

# Stem cells: current reality & future promise



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# Institute for Cellular and Molecular Medicine (ICMM)

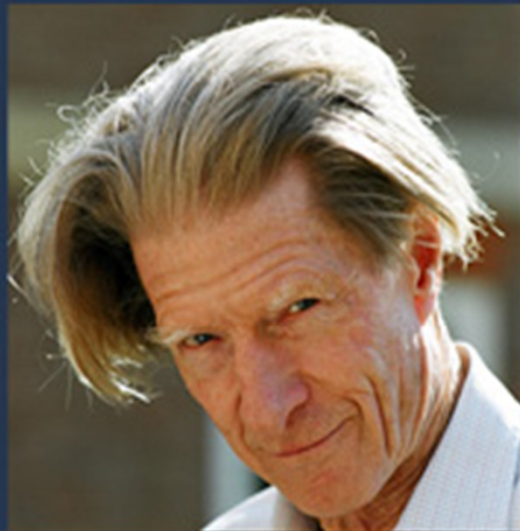
- Initiated in August 2008
- 2011 - Faculty Research Theme
- 2012 - Funding from UP Executive
- Faculties: HS, NAS, VS, Law, EMS, EBIT, Hum
- 16 groups
  - Pepper group
    - Cell-based therapy
    - Human genome

# Acknowledgements

- Funding
  - University of Pretoria
  - National Research Foundation
  - Medical Research Council
  - NHLS Research Trust
  - Ampath Trust

# The 2012 Nobel Prize in Physiology or Medicine

For the discovery that mature cells can be reprogrammed to  
become pluripotent



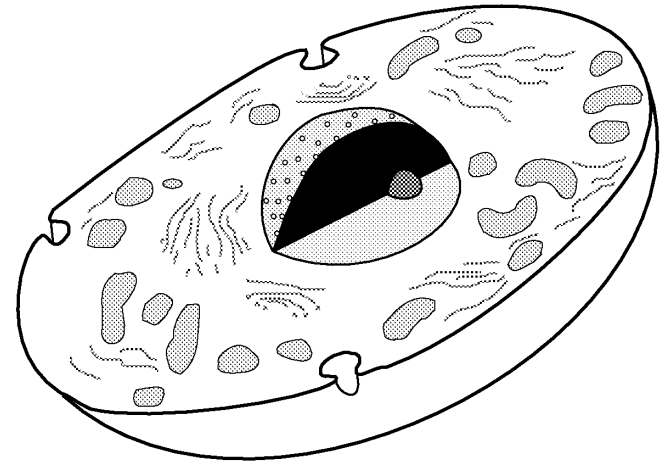
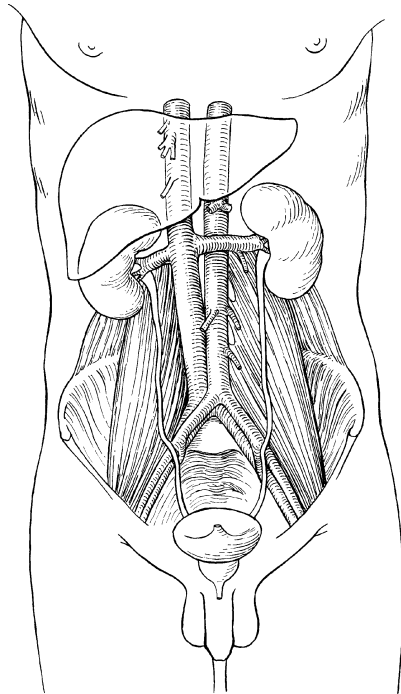
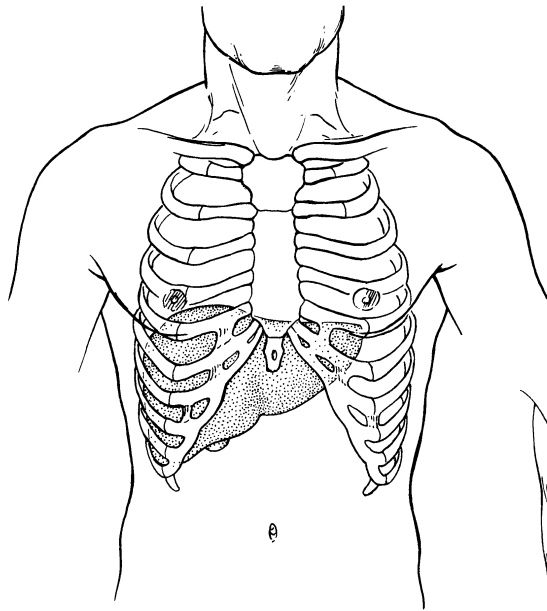
John B. Gurdon



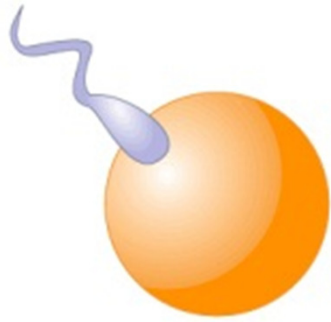
Shinya Yamanaka

# Stem cell principles

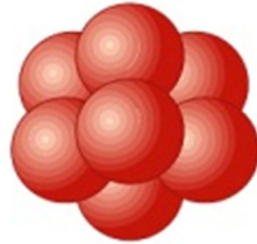
- Stem cell definition
- Stem cell differentiation potential
- Induced pluripotent stem cells
- Embryonic stem cells
- Somatic cell nuclear transfer
- Adult stem cells



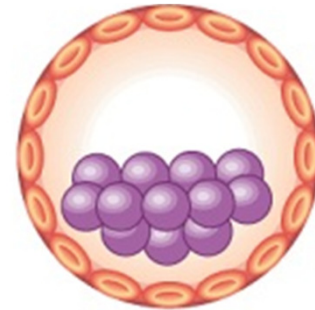
# In the beginning...



Zygote

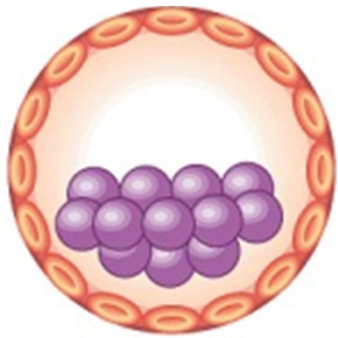


8-cell  
embryo



Blastocyst



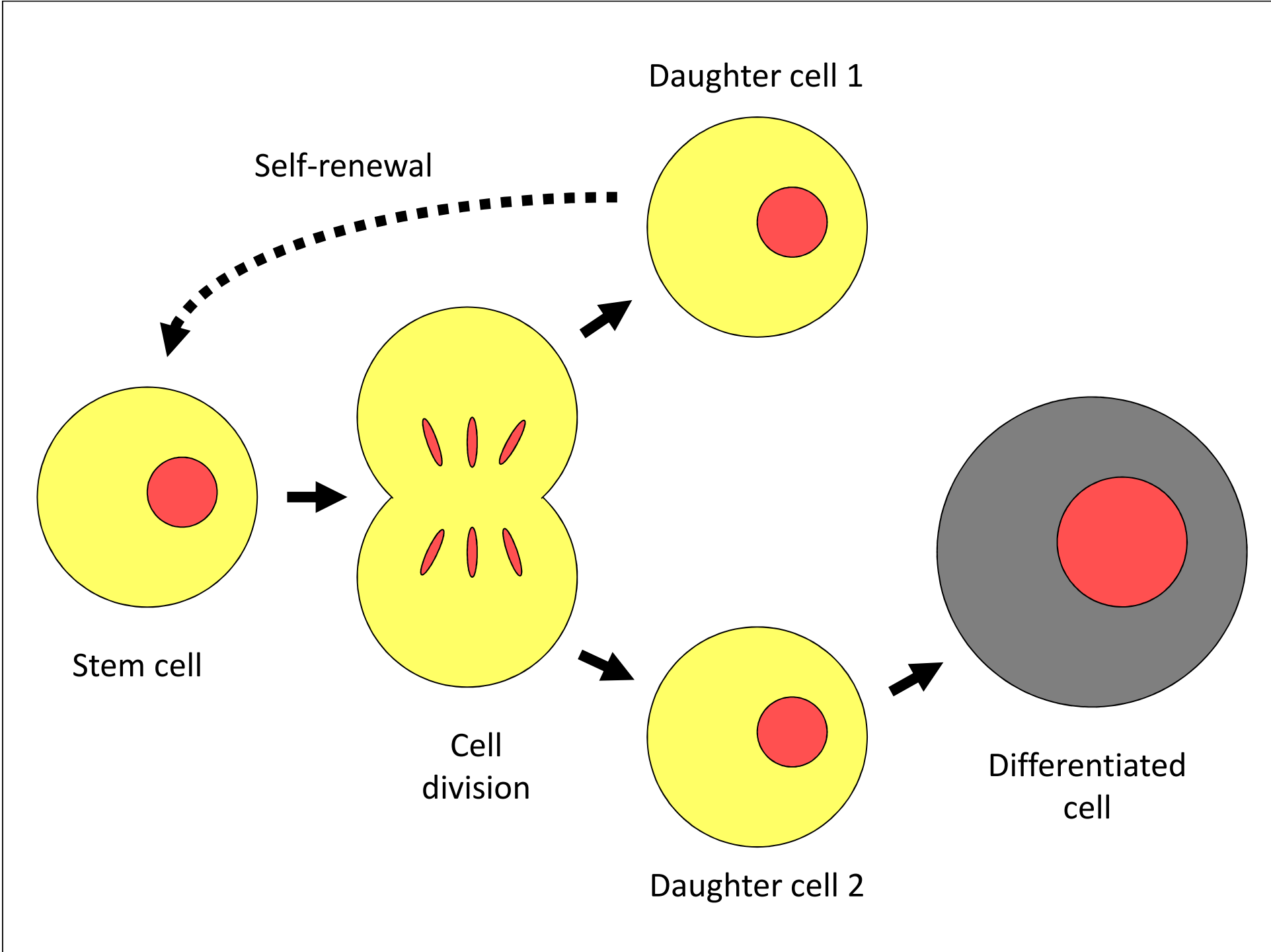


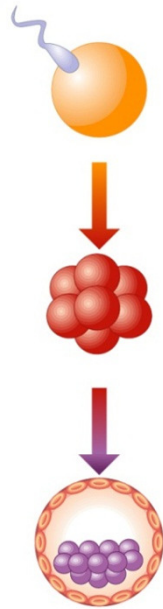
Blastocyst



Embryo







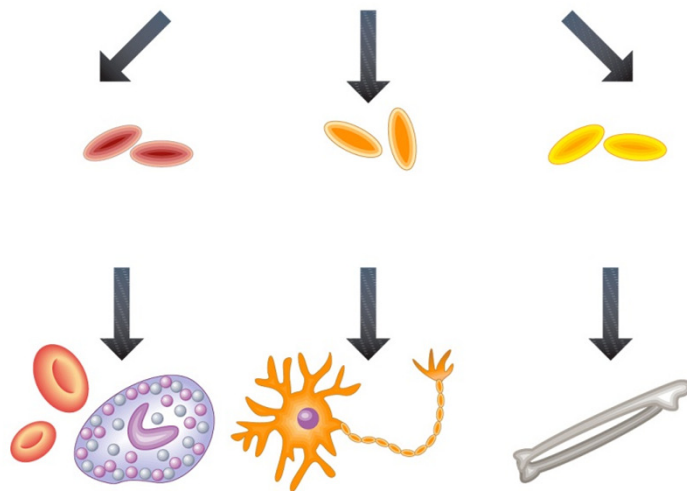
### Totipotent

- post-fertilization to morula
- all the cells of the human body

### Pluripotent

- inner cell mass of the blastocyst
- cells of all three germ layers

Embryo - germ layers:  
mesoderm, ectoderm, endoderm



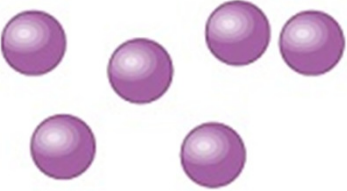
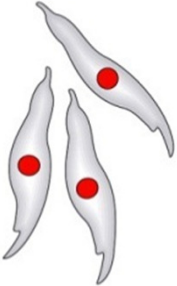
### Multipotent

- hematopoietic stem cells
- neural stem cells
- mesenchymal stem cells
- various specialized cells in a given tissue

# Induced pluripotent stem cells

4 transcription factors:

- Oct3/4
- Sox2
- Klf4
- c-Myc



Pluripotent stem cells

Differentiation factors



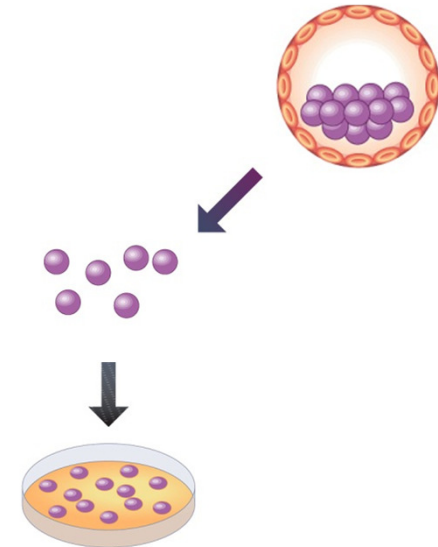
# Induced pluripotent stem (iPS) cells

- Somatic cells reprogrammed (dedifferentiated) to a pluripotent state using 4 transcription factors
  - Oct3/4, Sox2, Klf4, and c-Myc
- Done with cells from all 3 germ layers (e.g. fibroblasts, lymphocytes, liver, stomach, pancreatic  $\beta$  cells, neural stem cells, melanocytes, adipose stem cells and keratinocytes)
- Involves demethylation of pluripotency genes
- Sperm and egg cells generated from mouse iPS cells - used to generate mouse pups
  - primordial germ cells (precursors to sperm and eggs) generated from human iPS cells derived from human skin

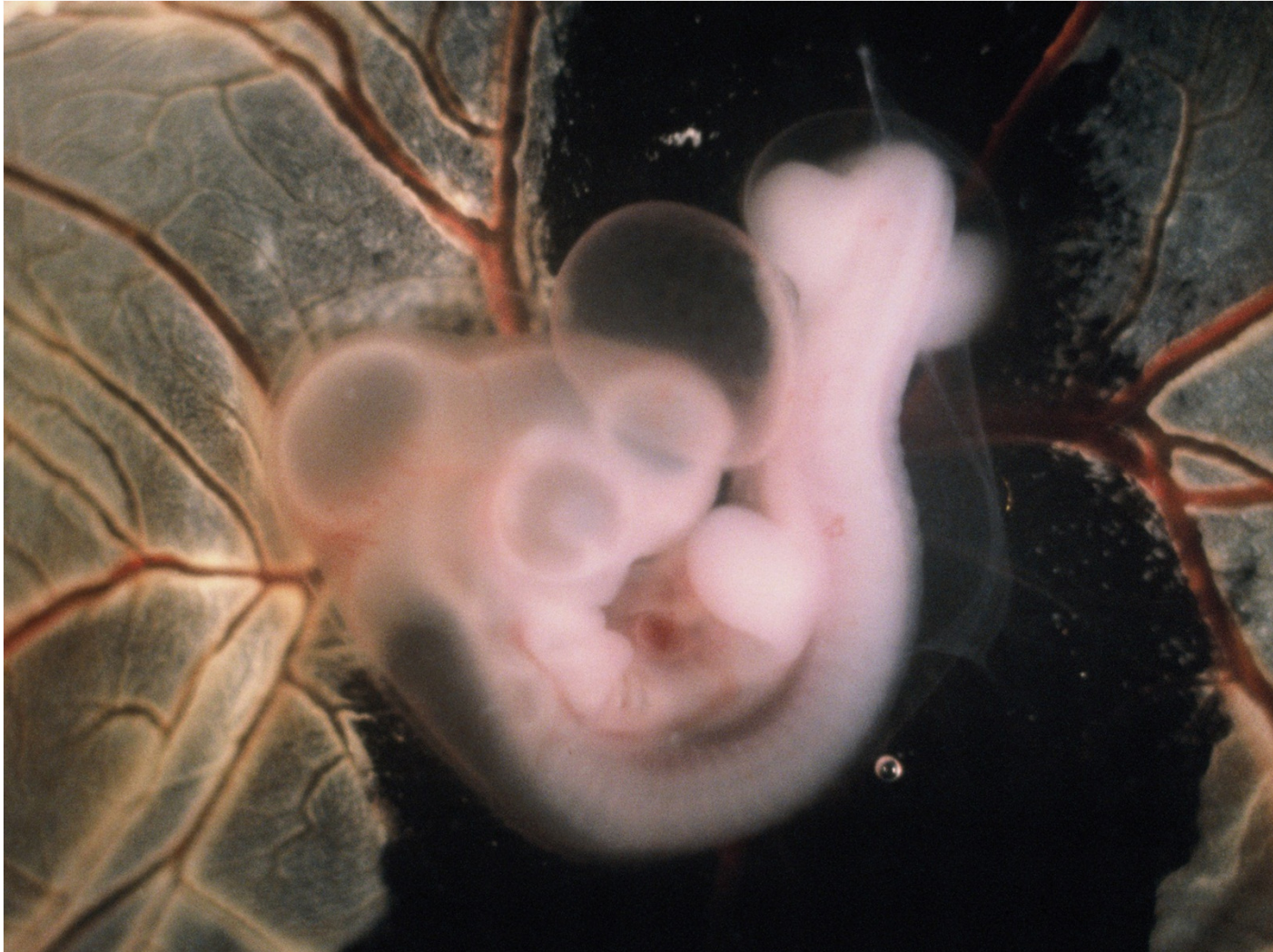
# Embryonic stem cells

Method of obtaining ES cells:

- Blastocyst
  - 5 day old embryo
  - approx 100 cells
- Remove inner cell mass
- Cultured cells = ES cells



# The beginning of life

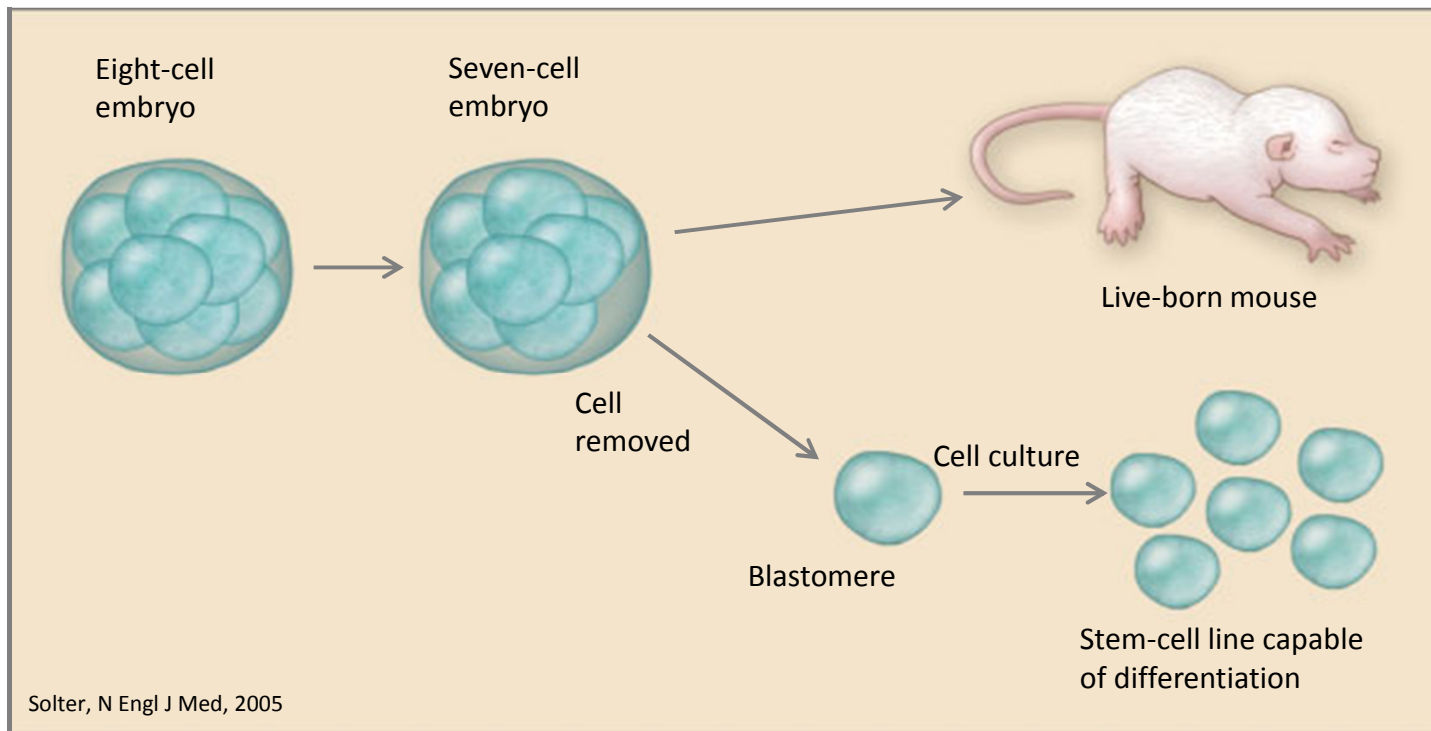


# Life begins...

- at the moment of fertilisation
- with the development of the first organ system (heart and blood vessels)
- at the moment of perceived consciousness
- from the moment the foetus is able to survive outside the uterus (22 - 24 weeks)
- .....

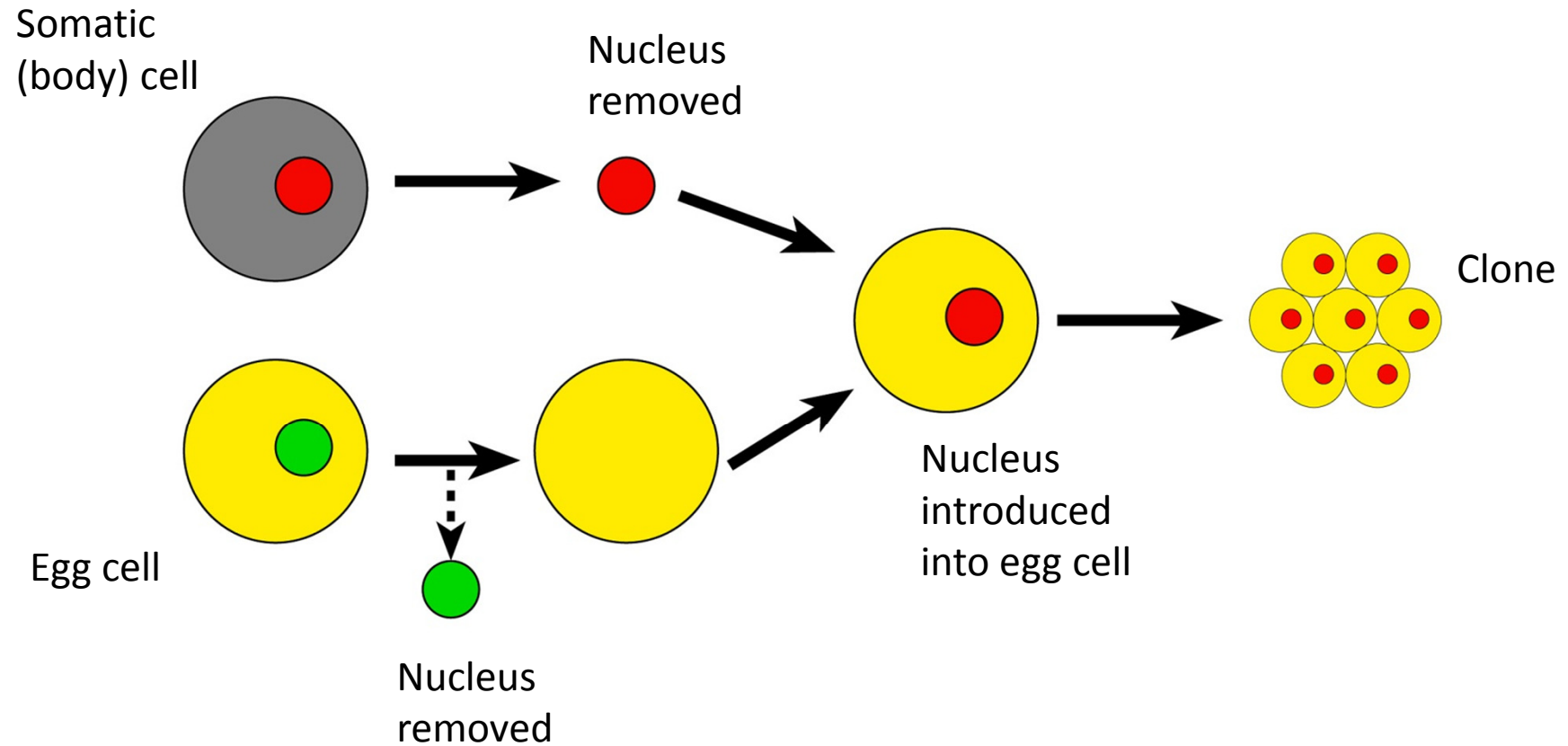
# A solution to the ethical debate?

Chung et al., Nature 2006; Klimanskaya et al., Nature 2006





# Somatic cell nuclear transfer

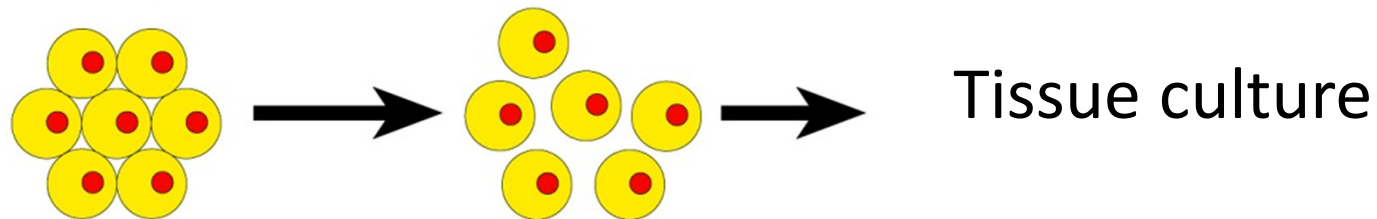


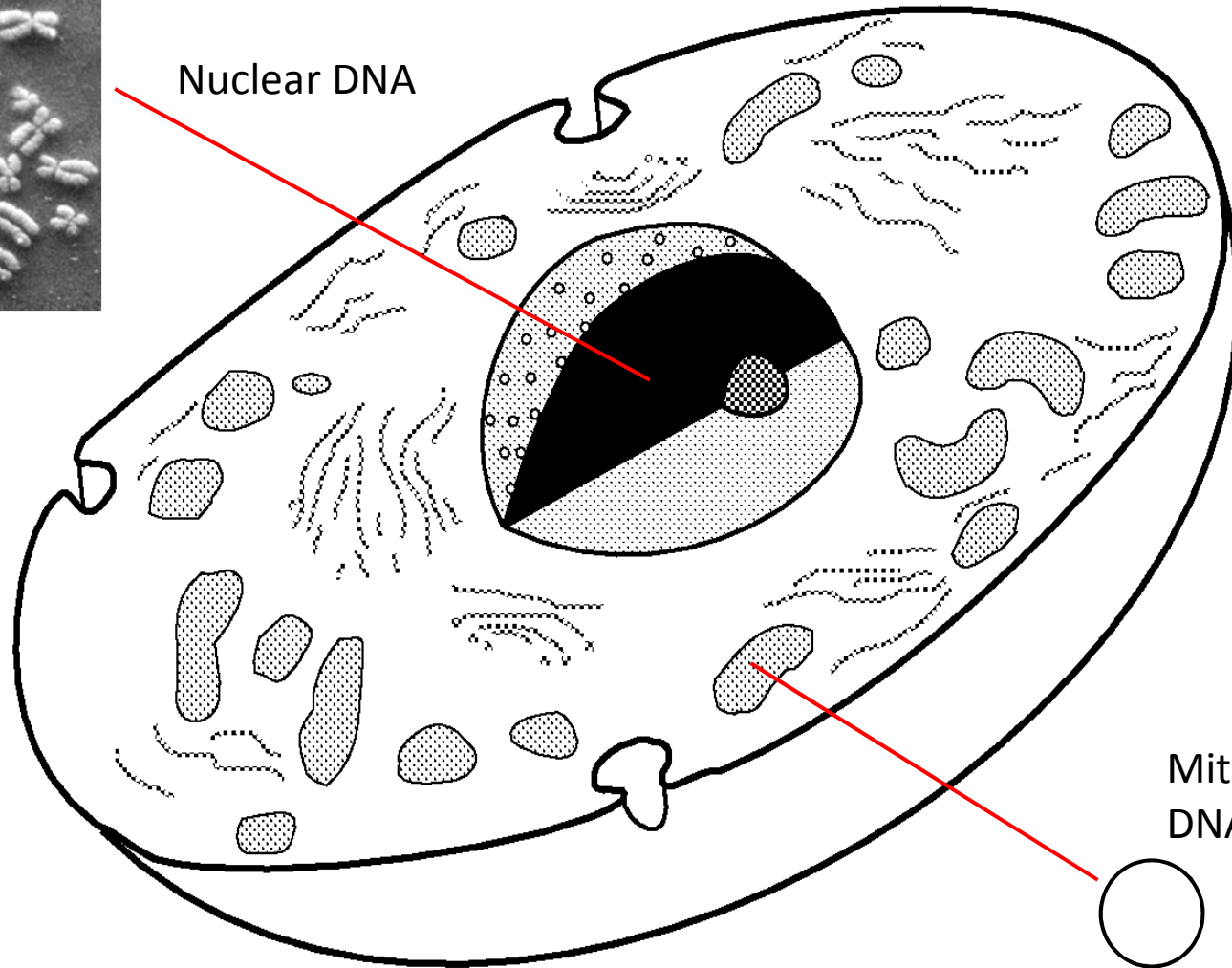
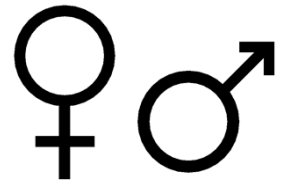
# Cloning

Reproductive cloning

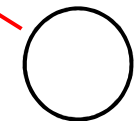


Therapeutic cloning



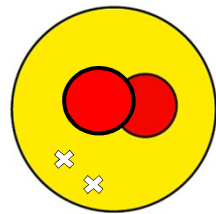


Mitochondrial DNA

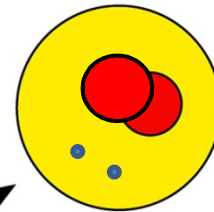
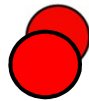


# Mitochondrial disorders

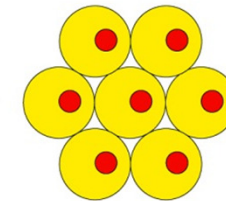
Fertilized egg – mutation  
in (maternal)  
mitochondrial DNA



Pro-nuclei  
removed

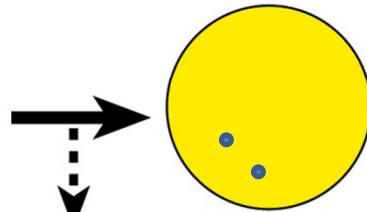
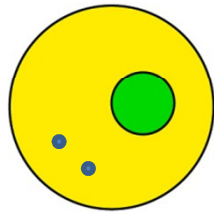


Pro-nuclei  
introduced  
into donor egg



Clone

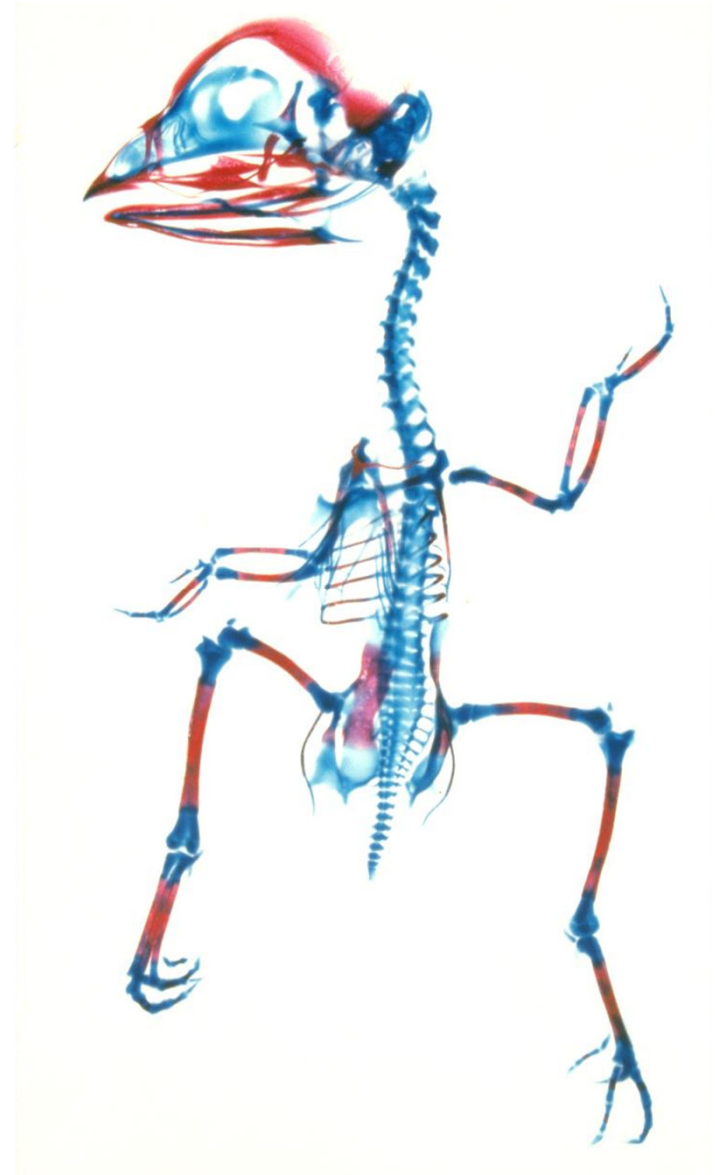
Donor egg – normal  
mitochondrial DNA



Nucleus  
removed

# Tissue-specific adult stem cells

- bone marrow
- gastrointestinal tract
  - liver, pancreas
- tooth
- skin, hair
- central nervous system
- kidney
- muscle (satellite stem cells)



# Pluripotent vs. adult stem cells

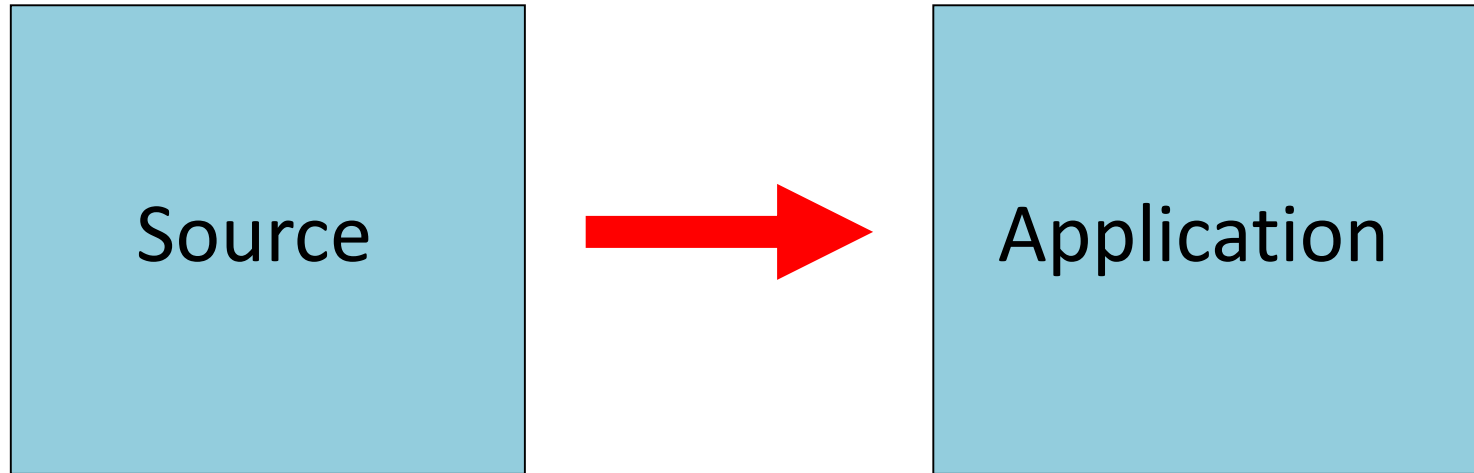
## Pluripotent

- Differentiation potential = all of the body's cell types
- Ethical issues
- Technically more difficult to obtain
- Potential for tumorigenesis
- Therapeutic value – remains to be determined
- Value: understanding disease processes, drug screening

## Adult

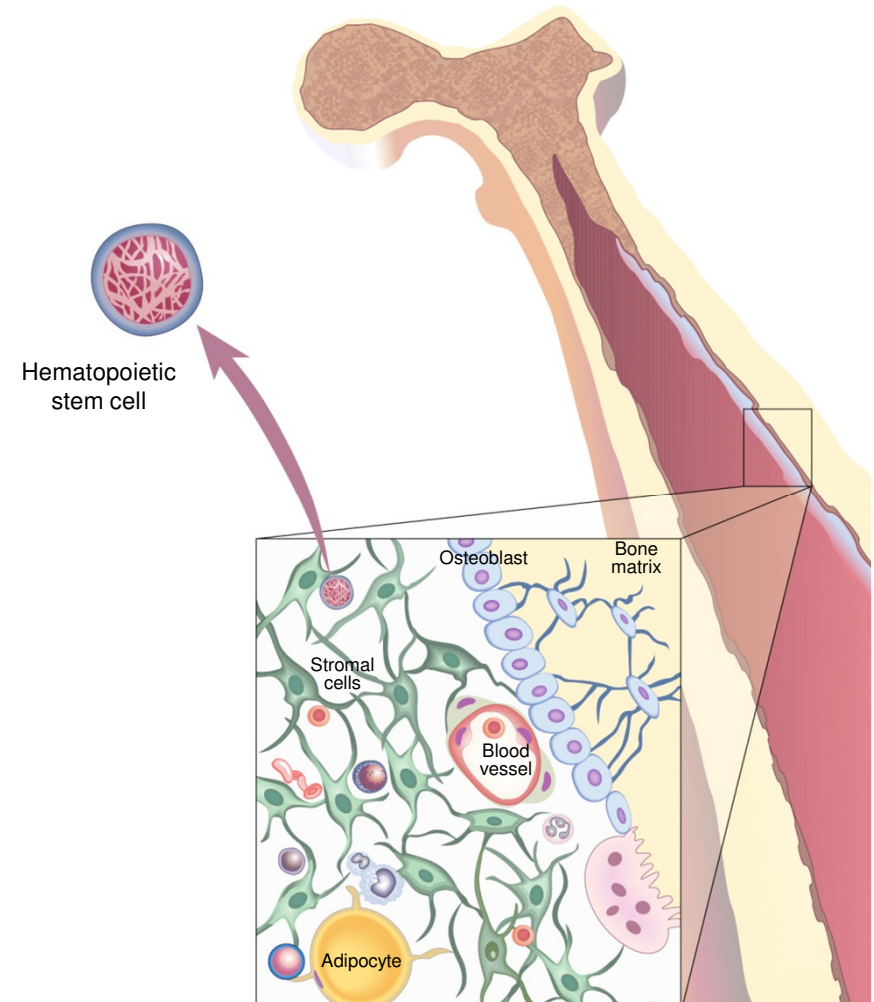
- Differentiation potential limited to cells of tissue in which they reside
- No ethical issues
- Readily available
- No evidence for tumorigenesis
- Therapeutic value well demonstrated:
  - HSCs for bone marrow transplantation
  - MSCs: approx 200 registered clinical trials

# Therapeutic applications



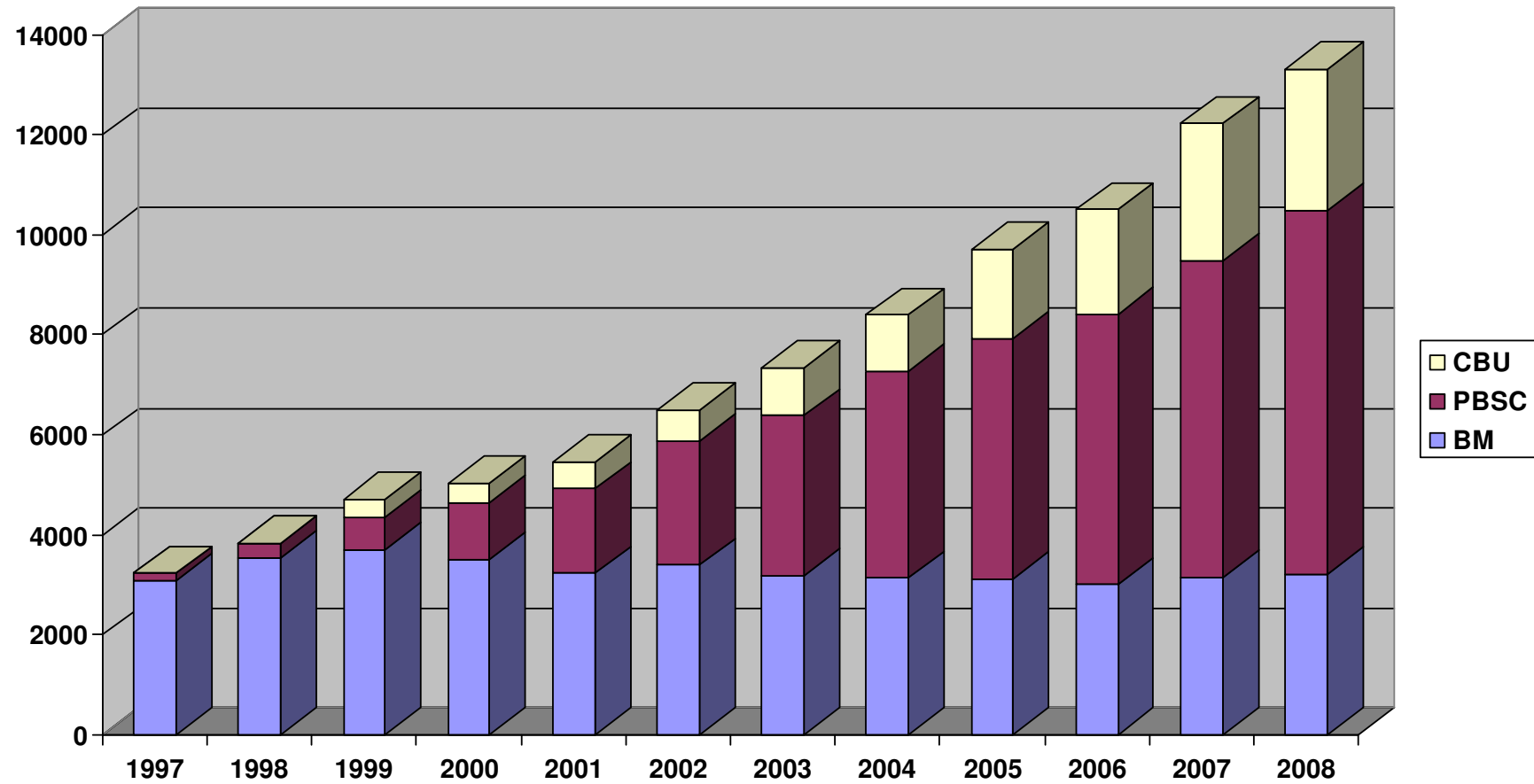
# Source

- Current
  - Bone marrow
  - Peripheral blood
  - Cord blood





# Sources of stem cells for unrelated bone marrow transplantation worldwide



# Source

- Future
  - ES cells
  - iPS cells
  - Adipose tissue
  - Other

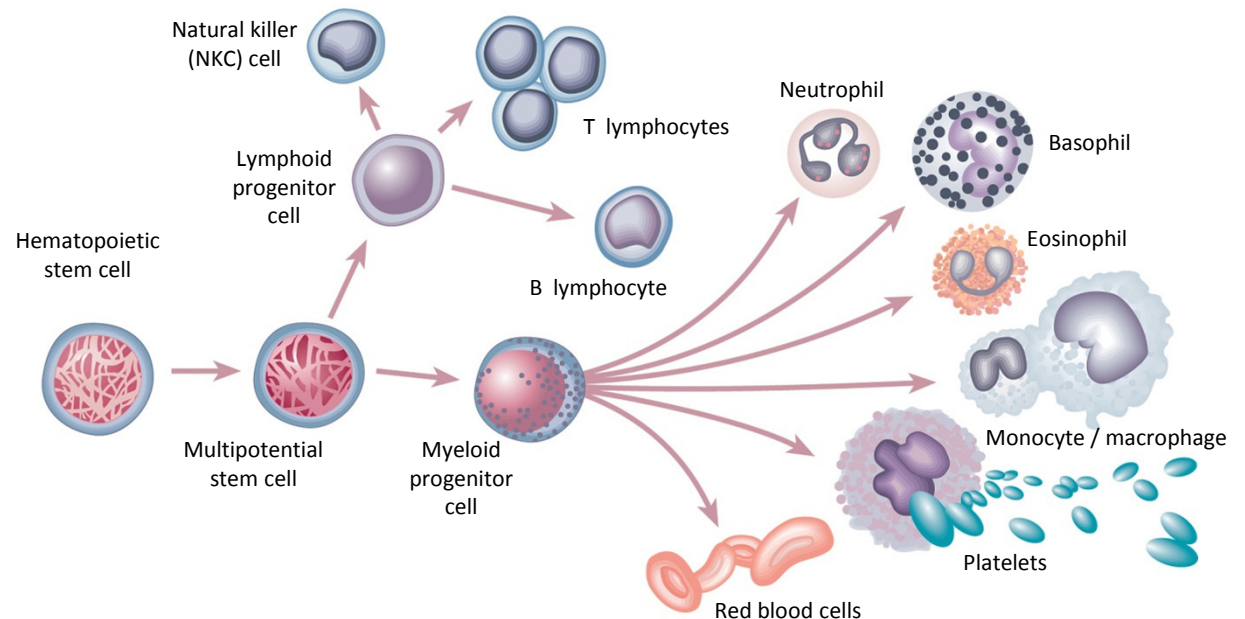


# Application

- Current
  - Bone marrow transplantation
  - Fractures, chronic wounds, burns
- Future
  - Myocardial infarction and heart failure
  - CNS disease (incl. spinal cord injury)
  - Diabetes (beta-cells)
  - Other

# Bone marrow transplantation

- Universally employed and approved
- Autologous or allogeneic
- Indications
  - cancer
  - blood disorders
  - genetic disorders



# Hematopoietic stem cell transplant – indications

## Leukemias

- Acute Leukemia
- Acute Lymphoblastic Leukemia (ALL)
- Acute Myelogenous Leukemia (AML)
- Acute Biphentotypic Leukemia
- Acute Undifferentiated Leukemia
- Chronic Leukemia
- Chronic Myelogenous Leukemia (CML)
- Chronic Lymphocytic Leukemia (CLL)
- Juvenile Chronic Myelogenous Leukemia (JCML)
- Juvenile Myelomonocytic Leukemia (JMML)

## Lymphomas

- Hodgkin's Lymphoma
- Non-Hodgkin's Lymphoma Burkitt's Lymphoma

## Plasma Cell Disorders

- Multiple Myeloma
- Plasma Cell Leukemia
- Waldenstrom's Macroglobulinemia

## Other cancers (Not originating in the blood system)

- Neuroblastoma
- Retinoblastoma

## Inherited Red Cell (Erythrocyte) Abnormalities

- Beta Thalassemia Major (also known as Cooley's Anemia)
- Blackfan-Diamond Anemia
- Pure Red Cell Aplasia
- Sickle Cell Disease

## Myelodysplastic Syndromes

- Refractory Anemia (RA)
- Refractory Anemia with Ringed Sideroblasts (RARS)
- Refractory Anemia with Excess Blasts (RAEB)
- Refractory Anemia with Excess Blasts in Transformation (RAEB-T)
- Chronic Myelomonocytic Leukemia (CMML)

## Other Disorders of Blood Cell Proliferation

- severe Aplastic Anemia
- Congenital Dyserythropoietic Anemia
- Fanconi Anemia (Note: the first cord blood transplant in 1988 was for this disease)
- Paroxysmal Nocturnal Hemoglobinuria (PNH)
- Pure Red Cell Aplasia
- Amegakaryocytosis / Congenital Thrombocytopenia
- Glanzmann Thrombasthenia
- Acute Myelofibrosis
- Agnogenic Myeloid Metaplasia (Myelofibrosis)
- Polycythemia Vera
- Essential Thrombocythemia

## Inherited Immune System Disorders

### Severe Combined Immunodeficiency (SCID)

- SCID with Adenosine Deaminase Deficiency (ADA-SCID)
- SCID which is X-linked
- SCID with absence of T & B Cells
- SCID with absence of T Cells, Normal B Cells
- Omenn Syndrome

### Neutropenias

- Kostmann Syndrome
- Myelokathexis

### Other

- Ataxia-Telangiectasia
- Bare Lymphocyte Syndrome
- Common Variable Immunodeficiency
- DiGeorge Syndrome
- Leukocyte Adhesion Deficiency
- Lymphoproliferative Disorders (LPD)
- Lymphoproliferative Disorder, X-linked (also known as Epstein-Barr Virus Susceptibility)
- Wiskott-Aldrich Syndrome

### Phagocyte Disorders

- Chediak-Higashi Syndrome
- Chronic Granulomatous Disease
- Neutrophil Actin Deficiency
- Reticular Dysgenesis

# Group projects

- Effect of HIV on hematopoiesis in vitro
  - Madelein-Meissner-Roloff (PhD student)
- HSC expansion and engraftment
  - Carlo Jackson (PhD student)
- AML diagnostic microarray
  - Dr Marco Alessandrini (post-doc)
- Leukemia & lymphoma immunophenotyping
  - Dr Chrisna Durandt (clinical research co-ordinator)

# Current situation in South Africa

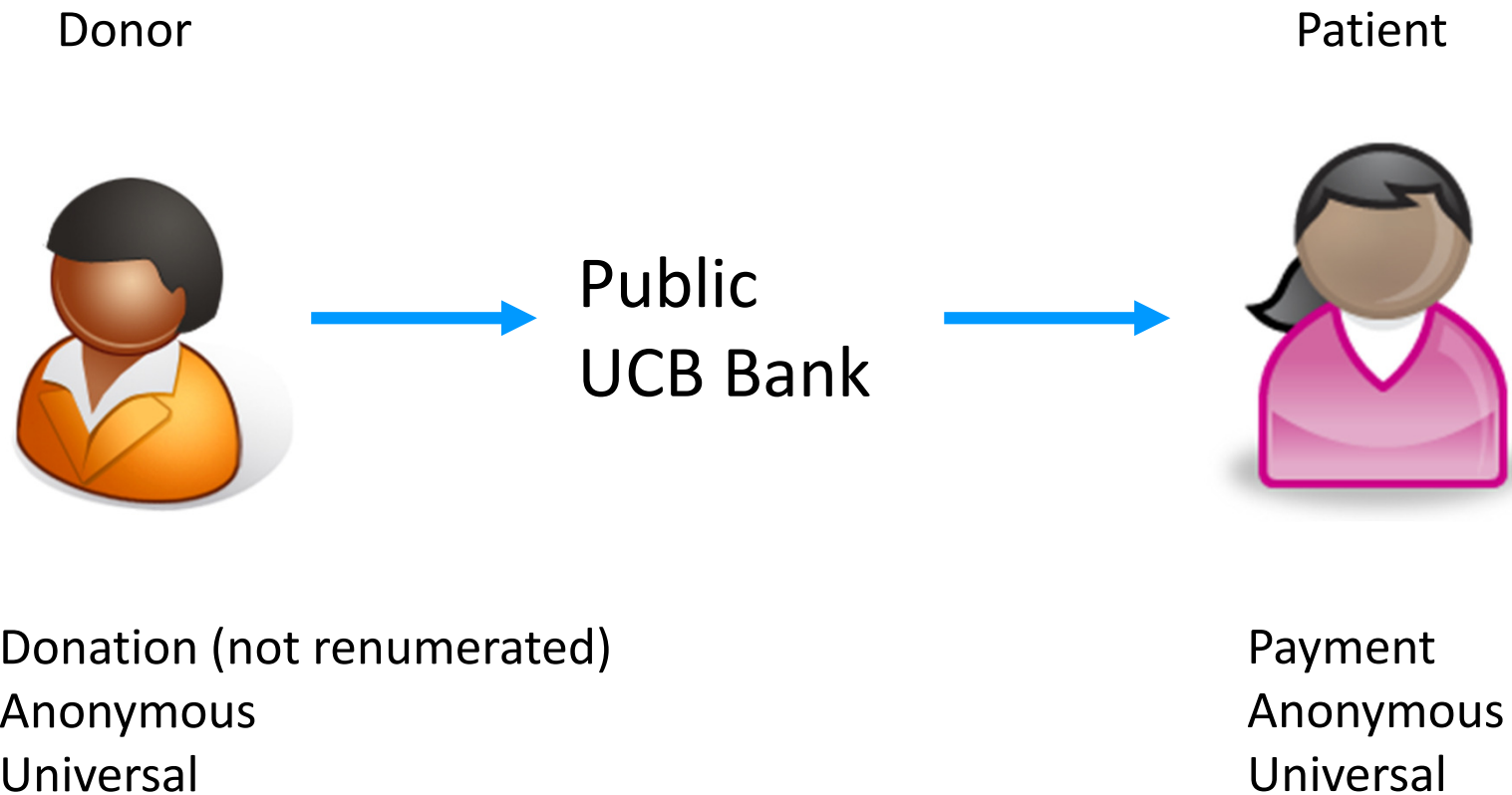
## Bone marrow transplantation (BMT)

- practiced successfully for many years
- many South Africans requiring a BMT remain untreated

## SA Bone Marrow Registry

- established in 1991
- >65,000 donors registered
- >250 transplants (matched unrelated donor)
- Donors: 25% local; 75% international
- majority of donors are Caucasian

# Cord blood banking - public





# Cord blood banking in SA

## Public cord blood bank

- none at present
- genetically-diverse population
- feasibility study
  - Mandated by DoH and SANBS
  - Funded by the MRC

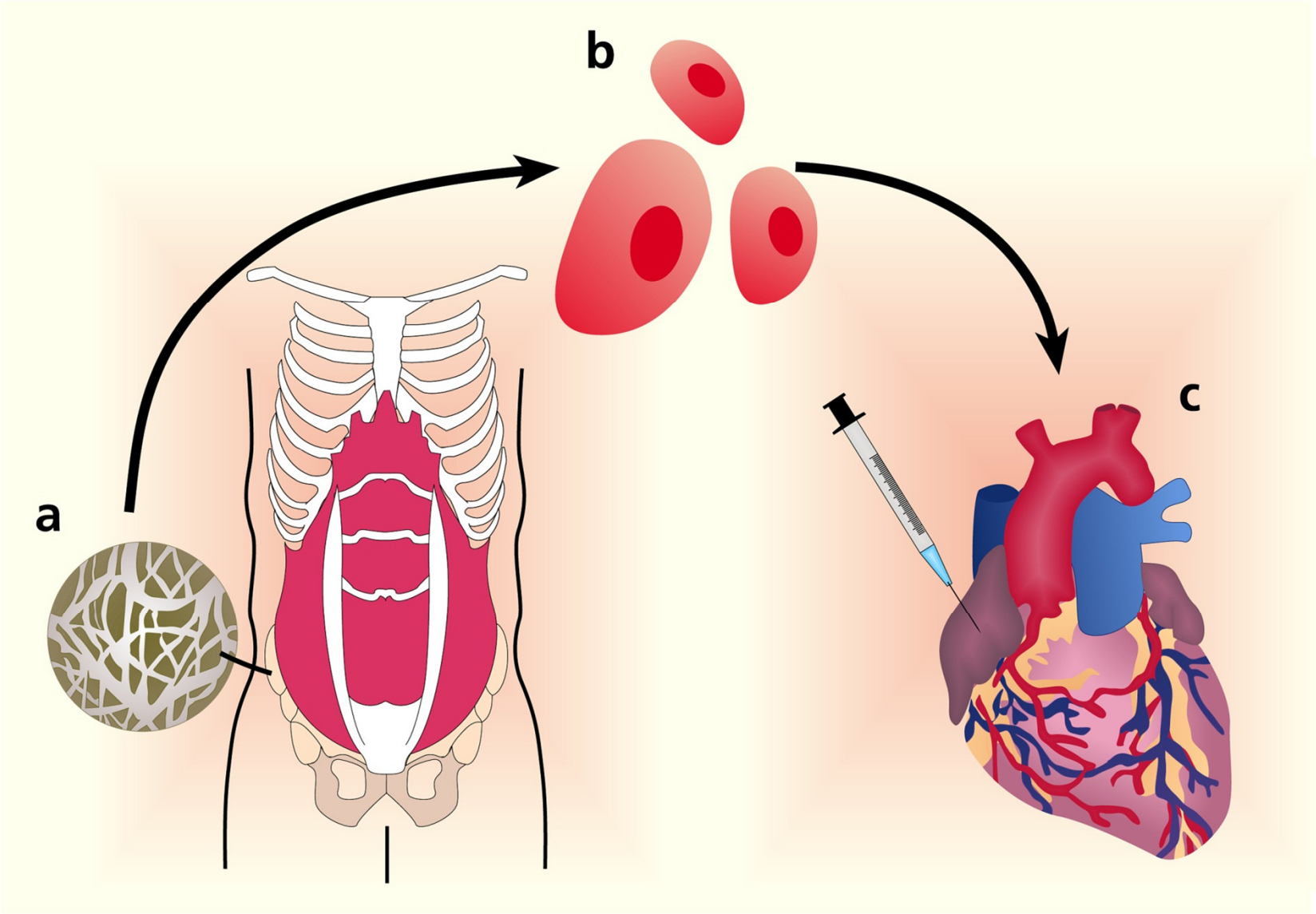
## Private cord blood banks

- 2 in South Africa

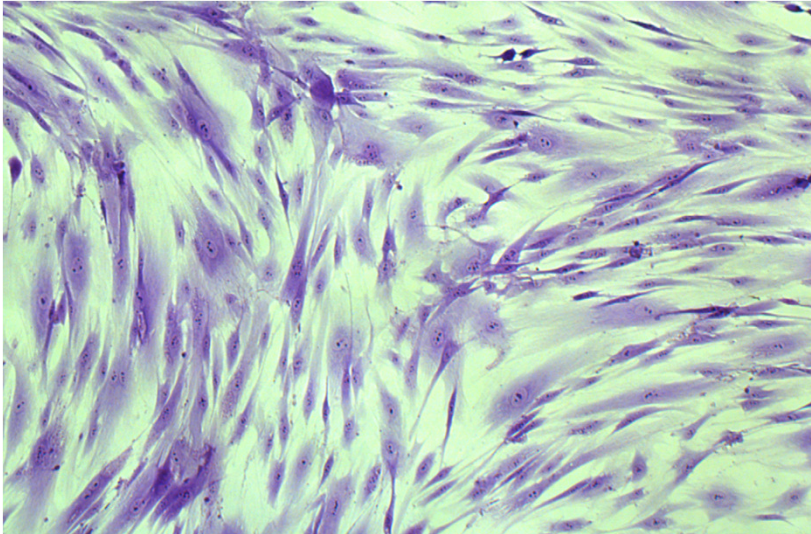
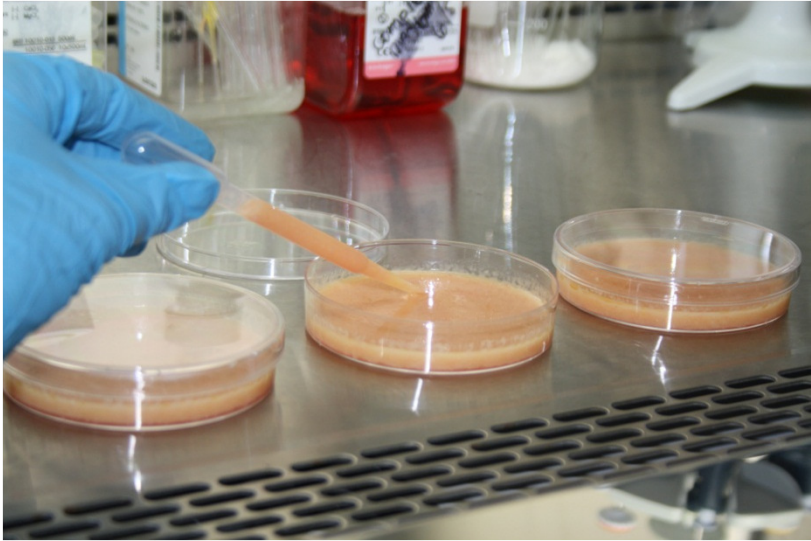
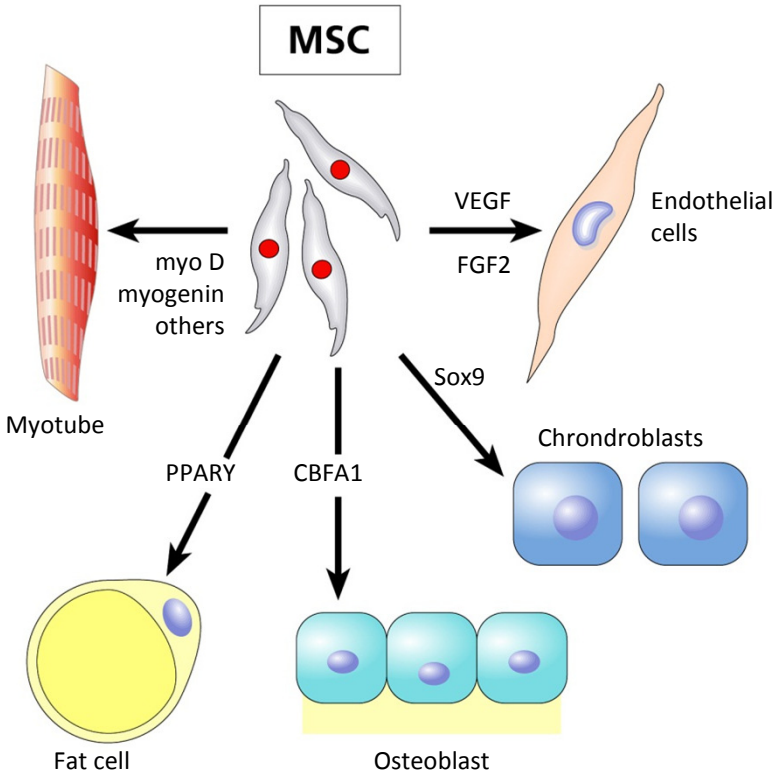
# Public bank feasibility study

- Survey of public opinion
  - Madelein Meissner-Roloff (PhD student)
- Composition of the bank
  - Juanita Mellet (MSc student)
- HIV testing
  - Madelein Meissner-Roloff (PhD student)
- Flow cytometry
  - Dr Chrisna Durandt (clinical research co-ordinator)
- Economic model (incl. sustainability)
  - Dr Marco Alessandrini (post-doc)
  - Isabella Rangaka (PhD student)

# Future applications



# Mesenchymal stem cells



# MSC clinical trials

- ClinicalTrials.gov
- December 2011
  - 188 registered MSC clinical trials
    - 80 completed
    - 108 on-going
- Almost 100 new trials initiated in 2010 and 2011 alone

# MSC clinical trials: indications

Indications (72 in total)	Completed*	Ongoing	Total
Diabetes (Type 1 and 2)	7	10	17
Liver cirrhosis	7	6	13
Graft vs host disease	7	5	12
Osteoarthritis	4	7	11
Myocardial infarction	3	6	9
Crohn's disease	2	5	7
Ischaemic stroke	0	7	7
Spinal cord injury	2	4	6
Heart failure	3	3	6
Multiple sclerosis	1	6	6
Organ transplantation	2	4	6
Other (4 or less)	42	45	88

\*Prior to 2012

# Group projects - MSCs

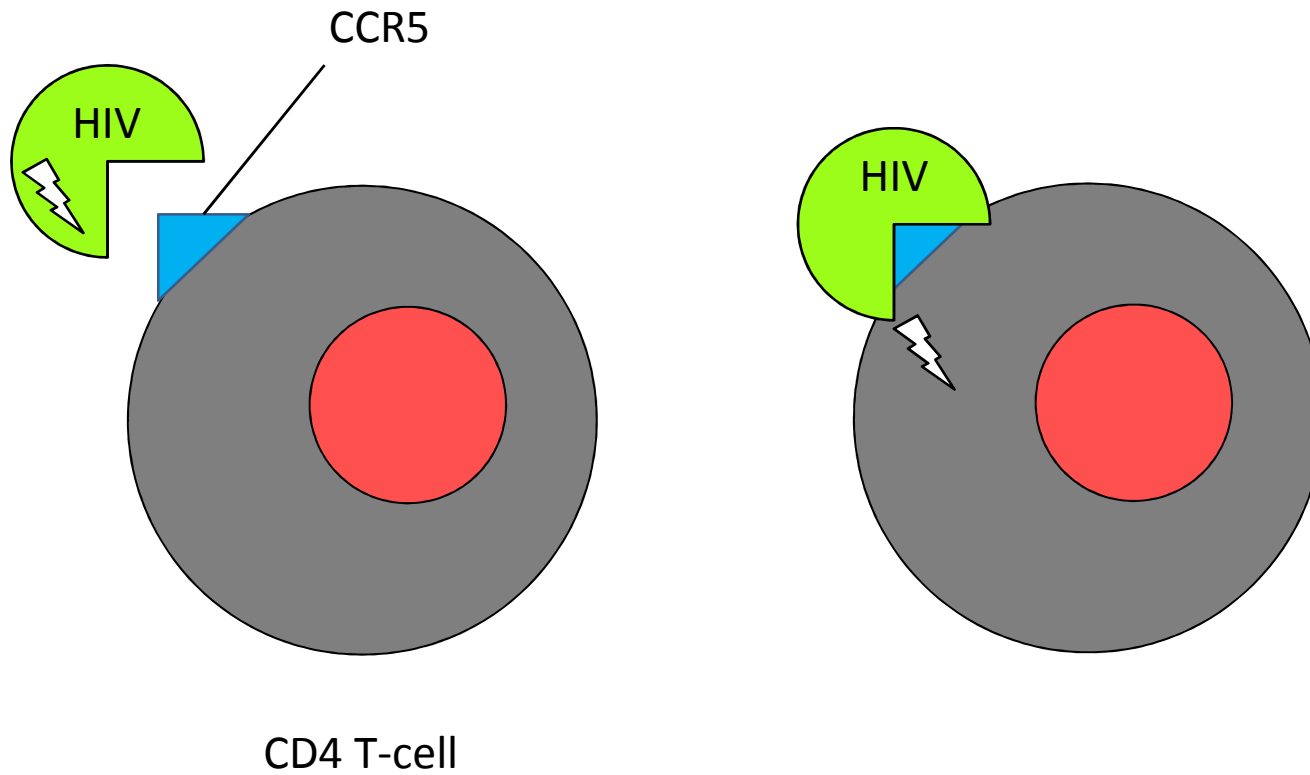
- Routine isolation and characterization
- Homing to sites of injury/inflammation
- Effect of ROS and hypoxia
- Clean room facility and clinical trials
- Team:
  - Dr Marnie Potgieter (post-doc)
  - Dr Marco Alessandrini (post-doc)
  - Dr Chrisna Durandt (clinical research co-ordinator)
  - Fiona van Vollenstee (MSc student)
  - Karlien Kallmeyer (MSc student)
  - Danielle de Villiers (MSc student)
  - Carla Dessels (MSc student)
  - Candice Honing (laboratory assistant)

# Stem cells in SA

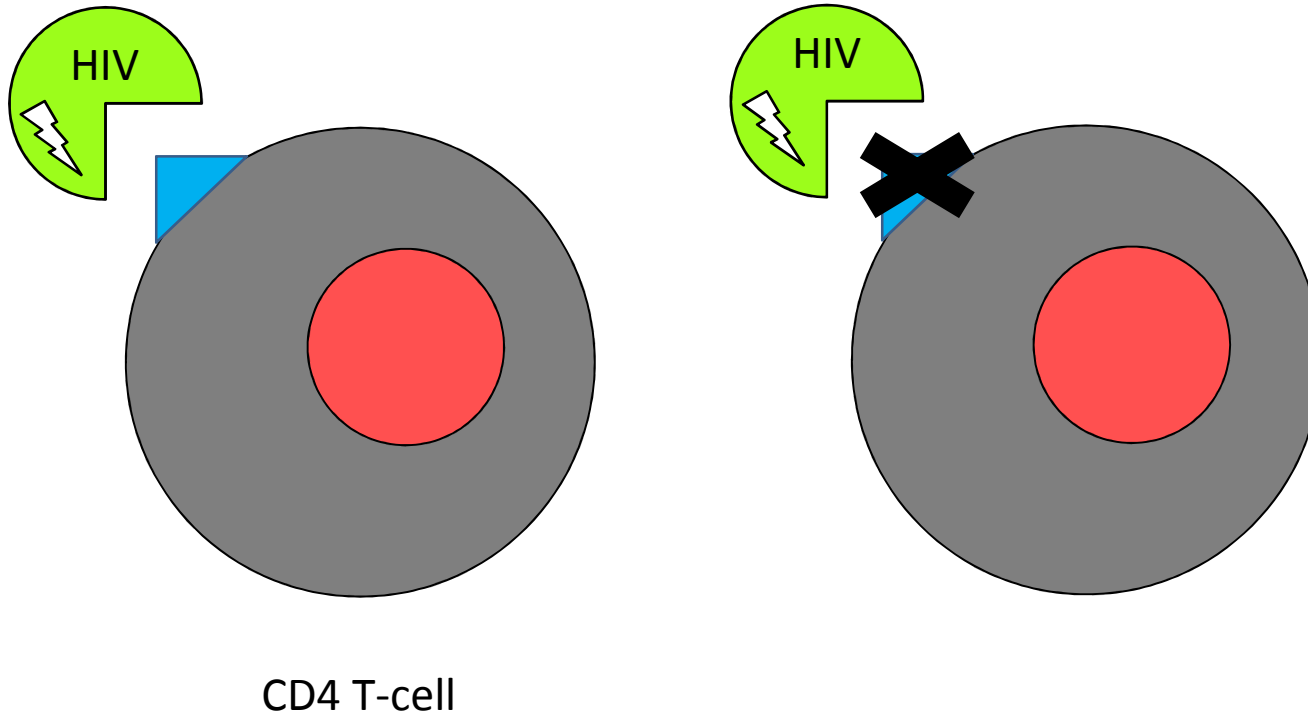
- Stem cell therapy and research are in their infancy in South Africa
  - potential to alleviate heavy burden of disease
  - communicable and non-communicable
- Role in cancer, hematological and inherited metabolic disorders well established but unable to meet demand
- Role in heart disease, spinal cord injury and others being assessed globally but not in SA
- Role in infectious diseases totally unexplored



# Gene-therapy for HIV

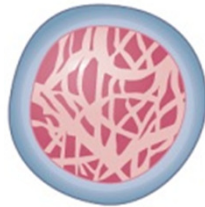


# Gene-therapy for HIV

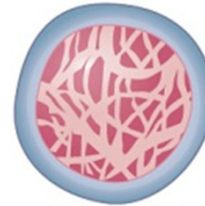




Harvest  
haematopoietic  
stem cells

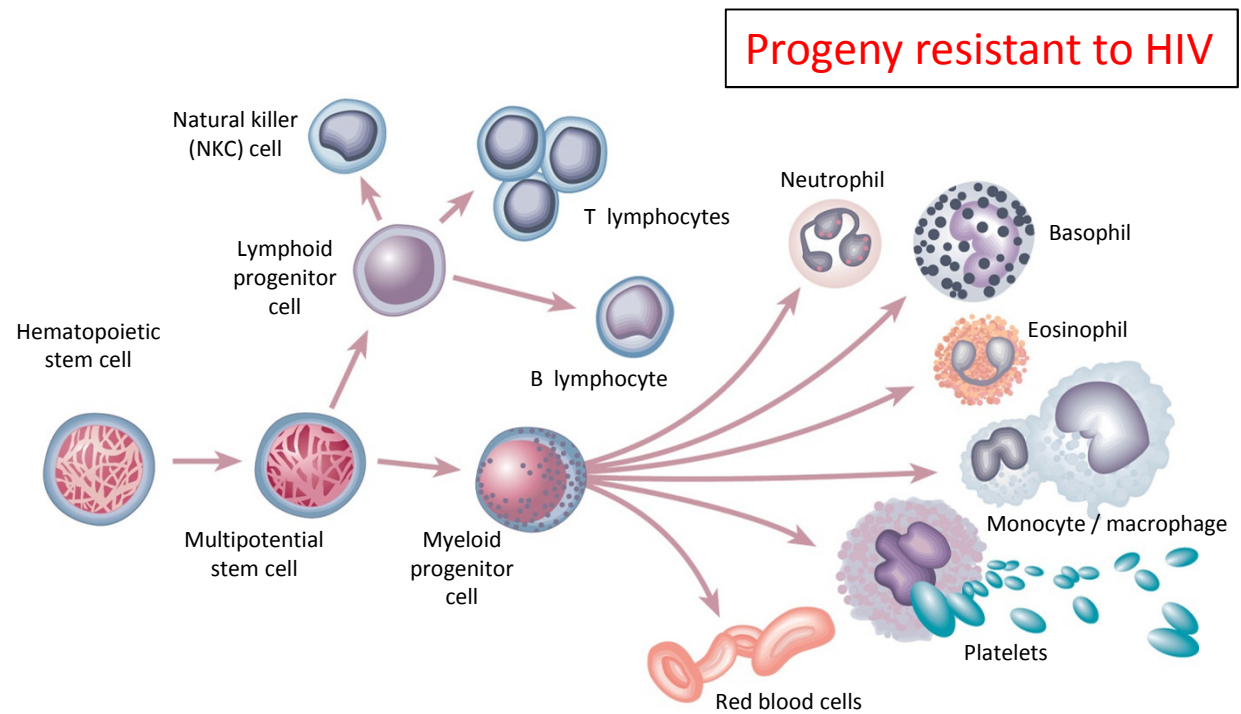


CCR5  
gene  
therapy



HIV-resistant  
hematopoietic  
stem cells

Transplant  
HIV-resistant  
hematopoietic  
stem cells



# HIV gene-therapy team

- University of Pretoria
  - Renier Myburgh (PhD student)
  - Carlo Jackson (PhD student)
  - Madelein Meissner-Roloff (PhD student)
  - Fatima Barmania (MSc student)
- University of Geneva
  - Prof Karl-Heinz Krause
  - Dr Patrick Salmon
- University of Zurich
  - Prof Roberto Speck
  - Renier Myburgh (PhD student)

# Human tissue legislation in South Africa

- Legislation incomplete and flawed
  - increasingly litigious society
  - importance of global standards
- Regulations
  - Redundancy/overlap
  - Lack of regulations
    - transplantation
    - cell-based therapy
- Definitions
  - not harmonized (NHA and regulations)

# Regulations: definitions

- Cell: “the basic structural and functional unit in people and all living things and is a small container of chemical and water wrapped in a membrane”
- Transgenic cells: cells derived from a species other than human
  - universally accepted definition of cells derived from other species is “xenogeneic”

# Human tissue legislation: towards self-regulation

Area	Professional body	Guidelines
Transplantation	Southern African Transplantation Society (SATS)	Yes <a href="http://www.sats.org.za/Guidelines.asp">http://www.sats.org.za/Guidelines.asp</a>
Assisted reproductive technology	Southern African Society of Reproductive Medicine and Gynaecological Endoscopy (SASREG)	Yes <a href="http://www.fertilitysa.org.za/TreatmentGuidelines/ReproductiveMedicine.asp">http://www.fertilitysa.org.za/TreatmentGuidelines/ReproductiveMedicine.asp</a>
Blood and blood products	National Blood Committee (not in operation since 2008)	Yes SANBS and WPBTS websites & other
Cell-based therapy	South African Stem Cell Transplantation Society (SASCTS)	Yes; none on website <a href="http://www.stemcell.org.za/index.htm">http://www.stemcell.org.za/index.htm</a>
Genetic Services	Southern African Society of Human Genetics (SAHGS)	Yes <a href="http://www.sashg.org/documents.htm">http://www.sashg.org/documents.htm</a>
Tissue banks	South African Tissue Bank Association (SATiBA)	Newly formed; in progress
Forensic pathology and medicine	National Forensic Pathology Services Committee	Yes No website
	National Clinical Forensic Committee	In progress

# Why legislation

## Reason no. 1

- Work involving material that will be (re)introduced into patients must be conducted in an accredited institution under strictly controlled conditions
  - to maximize normal structure and function of the material that is to be (re)introduced
  - to avoid the unintentional transfer into patients of harmful material (infectious and otherwise)



# Why legislation

## Reason no. 2

- To ensure that pre-clinical studies and well controlled clinical trials have been conducted prior to introduction of cells into patients
  - to ensure that the purported therapeutic effect is real
  - to ensure that there are no serious side effects

# Amariglio N. et al. PLoS Med, 2009

- Patient: 9 yr old boy with ataxia telangectasia
- Parents took him to Moscow
- 8-week human neural stem cells (aborted foetus?) harvested and expanded in culture for 2 weeks
- 50-100 million cells administered via intracerebellar and intrathecal injection
- 3 treatments over several years – 2001/2/4
- 2005 – recurrent headaches (Tel-Aviv)
- Developed a multifocal brain tumour
  - 2006: tumor removed surgically from cauda equina nerve roots
  - 2008: infratentorial tumour had doubled in mass
    - Patient stable and treated conservatively

# Why legislation

## Reason no. 3

- The absence of regulations permits (and even encourages) the emergence of medically-unsound and unethical practices that may be associated with the exploitation of emotionally vulnerable patients

# “Stem cells” for spinal cord injury

Melanie Skeen – MCs student

Patient Level of injury	Nature of stem cells Route of administration	Time after injury to Rx Type of injury	Treatment location	Cost Treatment (stem cells)	Travel and accomm.
Male, 47 yr T7 para	Autologous LP	10 mo Gunshot	India	R 231 000	R 36 000
Male, 32 yr T1 quad	Sheep SC (weekly x 6 mo) Orally (tds x 6 mo)	8 yr Gunshot	SA (Bloemfontein)	0	0
Male, 35 yr T8 para	Rabbit Subdural during spinal surgery	14 d Gunshot	Cells from Germany Given in SA at the time of surgery	R 200 000	0
Female, 38 yr C6/7 incomplete	Rabbit SC and LP	1 yr MVA	Germany	R 169 000 R 80 000	R 89 000
Male, 34 yr Locked-in syndrome	Rabbit SC and LP	3,5 and 5,5 yr MVA followed by CVA	Germany and SA	R 174 000 First procedure in Germany	R 54 000
Male, 34 yr C4/5 quad	Rabbit SC and LP	6 yr Fall	Germany	R 179 000 R 65 000	R 114 320
Female, 43 yr C5/6 incomplete	Sheep IM back or neck (x 8 mo)	7 yr MVA	SA (Bloemfontein)	R 8 000 (R 1 000 per treatment)	R 11 200
Male, 38 yr C6/7 quad	? type (not autologous) IV	27 mo MVA	Netherlands	R 142 000 R 76 000	R 66 000

# Current reality vs. future promise

- Currently only a few well-established indications for stem cell therapy
- Future applications provide a great source of hope for many patients
  - exploitation of emotionally-vulnerable patients
- Ethical considerations in pluripotent cells

*... it is tempting to wonder if  
this twisted sugar string of  
purine and pyrimidine base  
beads is, in fact, God*

James Watson

