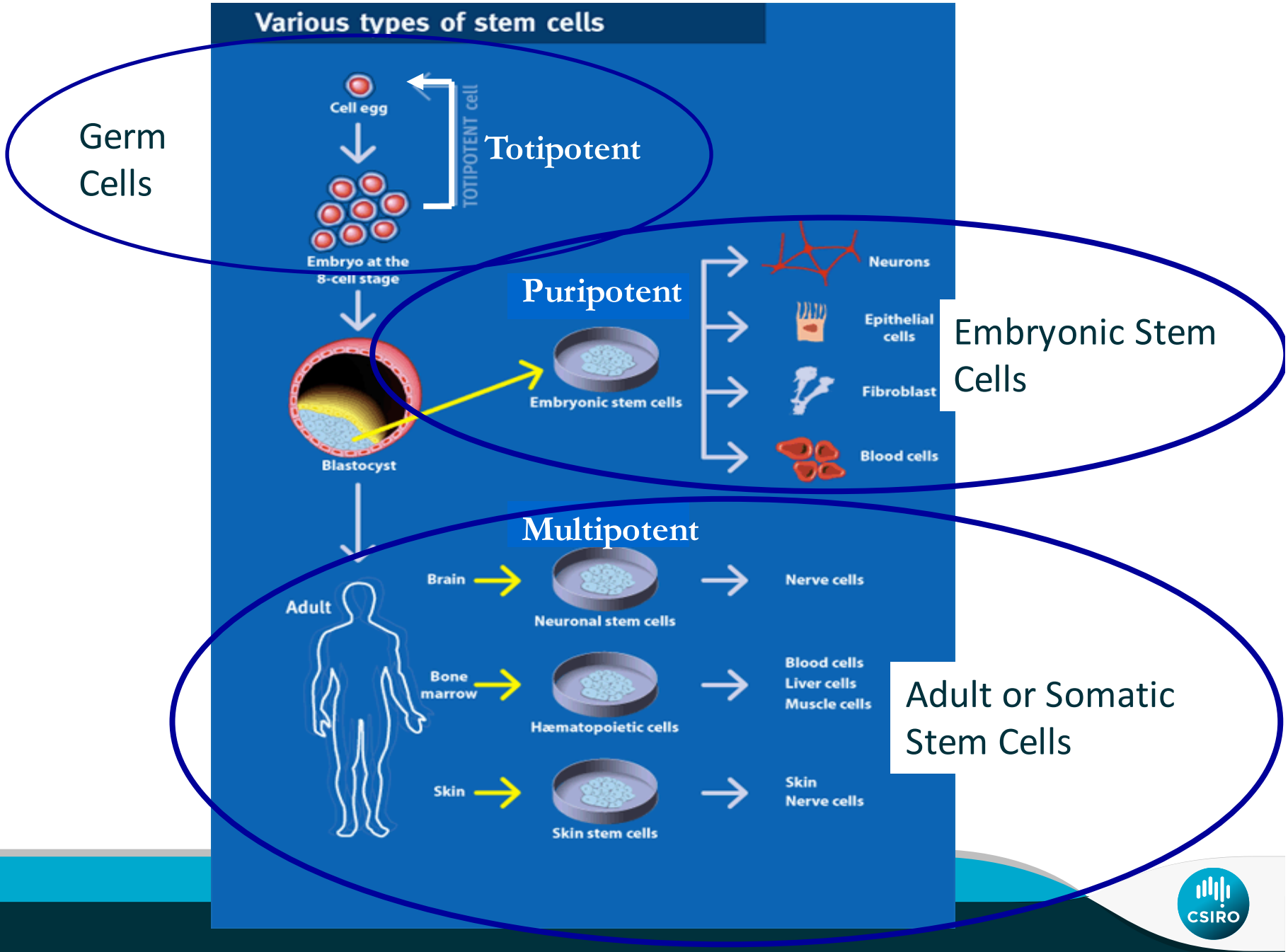
Embryonic stem cells (pluripotent)

Stem cells that can become only a few types of cells are termed **multipotent**



Tissue stem cells (multipotent)

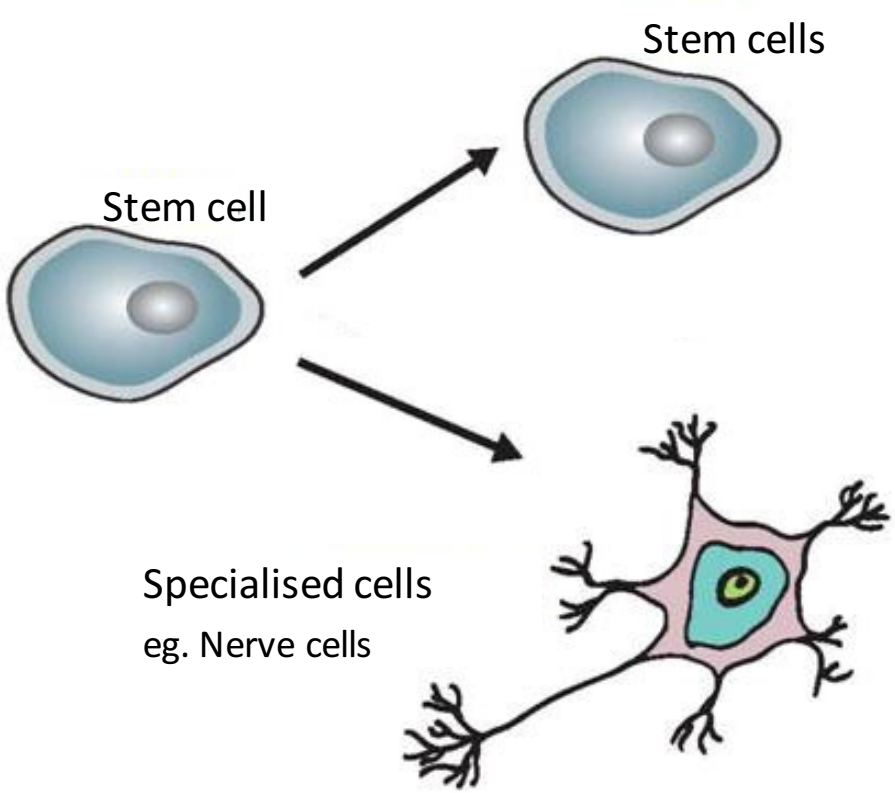
# Stem cells


Various types of stem cells
Germ Cells
Cell egg
TOTIPOTENT cell
Totipotent
Embryo at the 8-cell stage
Puripotent
Embryonic stem cells
Neurons
Epithelial cells
Fibroblast
Blood cells
Embryonic Stem Cells
Blastocyst
Multipotent
Adult
Brain
Neuronal stem cells
Nerve cells
Bone marrow
Hæmatopoietic cells
Blood cells
Liver cells
Muscle cells
Skin
Skin stem cells
Skin
Nerve cells
Adult or Somatic Stem Cells

# Why are stem cells special?

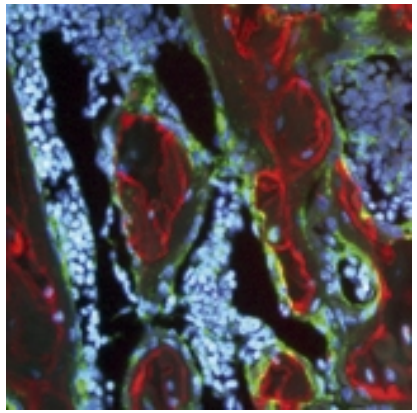
Stem cells can:

- Renew their population to make more stem cells
- differentiate into specialised cell types

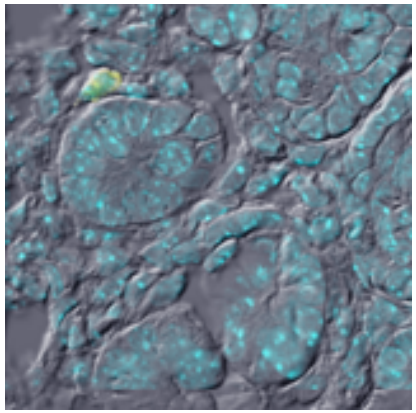


# Tissue stem cells

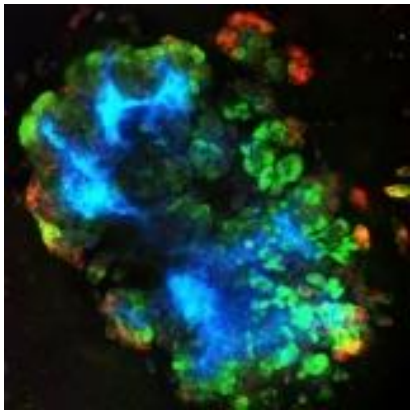
- often known as **adult** stem cells
- also includes stem cells isolated from fetal and cord blood
- reside in most tissues of the body where they are involved in repair and replacement



Bone marrow



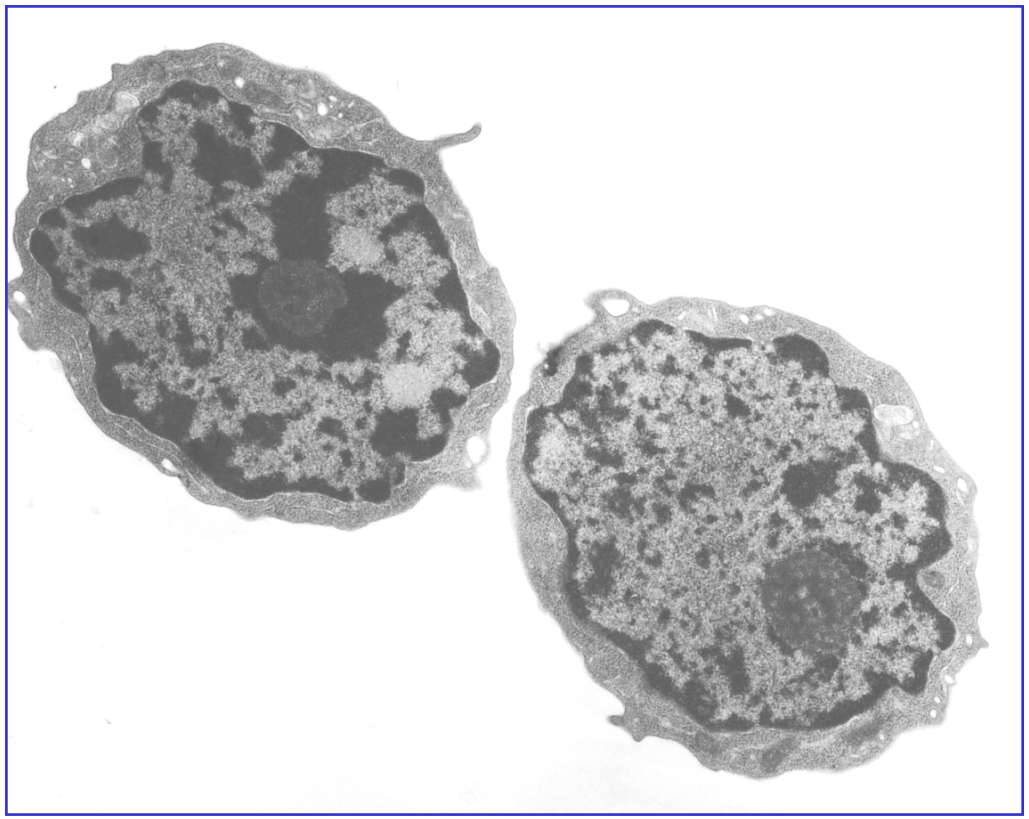
Kidney



Lung

- generally very difficult to isolate
- already used to treat patients (haematological malignancies, diseases of the immune system)

# Haemopoietic Stem Cells (HSC)



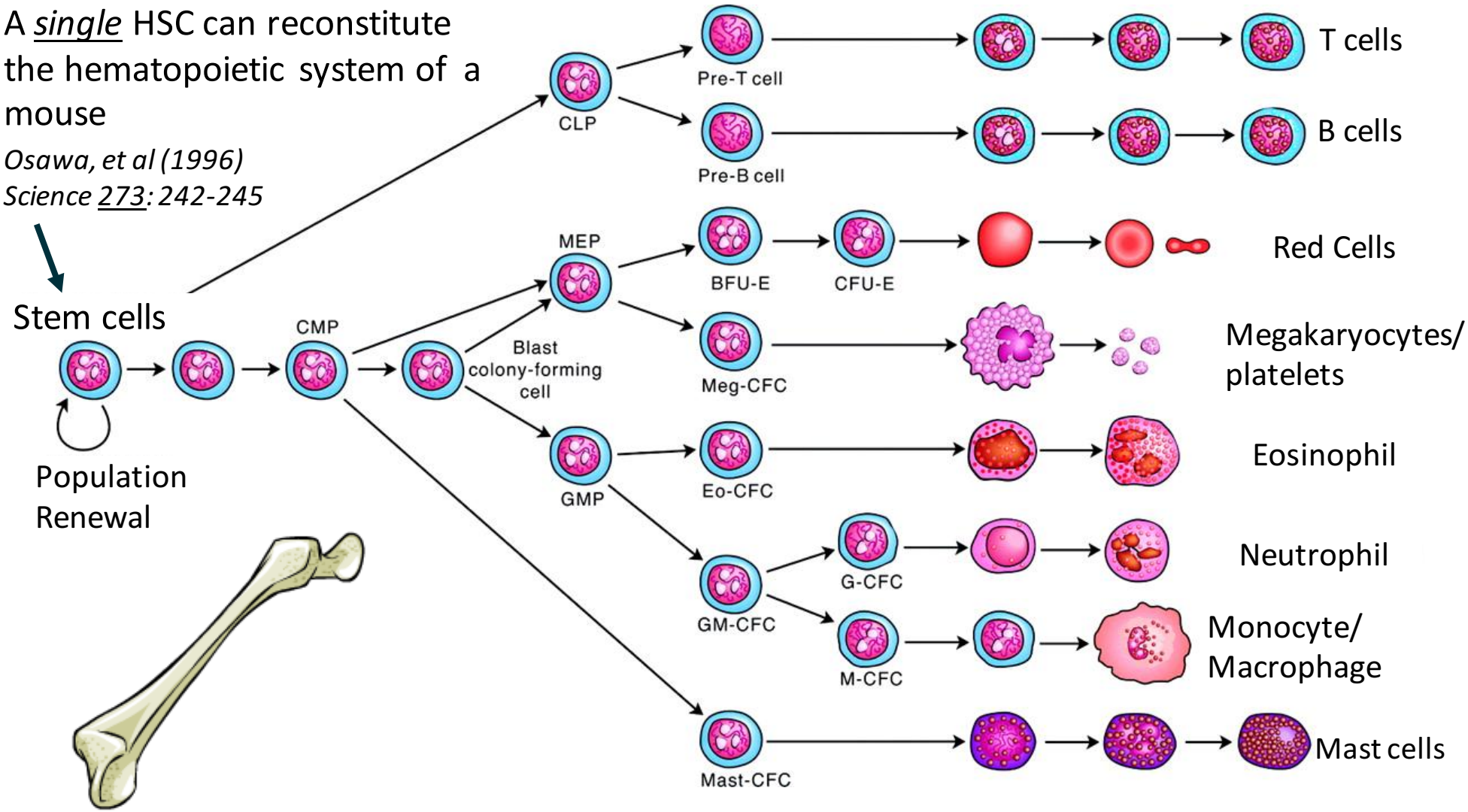
Human HSC isolated from Bone Marrow

The most extensively studied and well understood population of adult stem cells in vertebrates

# HSC: Hierarchical Adult Stem Cells

A *single* HSC can reconstitute the hematopoietic system of a mouse

*Osawa, et al (1996)*
*Science 273: 242-245*




we make 1 million mature blood cells per second

# HSC and Bone Marrow Transplantation: Adult stem cells – the potential

Adrenoleukodystrophy
Acute Lymphoblastic Leukemia
Acute Mast Cell Leukemia
AMFAcute Myelofibrosis
Acute Myelogenous Leukemia
Ataxia-Telangiectasia
Acute Undifferentiated Leuk-Mo
Beta-Glucuronidase Deficiency
Bare lymphocyte Syndrome
Breast Cancer
Beta Thalassemia Major
Cartilage-Hair Hypoplasia
Chronic Granulomatous Disease
Chronic Lymphocytic Leukemia
Chronic Myelogenous Leukemia
Chronic Myelomonocytic Leukemia
Central Nervous System Tumors
Di George Syndrome
Essential Thrombocythemia
Ewings Sarcoma
Fanconi Anemia
Gaucher's Disease
Glanzmanns Thrombasthenia
Hairy Cell Leukemia
Histiocytosis - X
Hodgkin's Lymphoma
Hemophagocytosis
Hunter Syndrome (MPS-II)
Hurler Syndrome (MPS-IH)
I-Cell Disease
Immune Deficiency + Neutropen
Juvenile Chronic Myelogenous
Kostmann's Agranulocytosis
Krabbe Disease
Leukocyte Adhesion Deficiency
Lesch-Nyhan
Lysosomal Storage Disease
Maroteaux-Lamy (MPS-VI)
Myelodysplastic Disorder
Myelofibro Myeloid Metaplasia
Multiple Myeloma
Morquio (MPS-IV)
Mucopolysaccaridosis
Neiman-Pick Disease
Neuroblastoma
Non-Hodgkins Lymphoma
Other Combined Immunodeficiency
Osteopetrosis
Plasma Cell Leukemia
Prolymphocytic Leukemia
Paroxysmal Nocturnal Hemoglobinuria
Polycythemia Vera
Refractory Anemia (RA)
Refractory Anemia-Excess Blasts
Pure Red Cell Aplasia
Severe Aplastic Anemia
Sanfillippo (MPS-III)
Sickle Cell Anemia
Severe Combined Immunodeficiency
SCID with ADA
Small Cell Lung Cancer
Absence of T&B Cells SCID
Absence of T, Normal B SCID
Wiskott Aldrich Syndrome
Waldenstroms Macroglobulinemia
X-Linked Lymphoproliferative

# Adult stem cells – limitations/problems

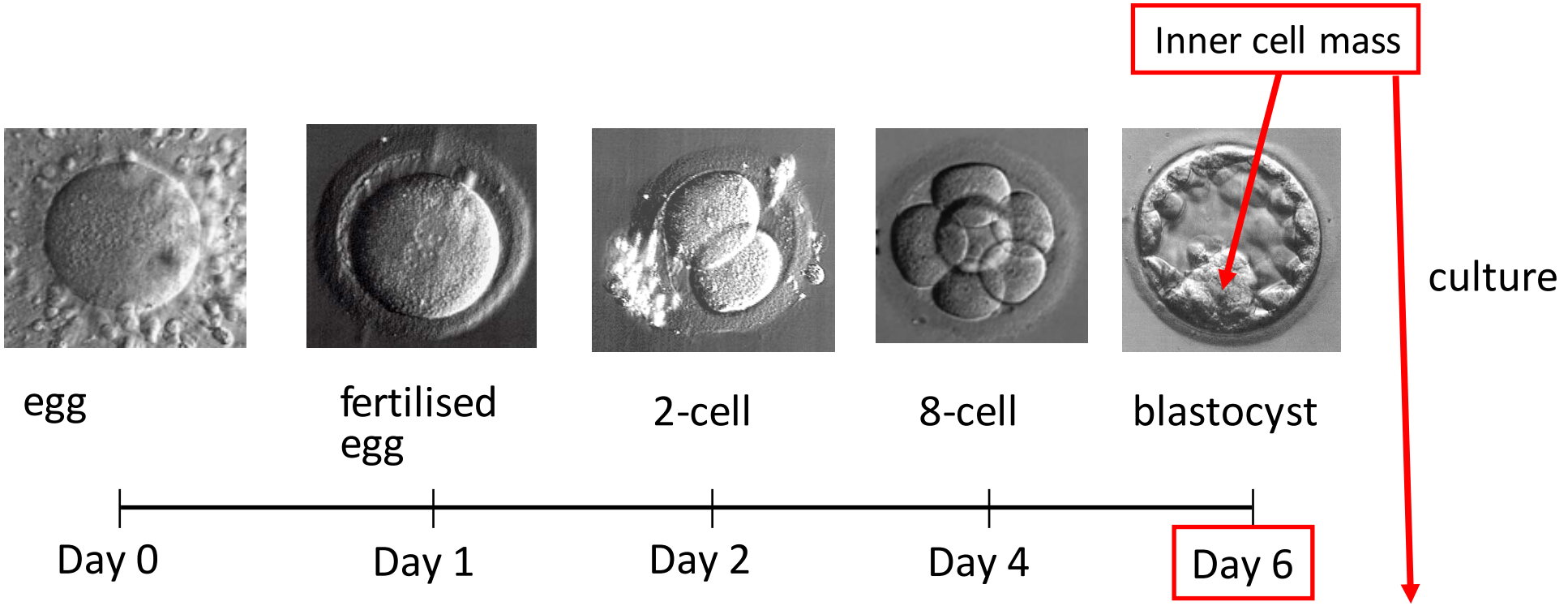
- **Rare, often difficult to identify**
  - Stem cells have not been identified in all adult tissues/organs
  - Location of stem cells unknown in most tissues
  - Difficult to isolate pure populations
  - Problems of accessibility

- **Conditions for growing adult stem cells *in vitro* have not been established for most stem cell types**
  - Poor growth in vitro; limited lifespan
  - Loss of stem cell properties due to spontaneous differentiation
  - Potential difficulties in obtaining sufficient numbers for therapy

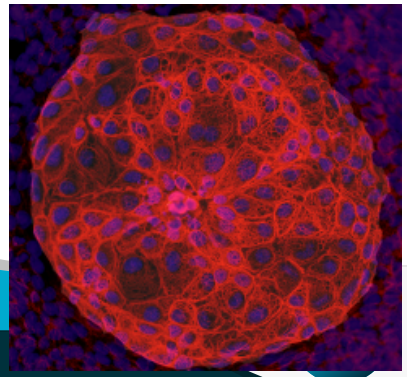
- **Effective means of transplanting adult stem cells in a clinical setting only exist for a limited number of stem cell types**
  - Await developments in tissue engineering and cell delivery systems
  - Immune-mediated transplant barriers

# Embryonic stem cells (ES cells)

- excess embryos from IVF clinics
- donated at the wish of and with full consent of parents




Embryonic stem cells (pluripotent)



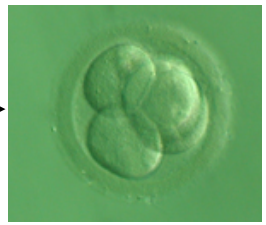
*Images from www.advancedfertility.com*

# Embryonic stem cell derivation in Australia

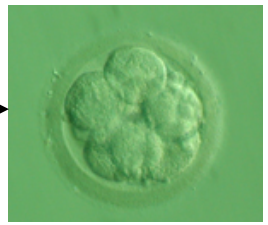
Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act 2006

**NHMRC licensed project**

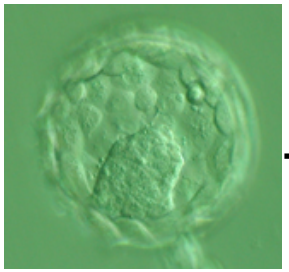
**Patient consent obtained for stem cell derivation**



donated frozen embryo

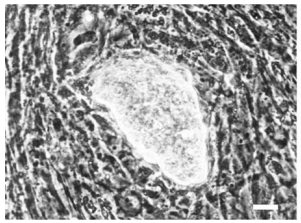


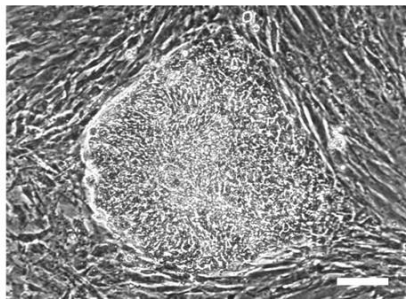
1 day



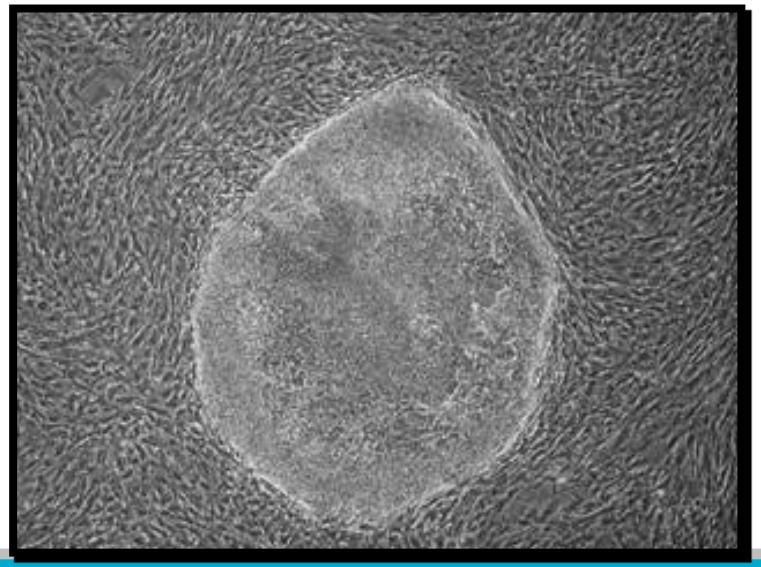
6 days

**Isolation of inner cell mass**



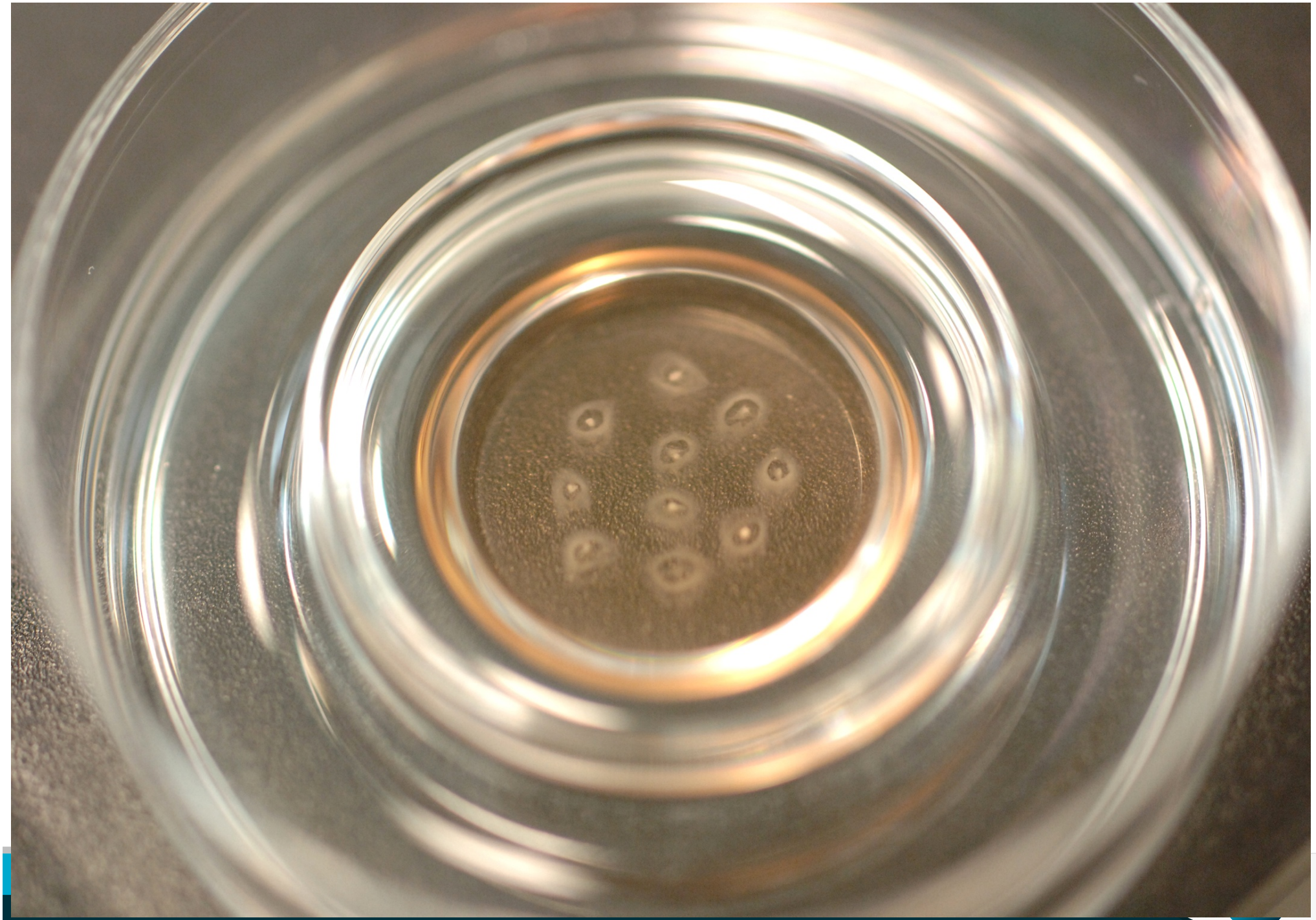


10-15 days in culture



**human embryonic stem cell line**

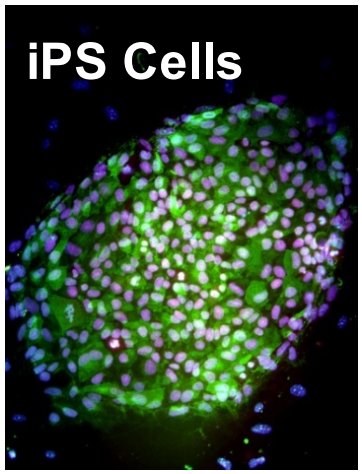
*Images from Melbourne IVF and Reubinoff et al, 2000*



# Induced pluripotent stem cells (iPS)

Starting cells from donor tissue

Induced change in gene expression



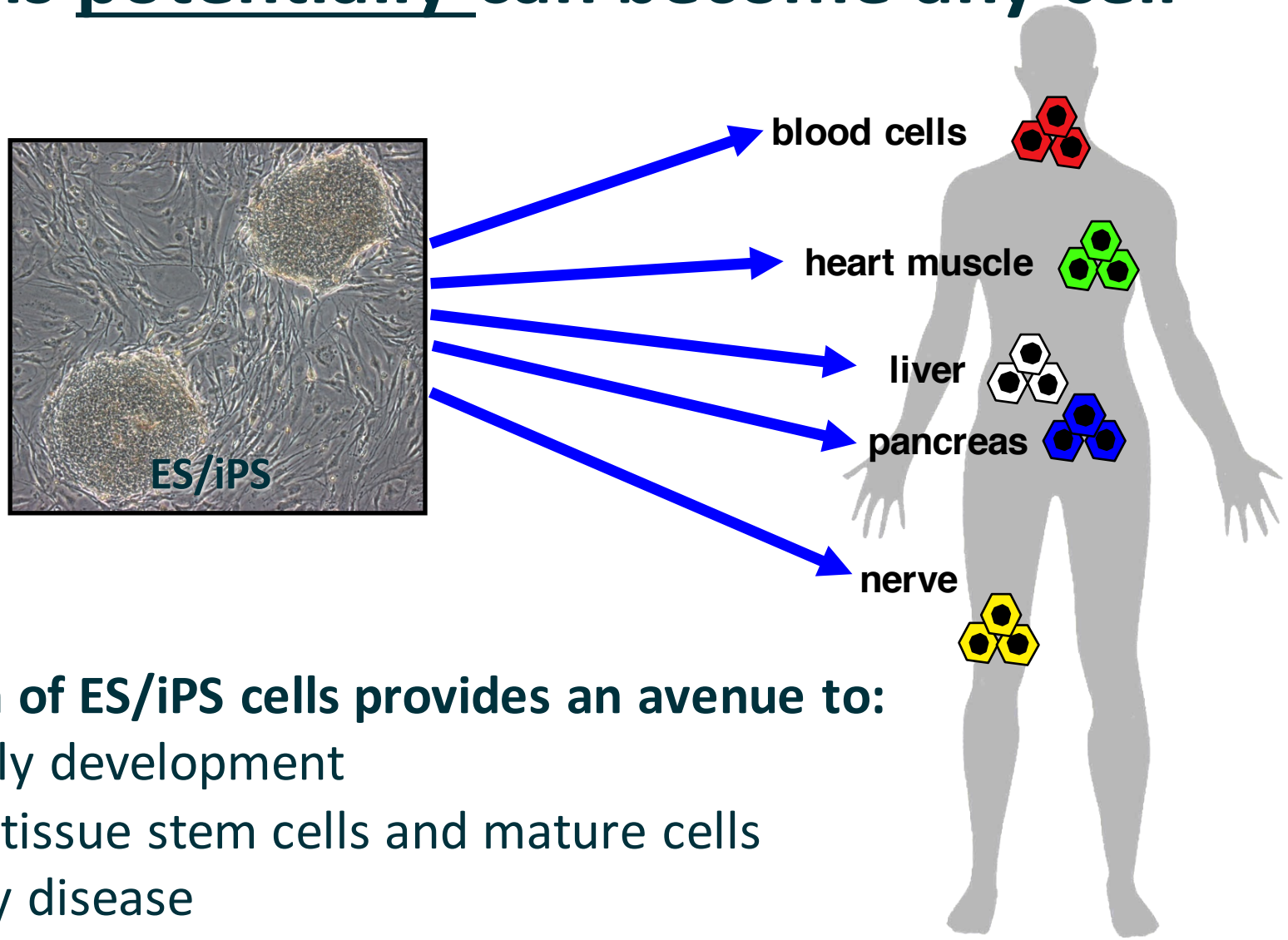

pluripotent stem cells

- derived from adult cells in 2007 - very recent discovery!
- can be grown indefinitely in culture in an undifferentiated state
- similar properties to embryonic stem cells– **pluripotent**

# iPS – continued

- Fewer ethical/legal problems because creation of iPS cells does not require the destruction of embryos
- As with embryonic stem cells, large numbers of cells can be grown and used to create disease - or patient - specific stem cell lines
- But many questions remain to be explored about iPS cells:
  - are they really equivalent to human embryonic stem cells?
  - are they stable over a long time?
  - will they be safe for treatment?
- Australian research into understanding iPS cells is ongoing

# ES/iPS cells potentially can become any cell




**Differentiation of ES/iPS cells provides an avenue to:**

- study early development
- generate tissue stem cells and mature cells
  to study disease
  to screen drugs/therapeutic agents for cell therapies

# Requirements for ES/iPS therapies:

- Must EFFICIENTLY make the required mature cell type
- Mature cells must be FUNCTIONALLY EQUIVALENT to endogenous cells
- Mature cells must be available in SUFFICIENT QUANTITIES and PURITY for therapeutic use
- Problems of IMMUNE REJECTION must be overcome
- Transplanted cells must be SAFE – free of INFECTIOUS agents and remaining stem cells that can cause TUMOURS

# STEM CELL therapies?

- Blood stem cell transplants using bone marrow, blood or umbilical cord blood have been in clinical use for >50yrs
- Mesenchymal stem cell therapies to assist bone and cartilage repair and blood stem cell grafting in trials
- Trials of nerve sheath cells (oligodendrocytes) grown from embryonic stem cells for acute spinal cord injuries
- Trials of retinal pigment cells grown from embryonic stem cells for some forms of blindness are ongoing
- More trials will continue to start…

# Some ethical questions to ponder re: ES and iPS Stem Cells

- are frozen fertilised embryos considered a life?
- couldn't these embryos be used for research because they are to be destroyed anyway?
- do the ends justify the means?
- given that iPS cells can potential make all cells in the body, can this technology be abused? Could you clone someone using iPS cells?

# Arguments for:

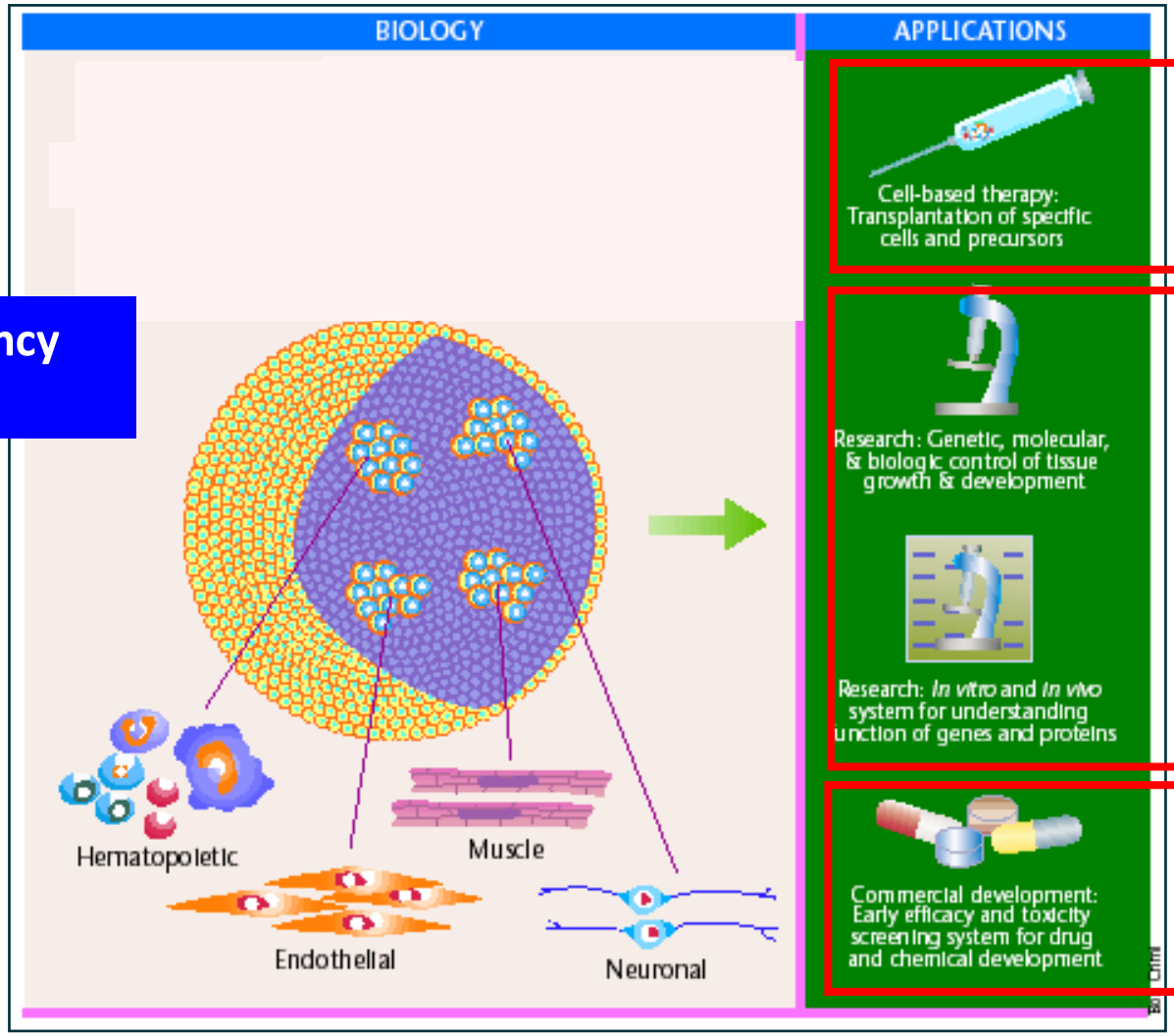
- ES and iPS cells have the potential to treat many terrible diseases and save many lives
- Frozen embryos are not life as they were made in a tube and are frozen in liquid nitrogen
- Informed consent was obtained from the couple for the extra embryos to be donated to research
- Most couples who have successfully used in vitro fertilisation (IVF) want their extra embryos used for research not destroyed

# Arguments against:

- Life begins at conception whether in a women or a test tube
- ES research involves the destruction of life and no life should be destroyed even if it is to save another
- Frozen embryos have successfully been implanted into women giving rise to healthy life

# What makes stem cells so valuable?

**Pluripotency essential**




**Cell Therapy**

**Research**

**New Drugs**

Modified from Keller & Snodgrass, Nat Med 1999

# What about cloning? Has cloning got anything to do with stem cell research?

Dolly the Sheep

Snuppy the Puppy

# Cloning

- Dolly was created by **somatic cell nuclear transfer**
- take an egg, remove its nucleus - the DNA – and add a new nucleus of a cell from the animal to be cloned
- the reconstructed egg is treated with a jolt of electricity and put in a dish to divide. The cluster of cells is either transplanted into a surrogate mother's uterus (**reproductive cloning**) or the inner cell mass removed and stem cells cultured (**therapeutic cloning**)
- reproductive cloning in humans is **illegal** in Australia and punishable by 15 years in prison

# Stem cells Tourism

- The promise of stem cells has caused multiple overseas clinics to offer unproven and experimental treatments for a high cost
- This has caused a new phenomenon: **Stem Cell Tourism**
- Stem Cells Australia receives daily enquiries regarding unregulated overseas experimental stem cell treatments

# What's the problem?

- Unregulated clinics with unproven methods often charging large sums of money
- Lack of clinical trials to test safety and efficiency
- Patients can return home with infections, more pain, no medical records or follow-up care and in some cases cancer
- Need public education to increase knowledge of these issues to stop stem cell tourism for vulnerable patients

# Stem cell tourism:

## promising instant results for incurable diseases

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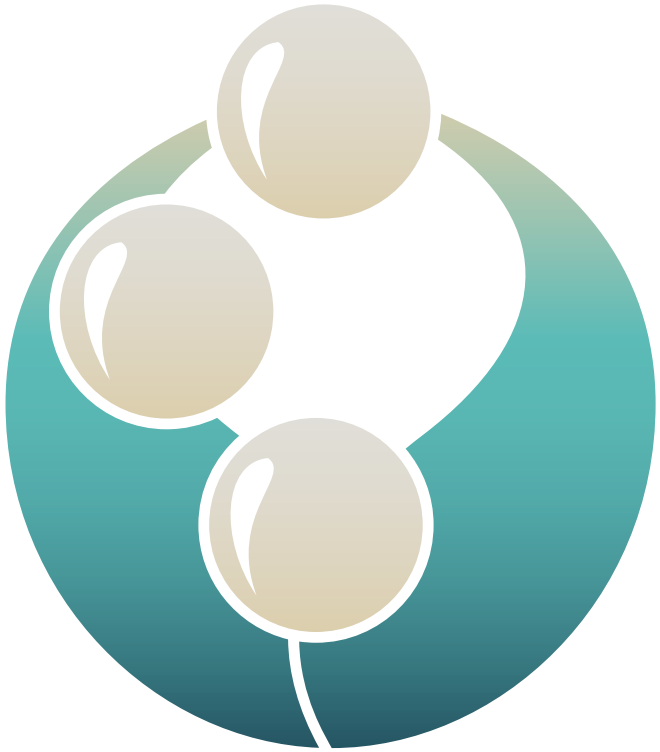
## promising instant results for incurable diseases



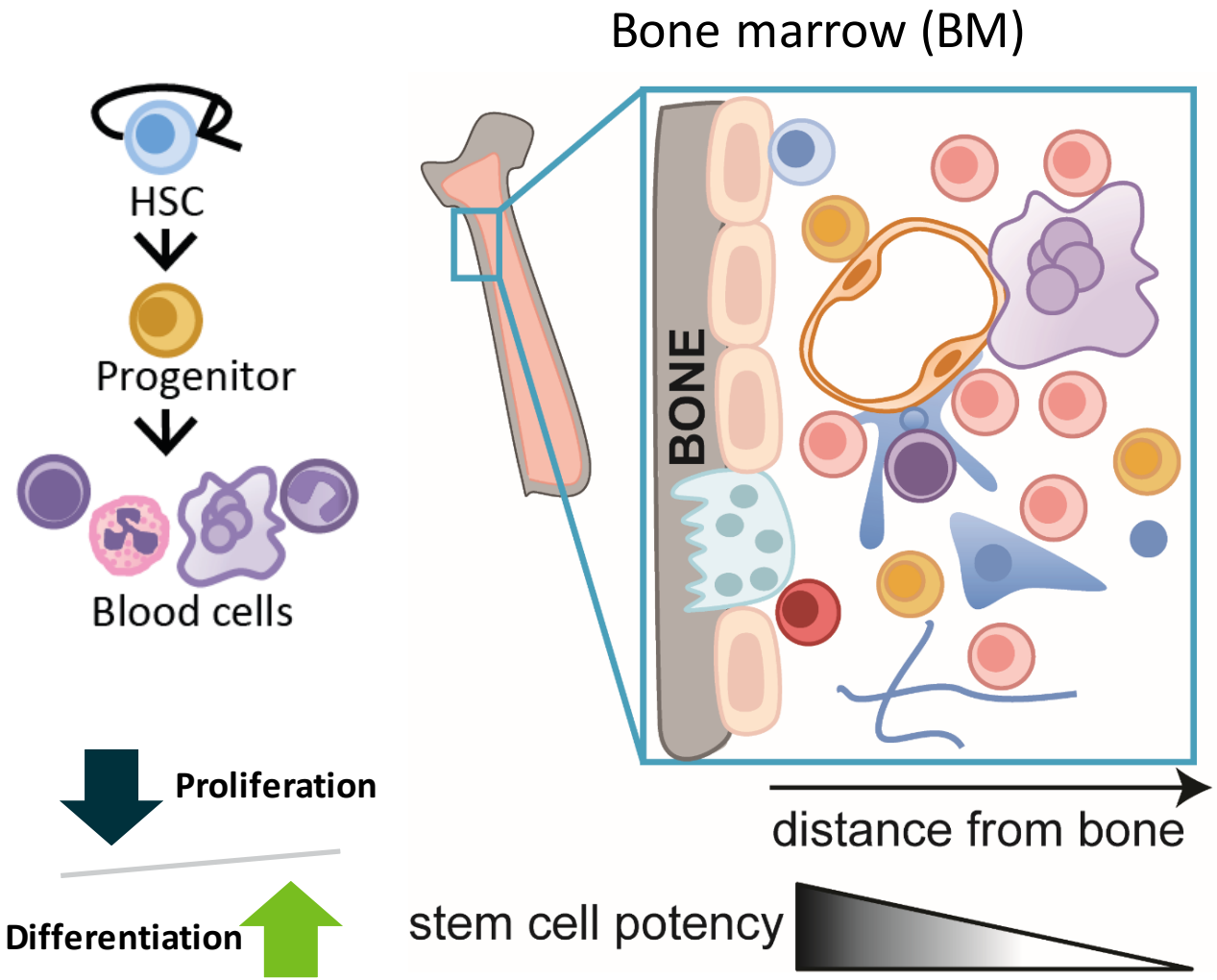
OPENED

Cells4health

# Stem cell tourism:

## promising instant results for incurable diseases

# Haemopoietic stem cells (HSC) and their microenvironment




**Cellular**
Megakaryocytes
Endothelial

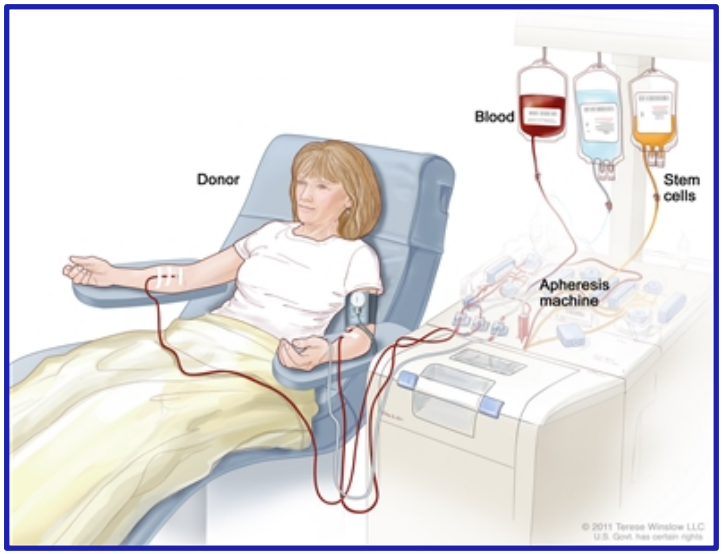
**Extracellular**
Matrix
Cytokines

# HSC for stem cell transplants

**Then** → **Now**

From Bone Marrow

From Peripheral Blood

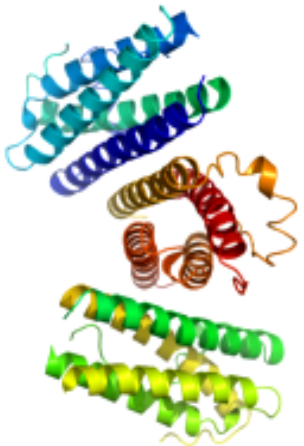



**Problem:** Peripheral blood HSC are extremely rare

**Solution:** **Stem cell mobilisation agents** increase blood HSC numbers

# Clinical mobilisation agents:

## *G-CSF (Filgrastim; marketed as Neupogen®)*



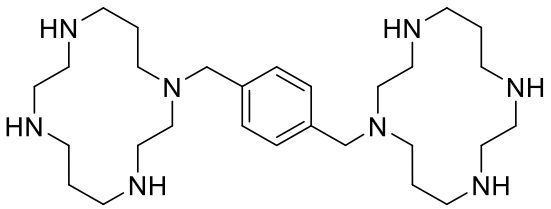
• Stimulates division and mobilisation of marrow cells and HSC

**Mechanism:**

- activation of proteases
- downregulates expression of adhesion proteins

**Drawbacks:**

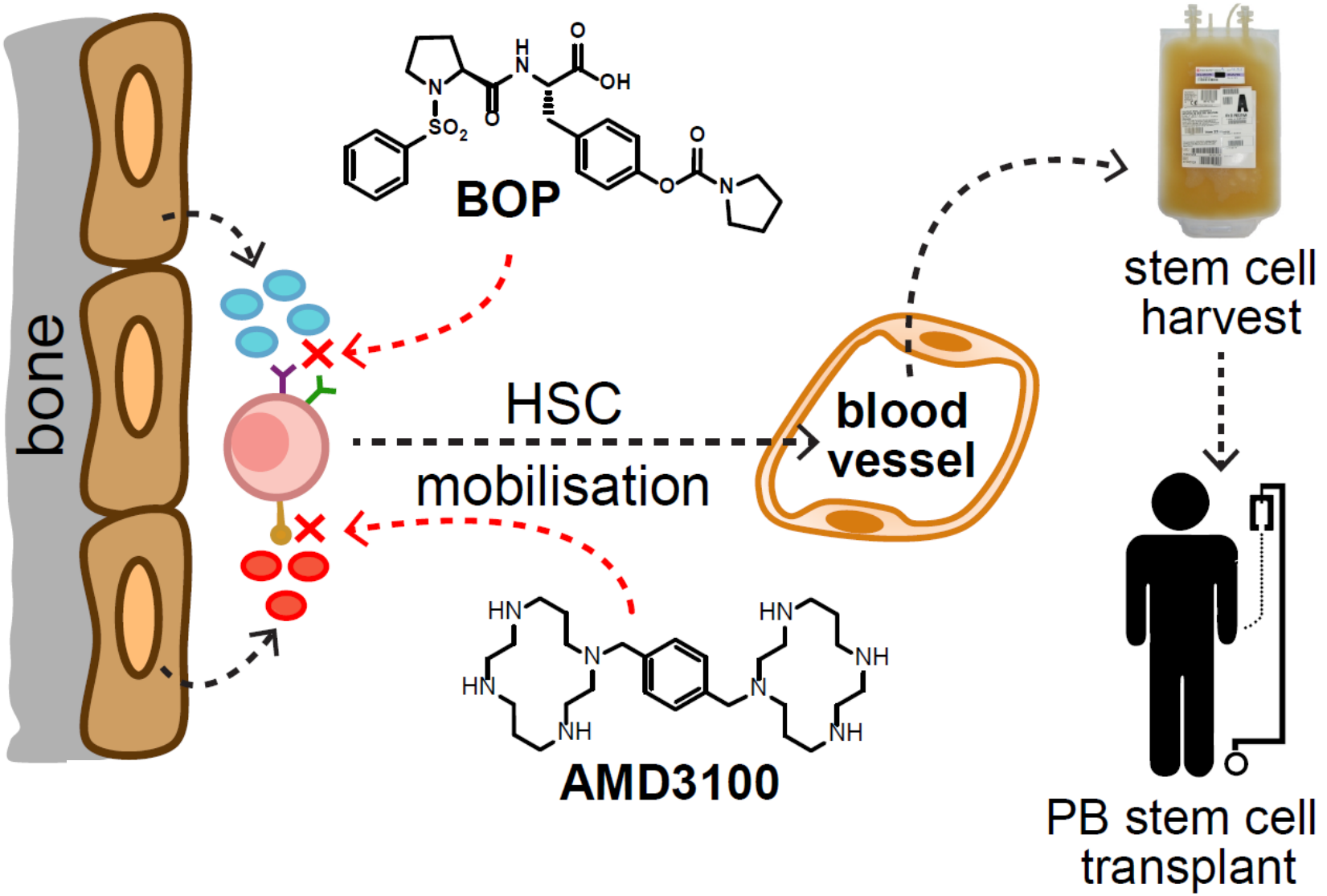
- bone pain, splenomegaly, alters bone marrow microenvironment
- repeated daily doses over 4-5 days




## *AMD3100 (Plerixafor; marketed as Mozobil®)*

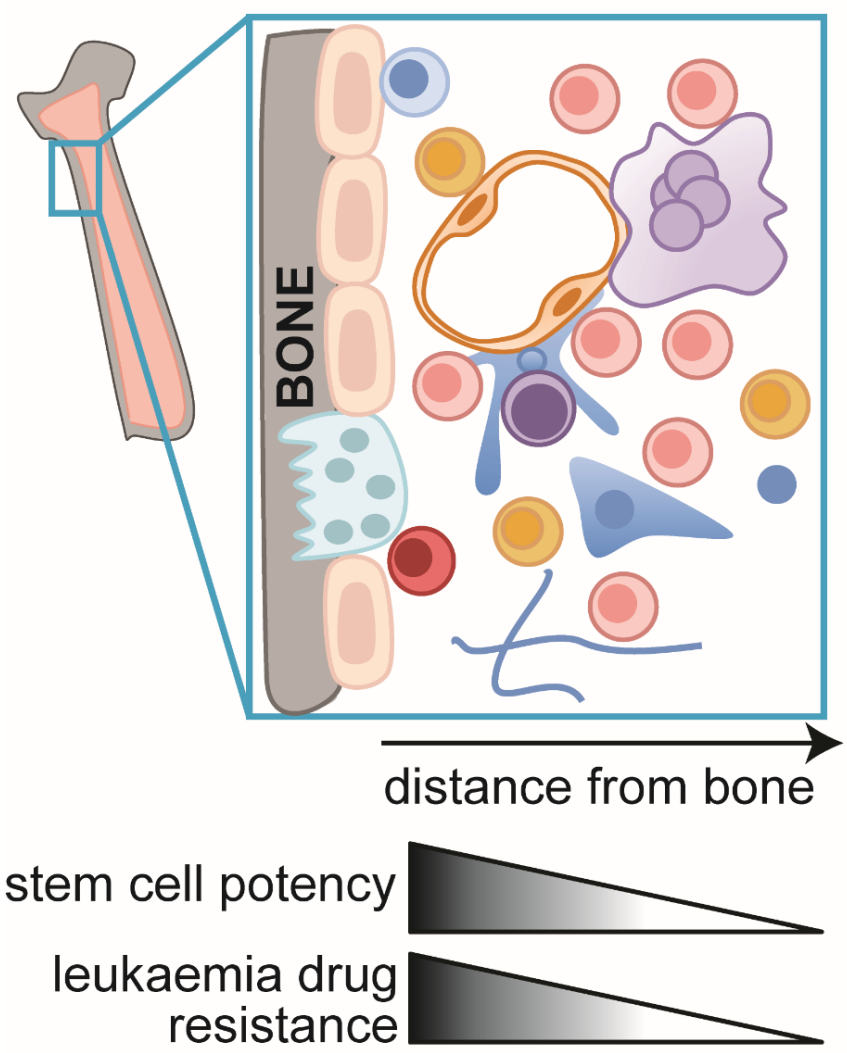
- Selective for one receptor
- inhibits a key HSC microenvironment interaction
- Rapid mobilisation of HSC and marrow cells
- Clinical use is dependent on G-CSF

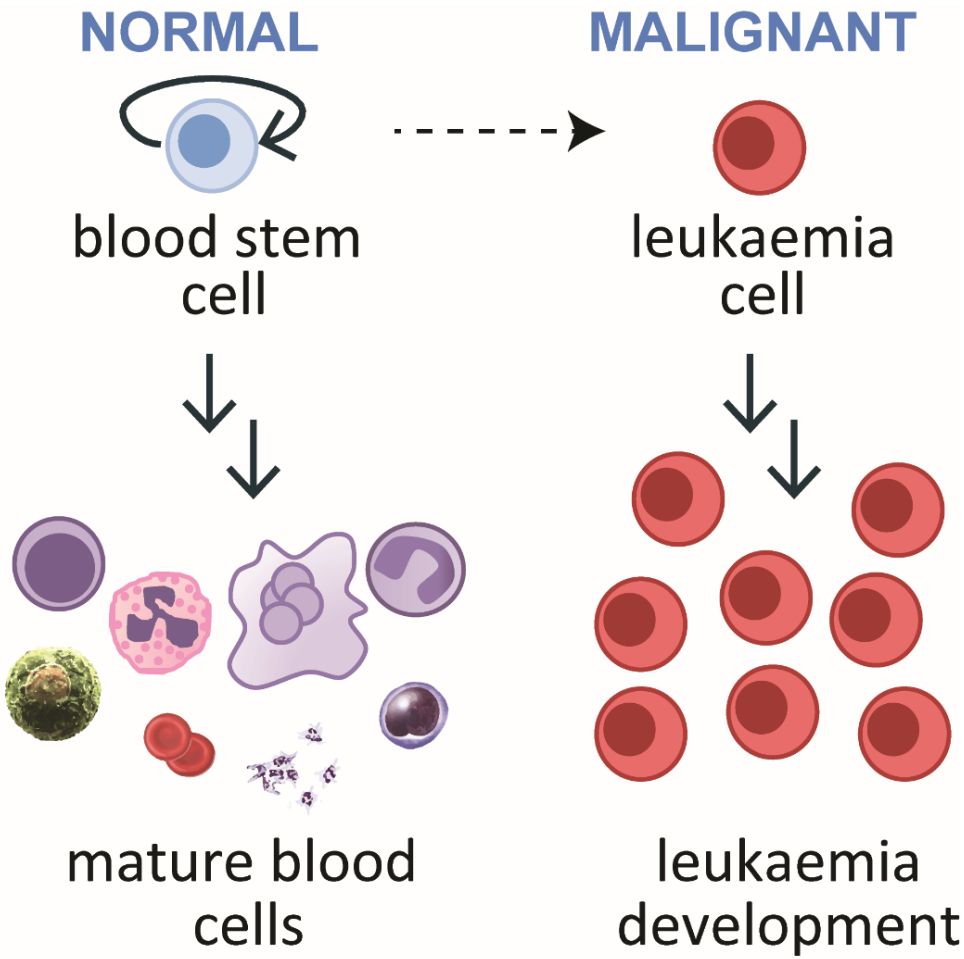
# HSC mobilisation using BOP



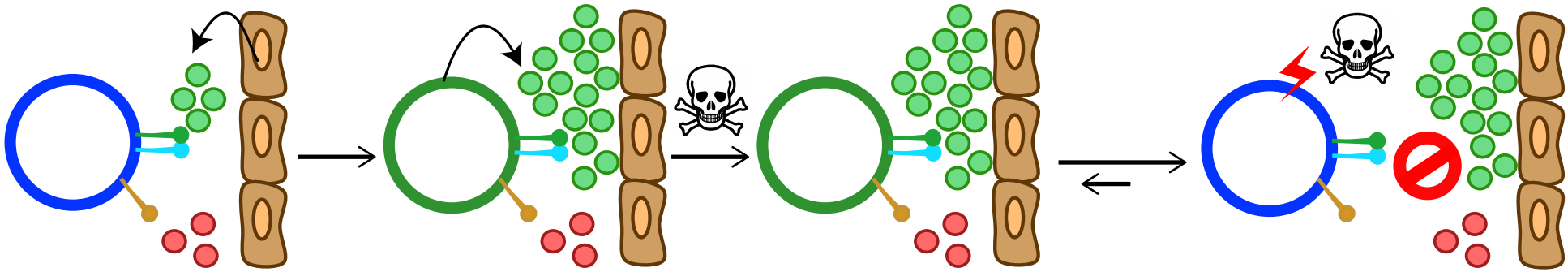

*Cao et al. Nature Communications 2016*

# Malignant blood stem cells



# Chemosensitisation:



LSC migrate to and anchor near bone

LSC make a protein that makes them stop going through cell division

Chemotherapy fails to kill LSC

Dislodging LSC from microenvironment drives them into cell cycle and makes them susceptible to chemo

# Chemosensitisation using BOP: