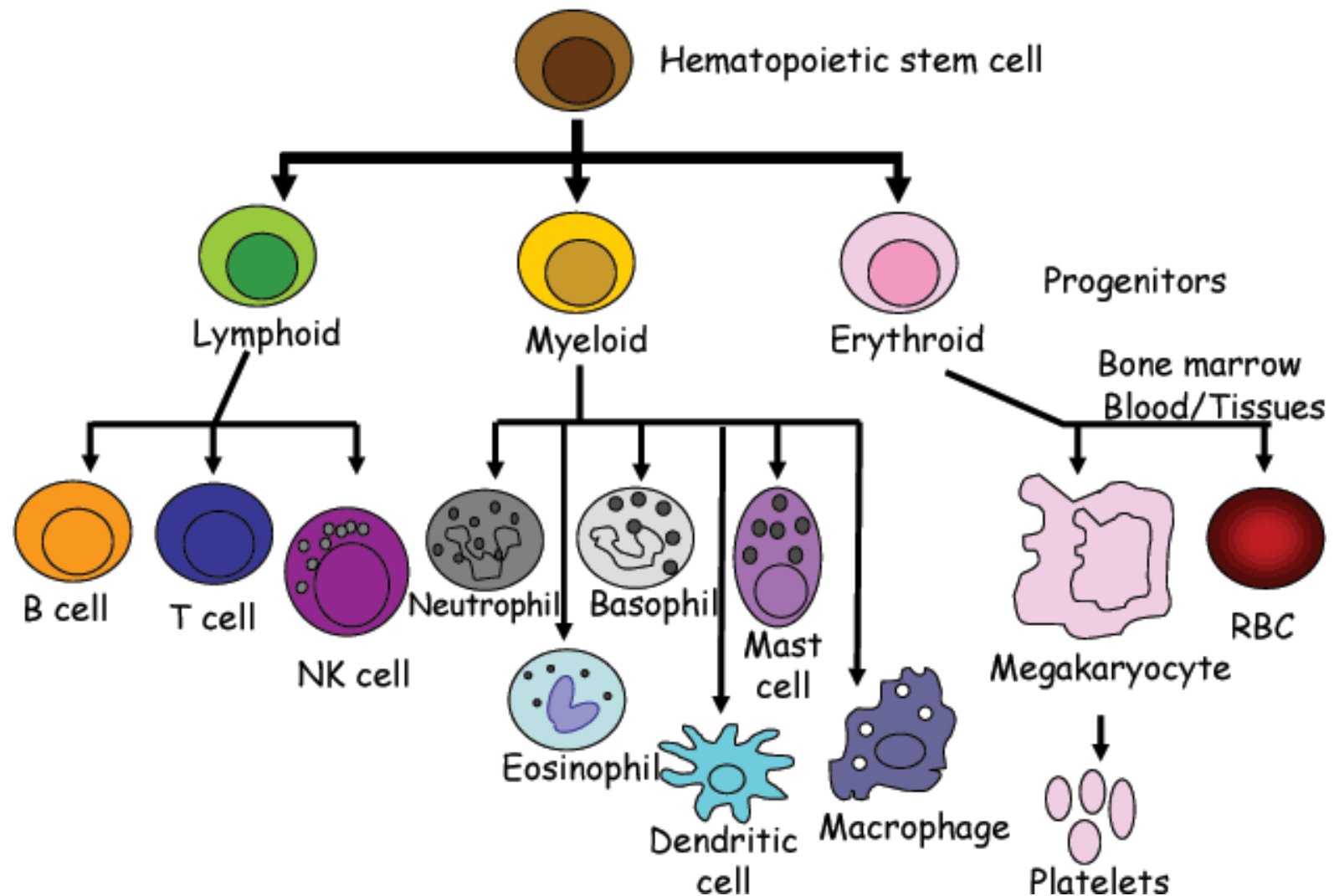


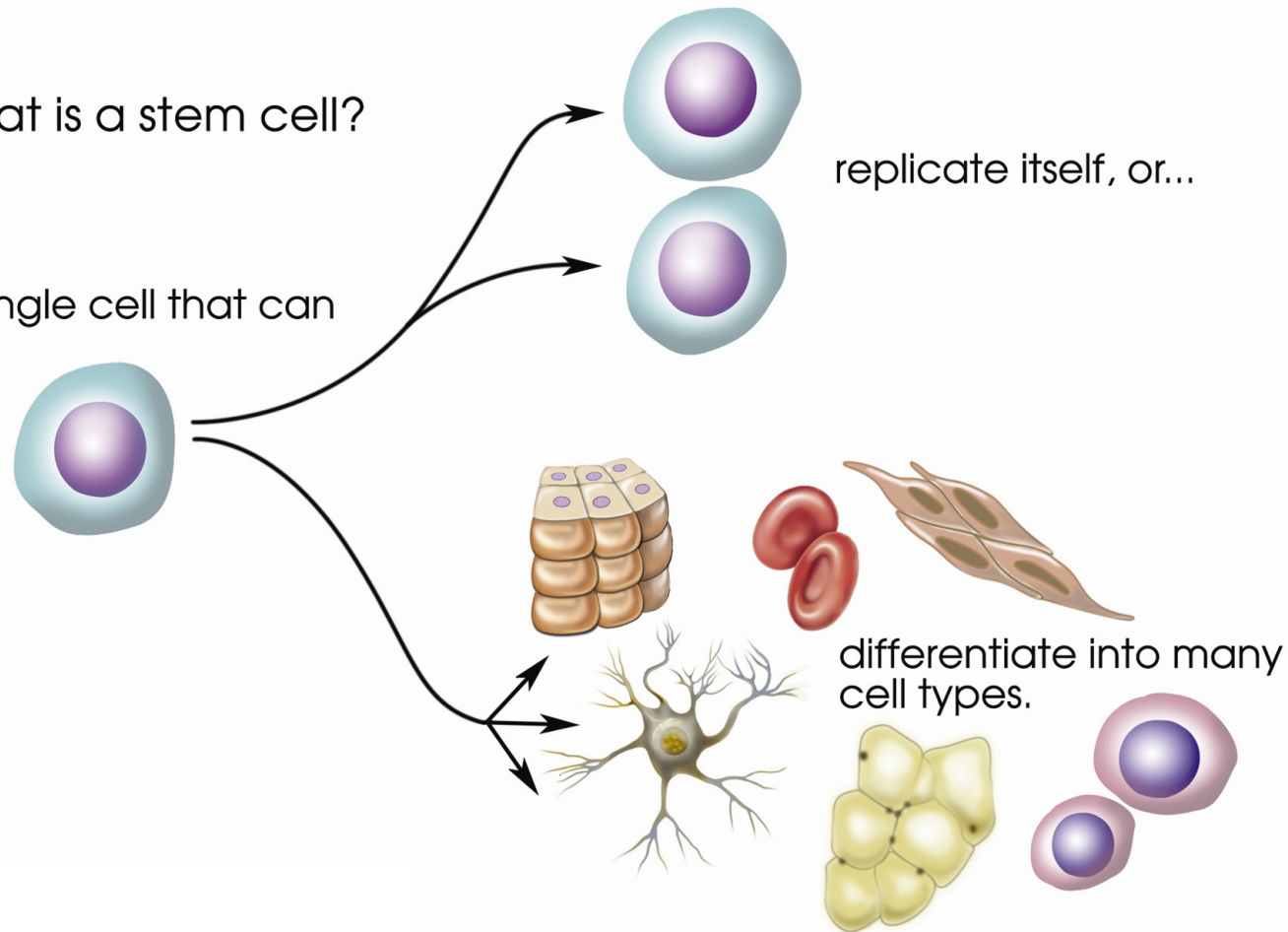
Introduction to Stem Cells
HC70A Spring 2015
Dr. Pei Yun Lee

Immune cell development: Hematopoiesis



What is a stem cell?

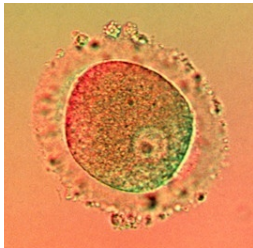
A single cell that can



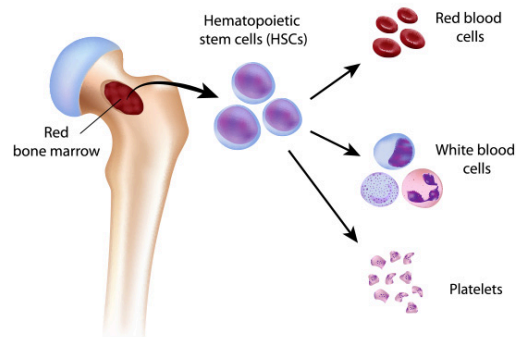
Stem cells: unspecialized cells that can differentiate and self renew

e.g. hematopoietic stem cell, embryonic stem cells, induced pluripotent stem cells

What are the Normal Uses of Stem Cells?



Development

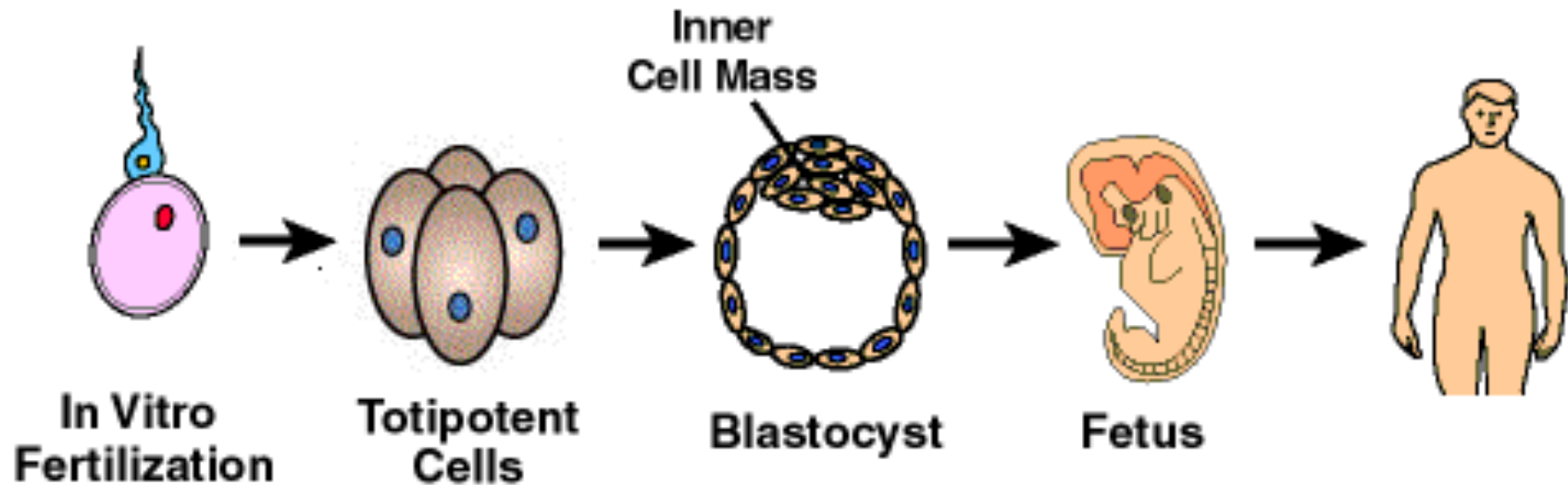


Tissue homeostasis

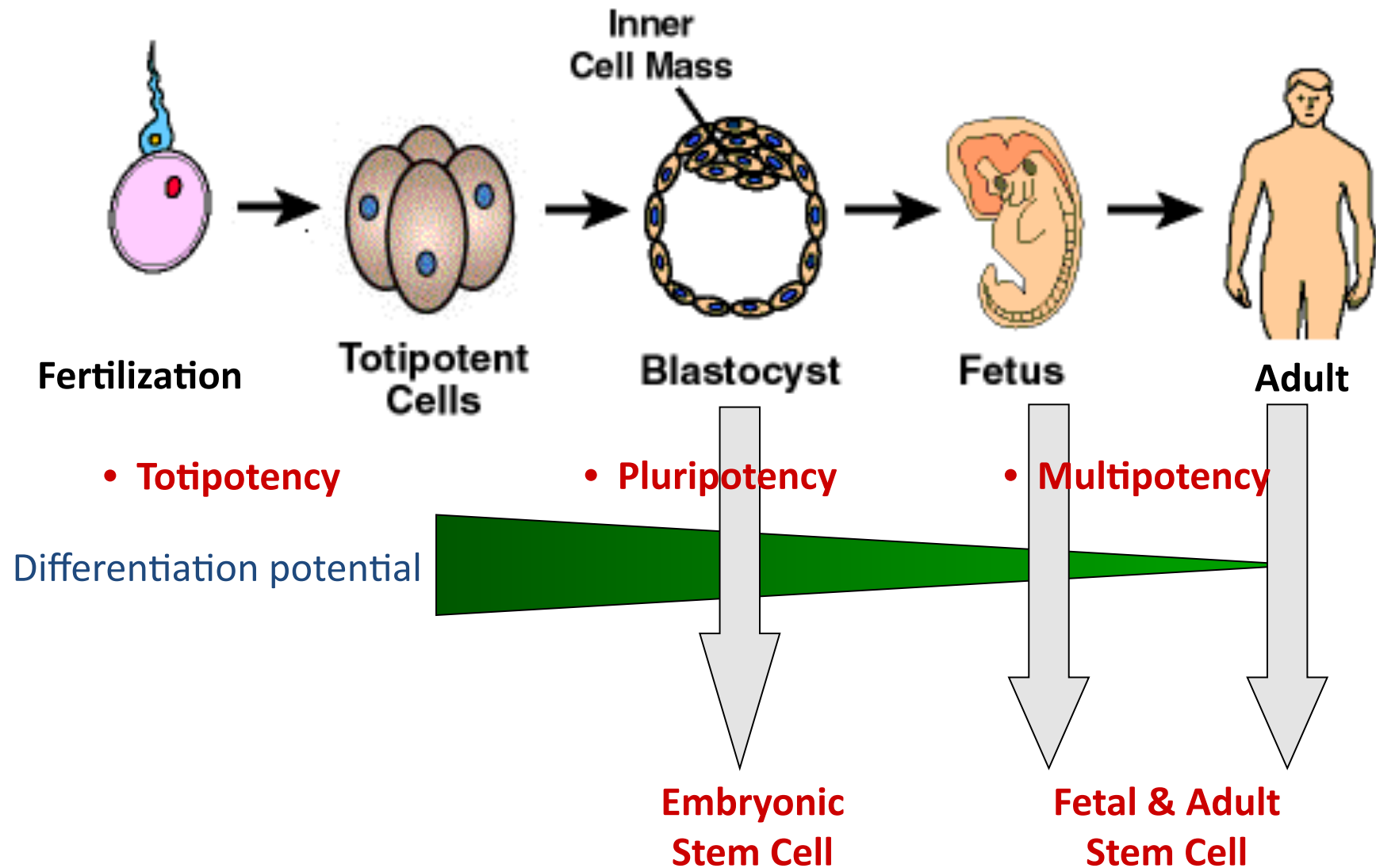


Tissue repair following injury

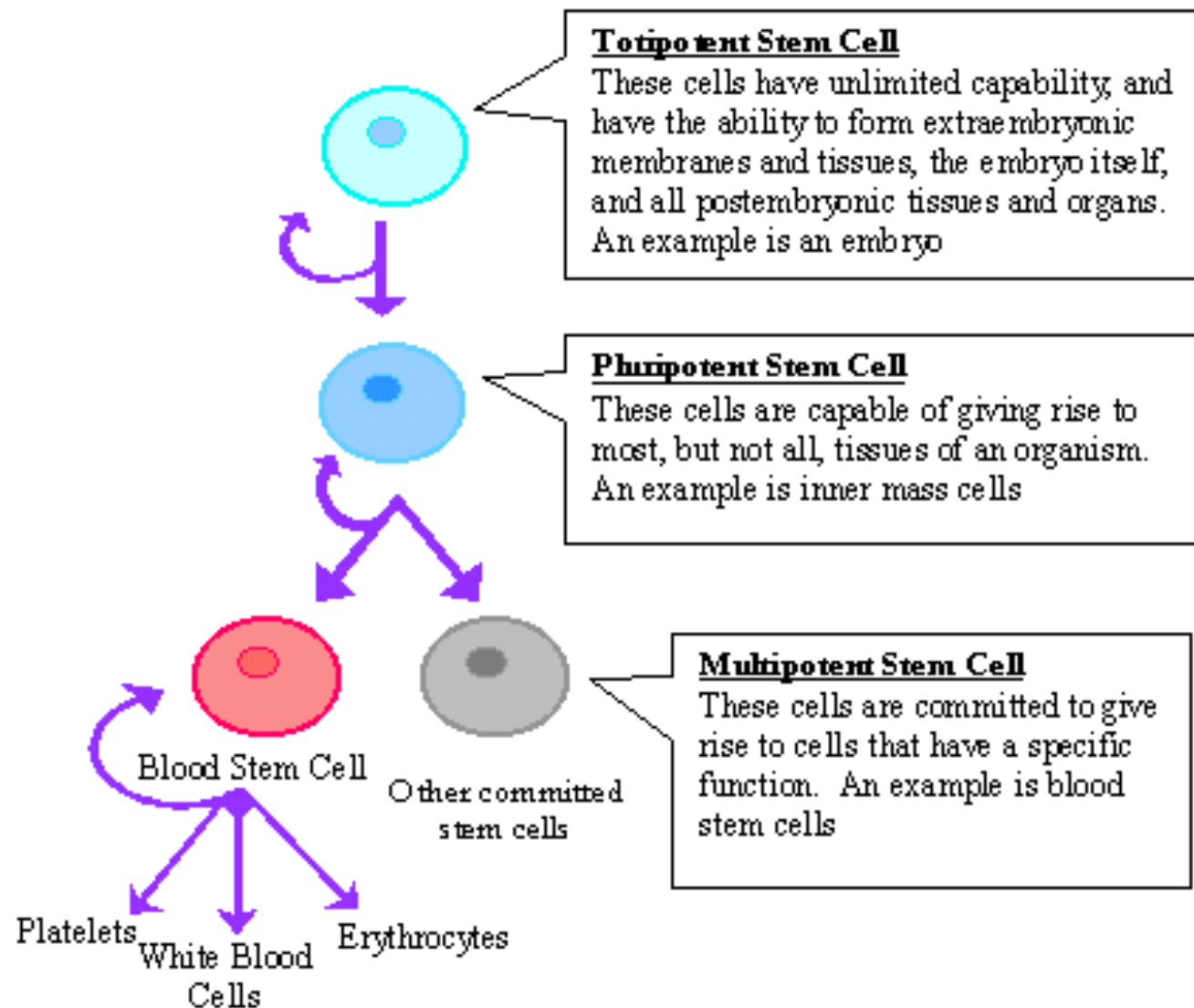
Where can stem cells be found?



Progressive Restriction of Developmental Potential



Stem cells have different developmental potentials

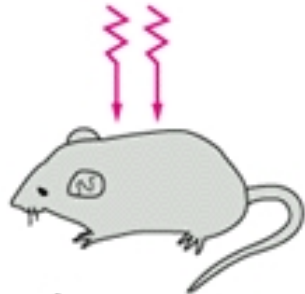




Alexander Maximow 1909

Unitarian theory of hematopoiesis (blood formation)
-all blood cells descended from a common
precursor “stem” cell

x-irradiation halts blood cell production; mouse would die if no further treatment was given



INJECT BONE MARROW CELLS FROM HEALTHY DONOR



mouse survives; 2 weeks after infection, many newly formed blood cells are in circulation



EXAMINATION OF SPLEEN REVEALS LARGE NODULES ON ITS SURFACE



each spleen nodule contains a clone of hemopoietic cells, descended from one of the injected bone marrow cells

McCulloch and Till were the first to demonstrate the existence of multipotent stem cells!



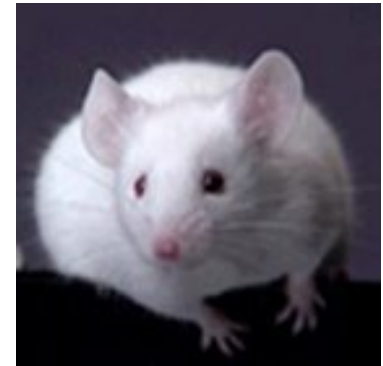
Analysis of the cells in the spleen demonstrated that the cells in each nodule were clones



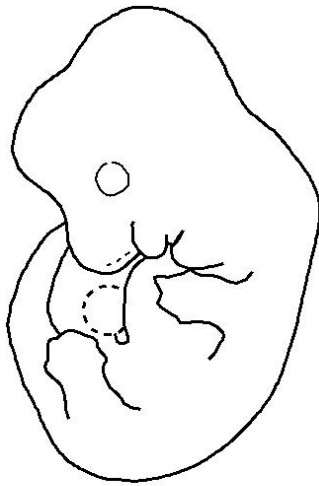
Leroy Stevens



Mouse lines are inbred and
therefore genetically identical!

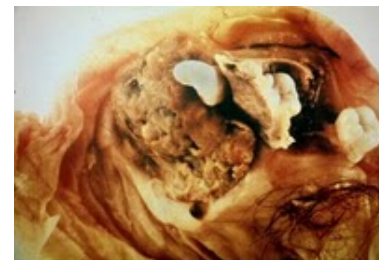
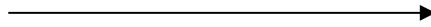


Strain 129!



Isolate cells from mouse embryos

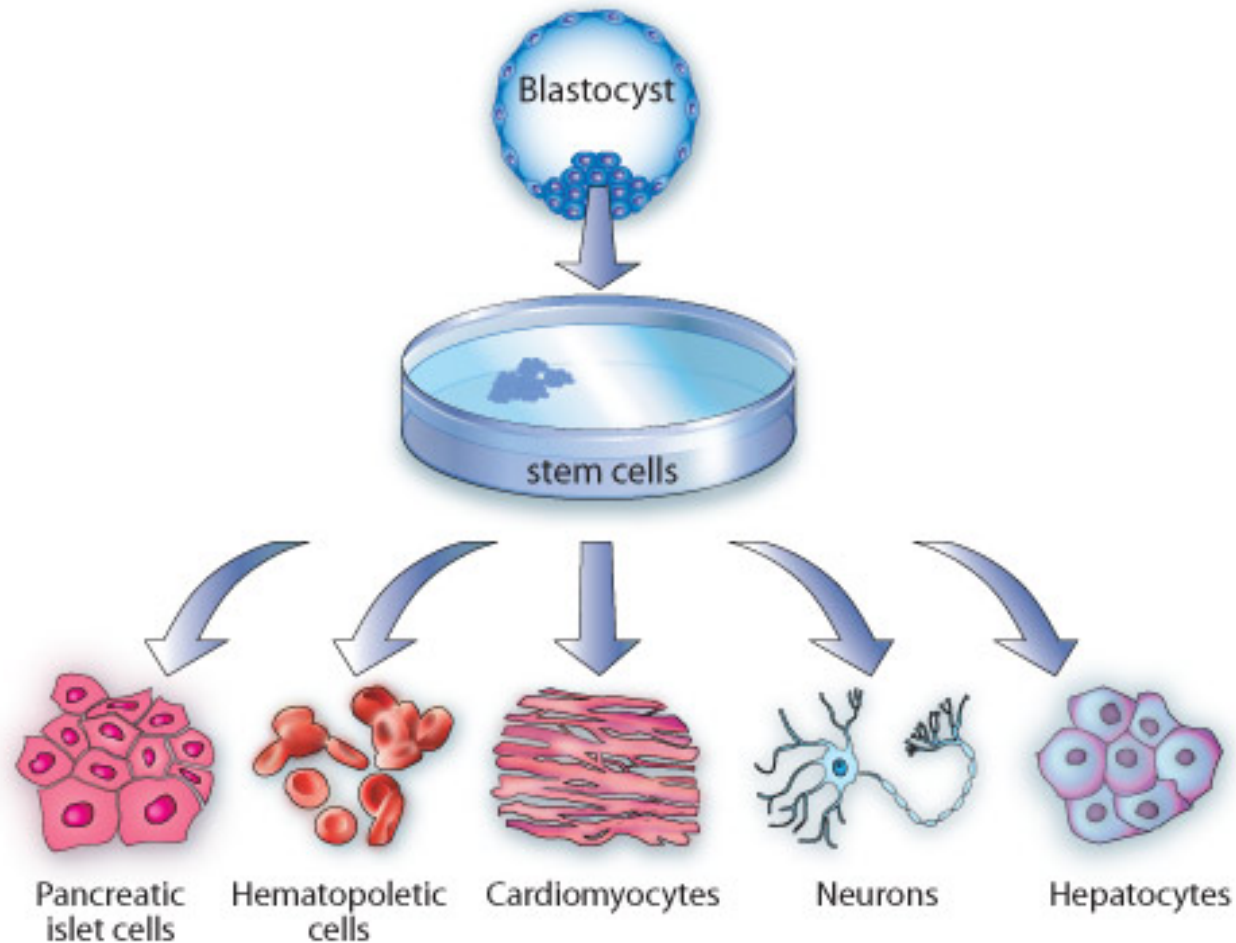
Transplant into adult
mouse testes



Tumors develop from pluripotent stem cells in
the mouse gonad

Teratomas!

Pluripotent stem cells have the potential to develop into any cell in the human body!



ES cells are pluripotent stem cells!

Targeted ES cells
are injected into
blastocysts



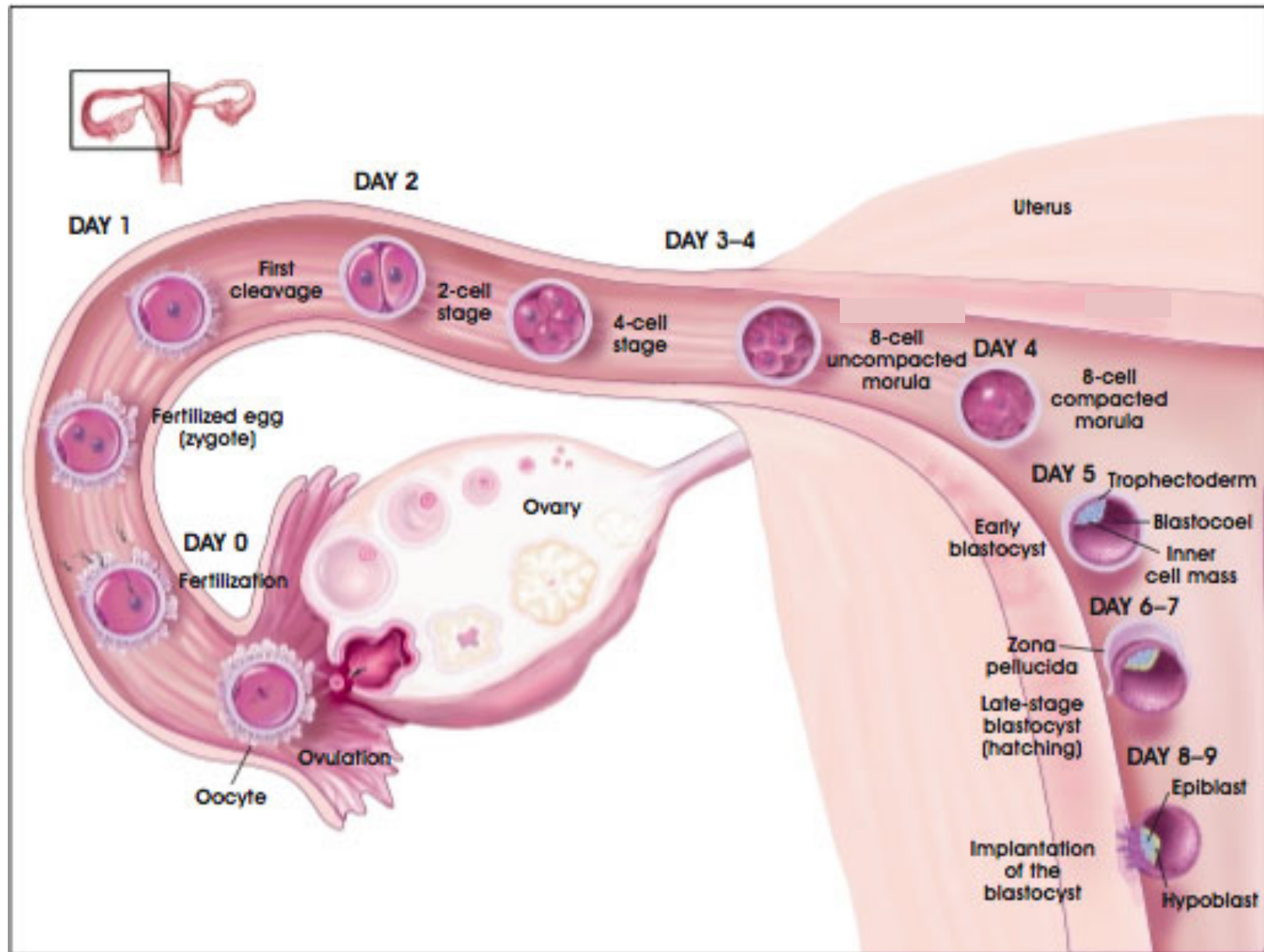
Blastocysts are
implanted into mice
foster mothers



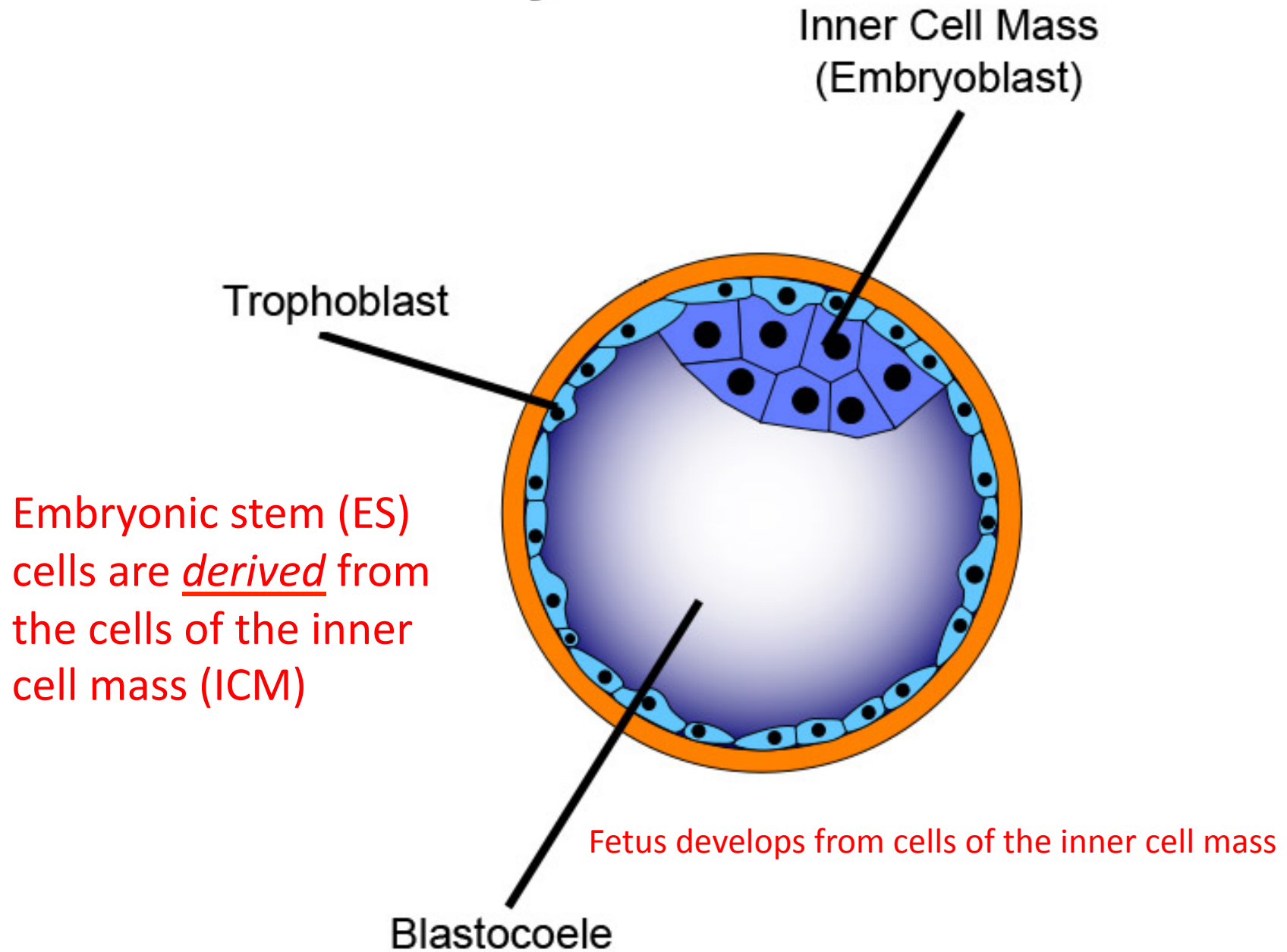
Chimeric mice
offspring



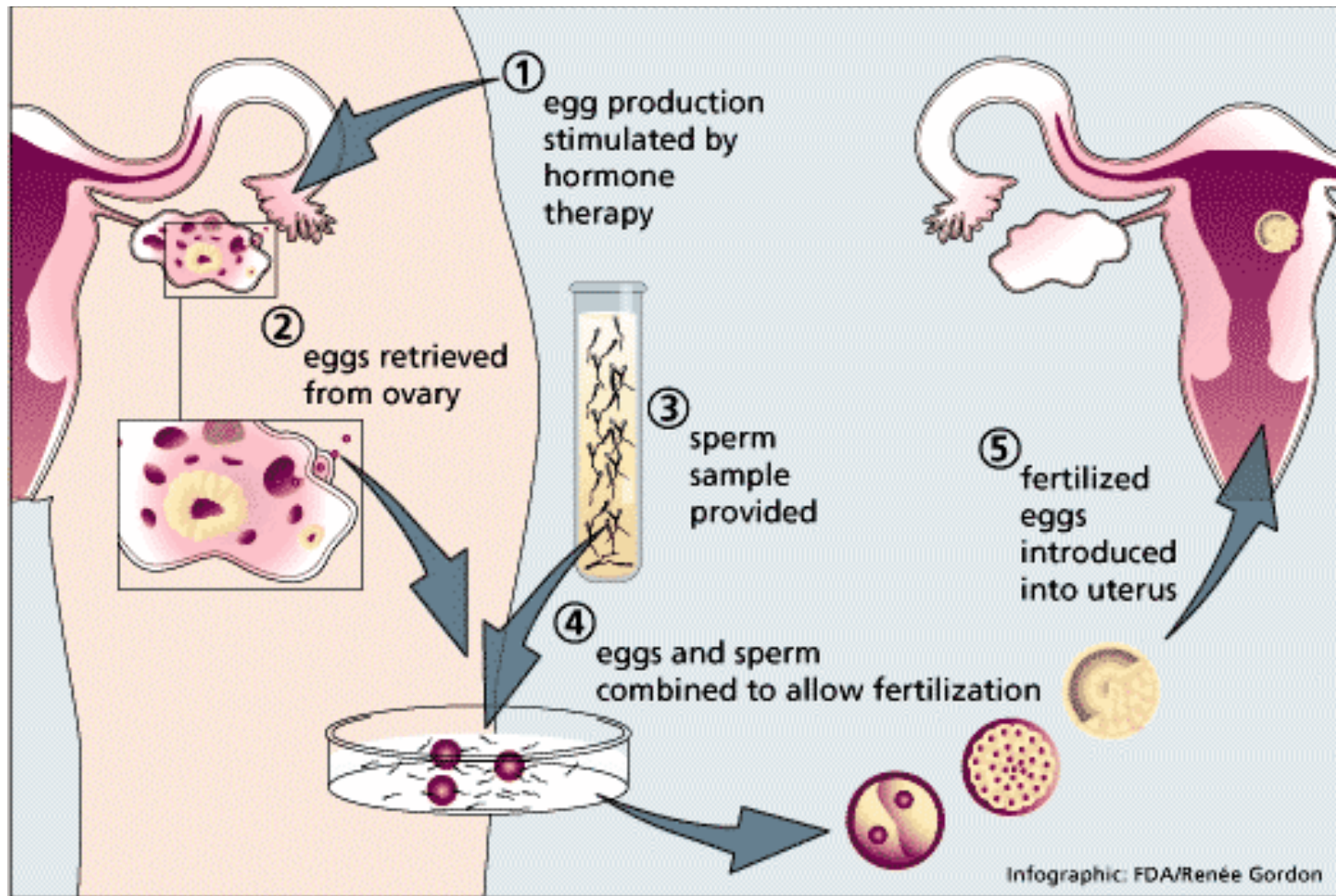
What developmental stage is a blastocyst?



The Blastocyst



Embryos for ES cell research are obtained through *in vitro* fertilization



Accounts for 99% of ART procedures
Estimated 3 million worldwide (2% of all US births)

- 1 EMBRYO**
An egg is fertilized or cloned to form an embryo. The embryo begins to divide

- 2**
1 TO 5 DAYS
The embryo divides again and again and takes shape as a sphere called a blastocyst

- 3 5 TO 7 DAYS**
By this time embryonic stem cells are visible and are capable of developing into any tissue in the body

- 4 STEM LINE**
The cells are removed and grown in a Petri dish. As they divide, they create a line of stem cells

- 5 TISSUE PRODUCTION**
Using various recipes of nutrients and other factors, scientists hope to turn stem cells into any of the body's more than 200 tissues, such as:

PANCREATIC ISLET CELLS
Could provide a cure for diabetes

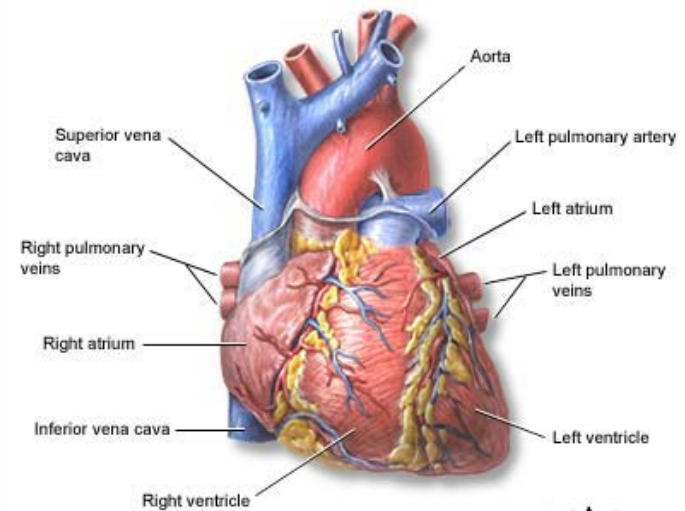
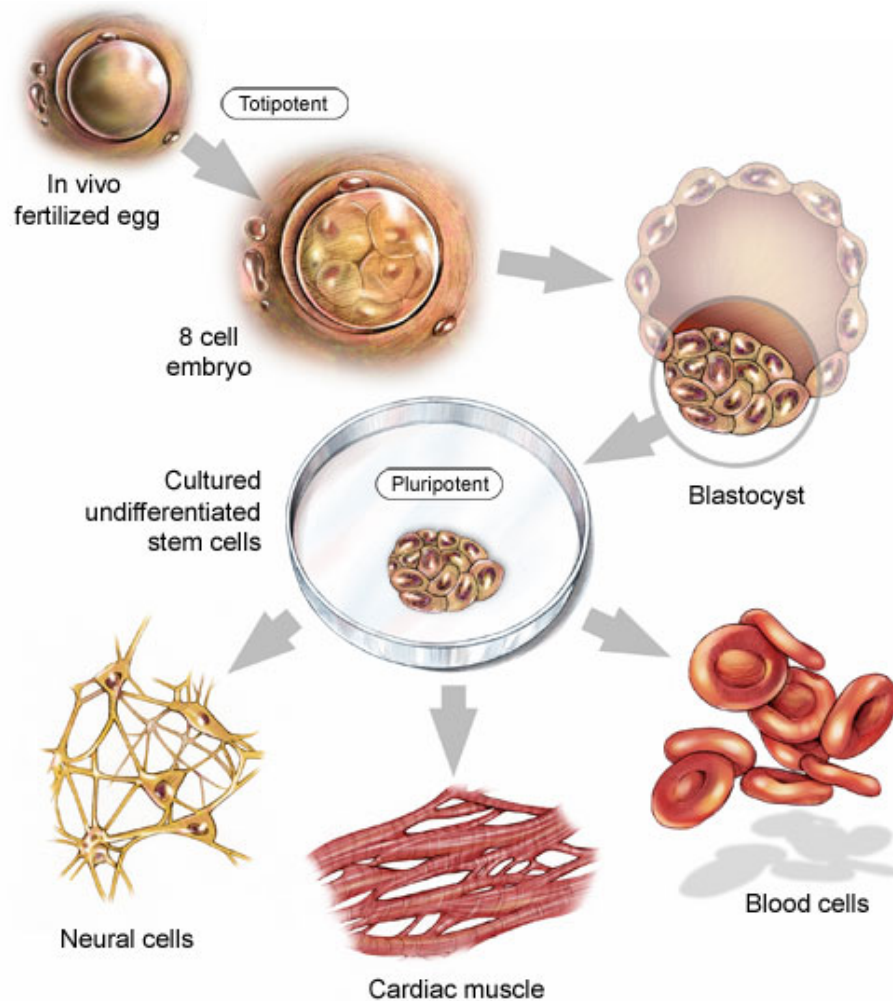
MUSCLE CELLS
Could repair or replace a damaged heart

NERVE CELLS
Could be used to treat Alzheimer's and Parkinson's diseases and repair spinal cord injuries

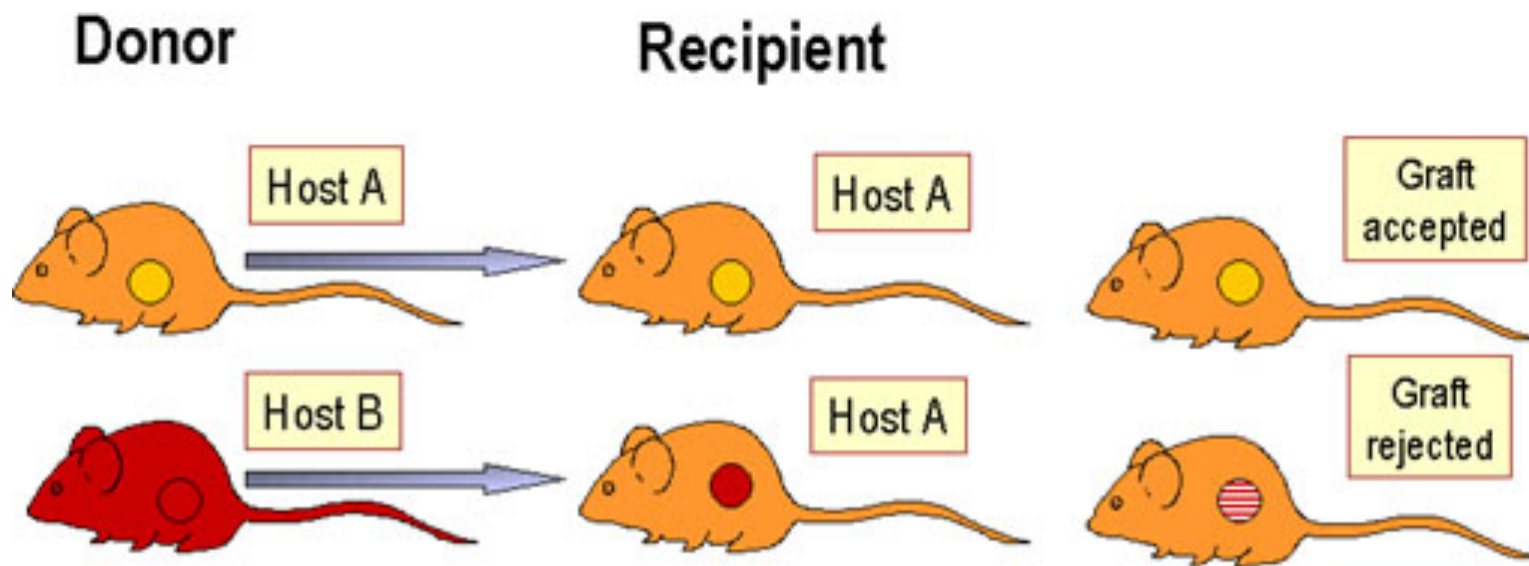
HOW IT WORKS From Embryo to Stem Cell

TIME Graphic by Lon Tweeten

Beating heart muscle from ES cells!



Our bodies can recognize self vs non-self



What confers this ability?

Human MHC genes are highly polymorphic

Table 17.3

MHC Class II Alleles

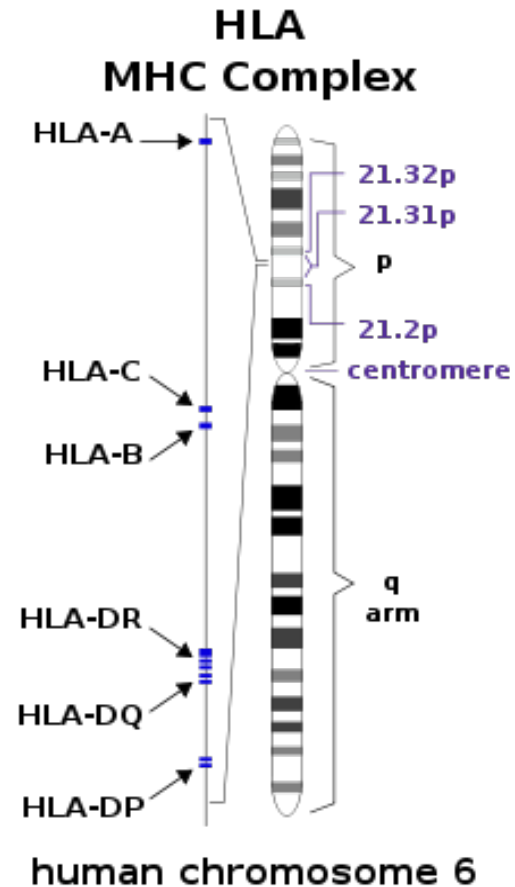
Locus	Number of Alleles
HLA-DRA	3
* HLA-DRB	542
HLA-DQA	34
HLA-DQB	73
HLA-DPA	23
HLA-DPB	125
HLA-DMA	4
HLA-DMB	7
HLA-DOA	12
HLA-DOB	9

MHC Class I Alleles

Locus	Number of Alleles
* HLA-A	479
* HLA-B	805
HLA-C	257
HLA-E	9
HLA-F	20
HLA-G	7

Note: Several other class I alleles are not listed.

Table 17-3 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



What is the probability that a full sibling will be a genetic match?

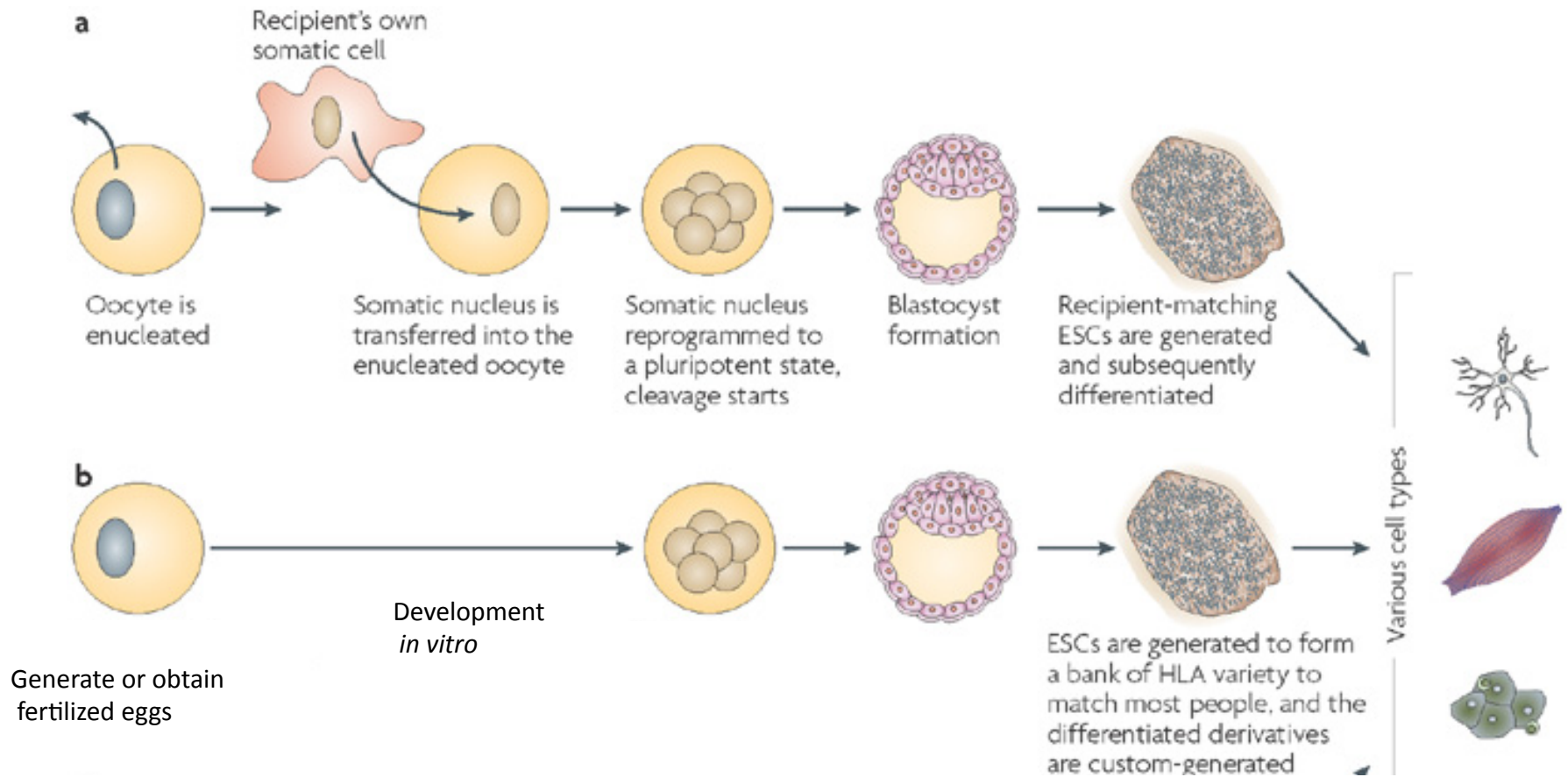
Probability of finding bone marrow match in the general population

	Caucasian	African-American	Asian	Hispanic
Caucasian	1/8,000			
African-American	1/133,000	1/127,000		
Asian	1/270,000	1/2,000,000	1/37,000	
Hispanic	1/45,000	1/370,000	1/370,000	1/39,000

Probability of finding a match is greatest within own ethnic group

Mixed-race individuals face greater challenges

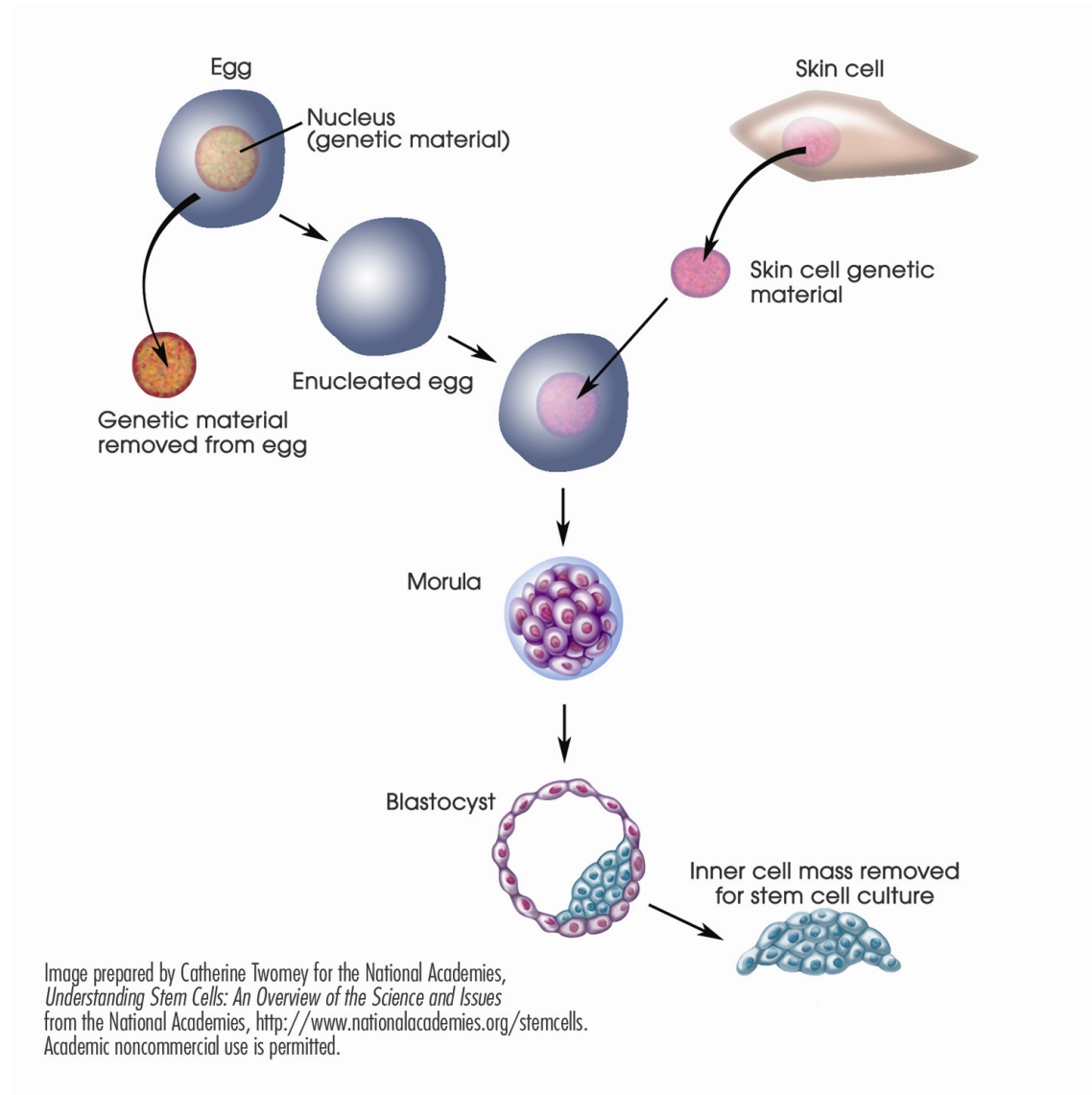
SCNT vs. ES cell bank



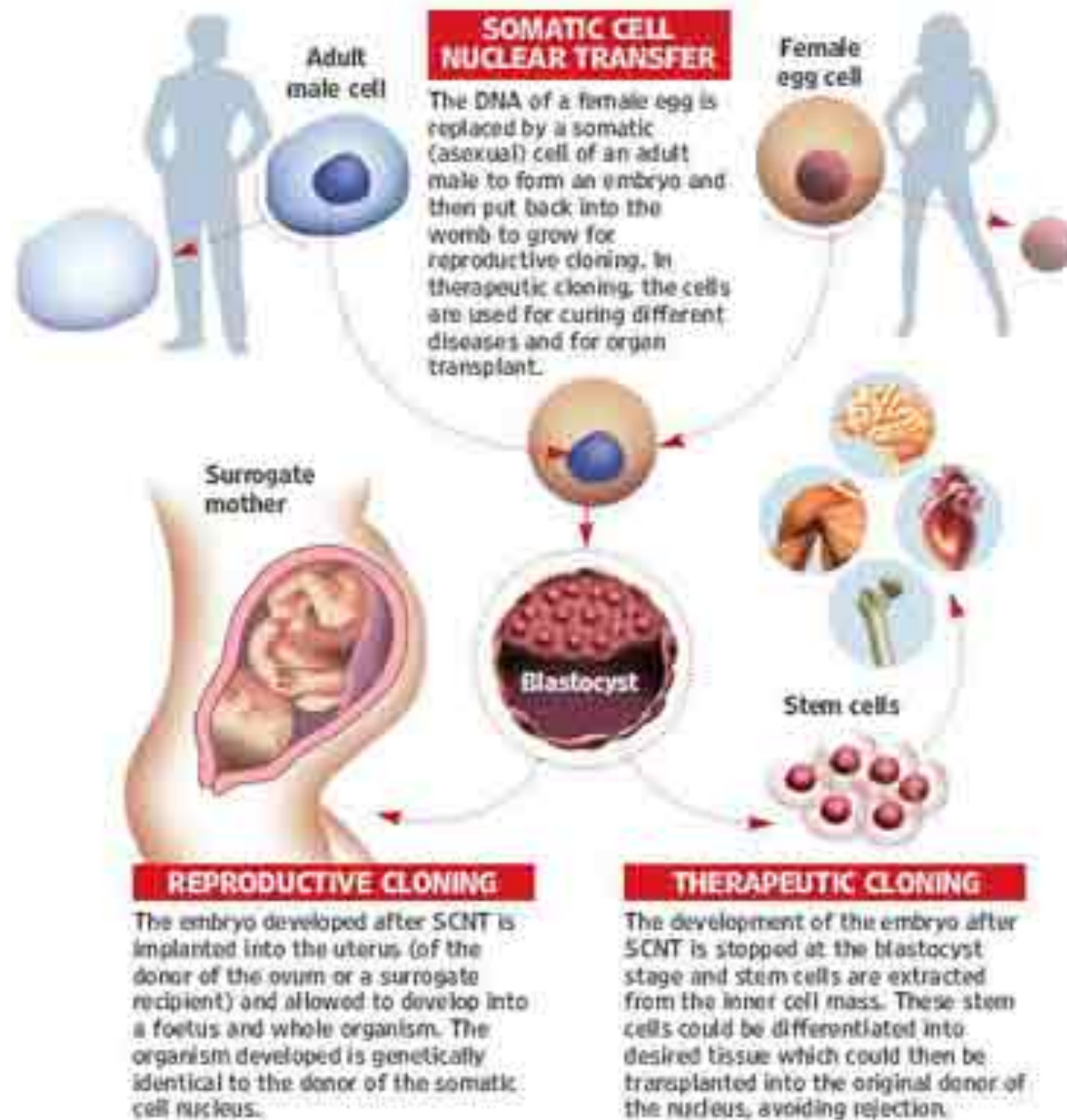
Solutions to the Tissue Rejection Problem

- Have a bank of ES cells of different HLA haplotypes representative of different populations
 - similar to bone marrow registry where chances of a match is related to what is available in the bank
- Obtain ES cell lines for each individual through somatic cell nuclear transfer (SCNT) or cloning
 - Personalized medicine!

How do you “clone” a human embryo?



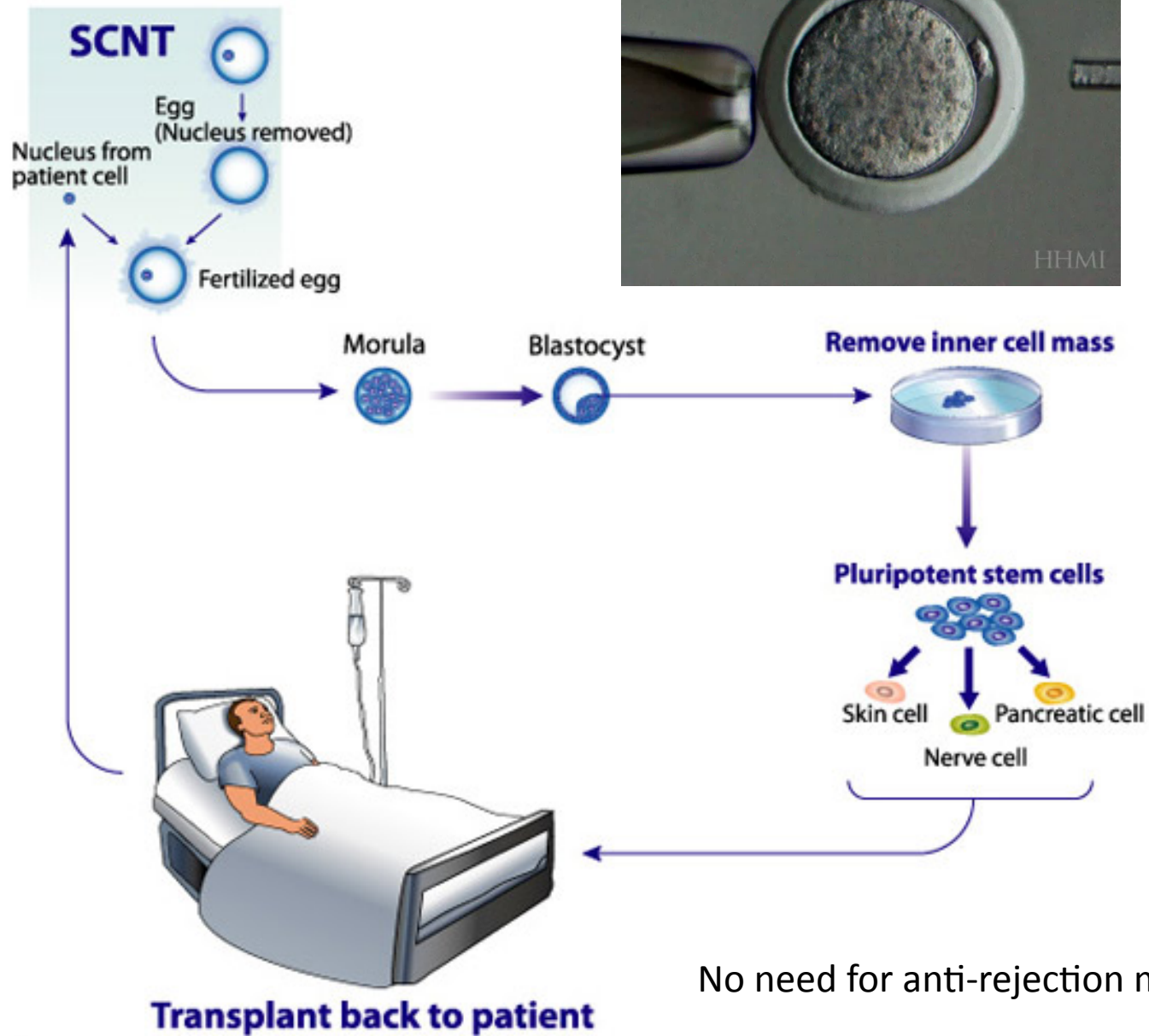
Reproductive vs. Therapeutic Cloning



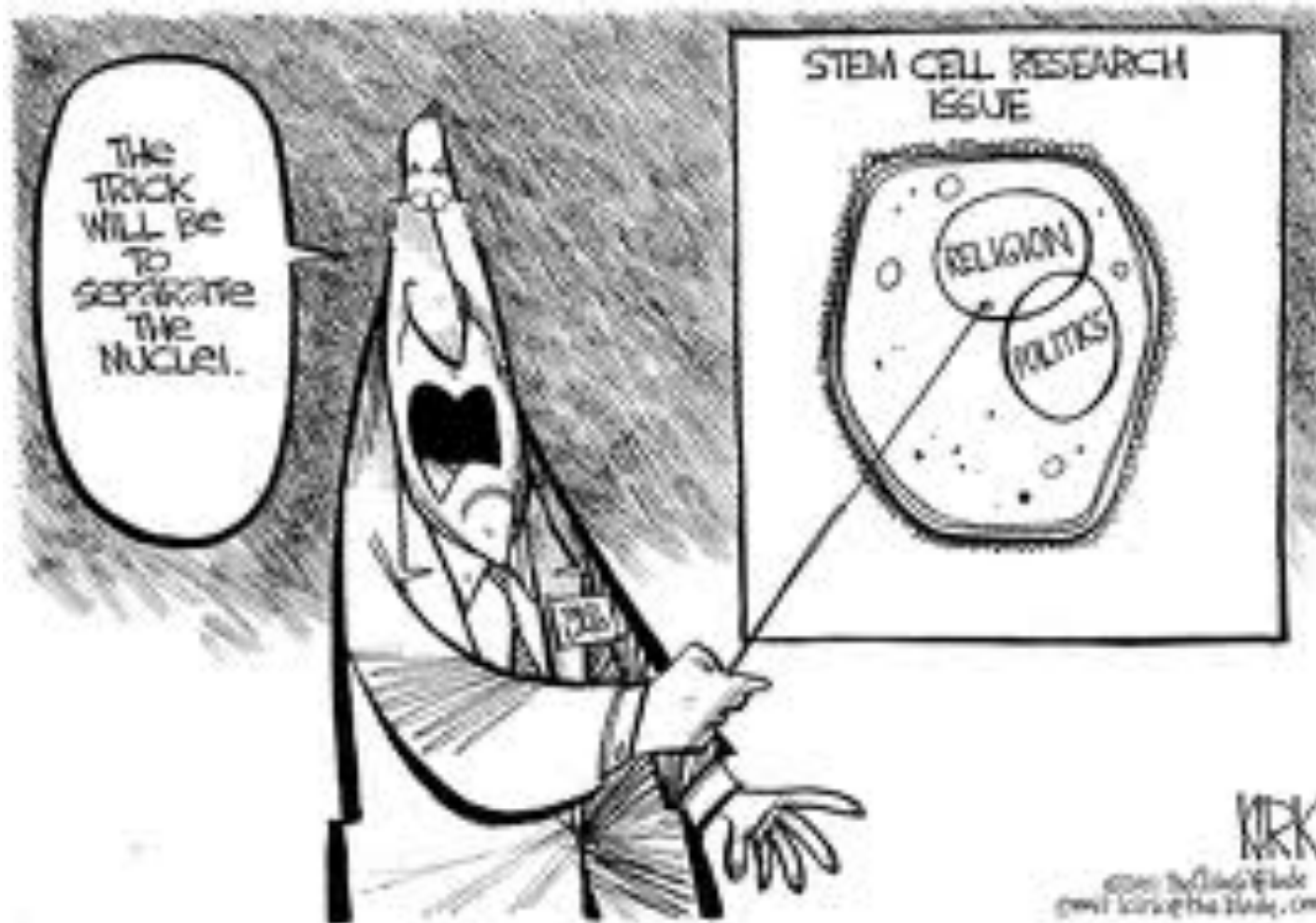
Human Cloning Laws

Reproductive Cloning Ban	Reproductive and Therapeutic Cloning Ban	Ban on State Funding for Cloning Related Research	State Funding Allowed for ES Cell Research (IVF and Cloning)
California, Connecticut, Illinois, Iowa, Maryland, Massachusetts, Missouri, Montana, New Jersey	Arizona, Arkansas, Indiana, Michigan, North Dakota, Oklahoma, South Dakota, Virginia	Arizona, Indiana, Louisiana, Michigan	California, Illinois, Missouri, Maryland, New York

There is no federal law banning reproductive cloning



Why is there controversy surrounding human ES cell research?



Moral Status

- Have protection afforded by moral norms
 - Owe obligation to such individuals
- Who should have moral status?
- What criteria should be used to determine whether a person has moral status?

Roe v. Wade (1973)

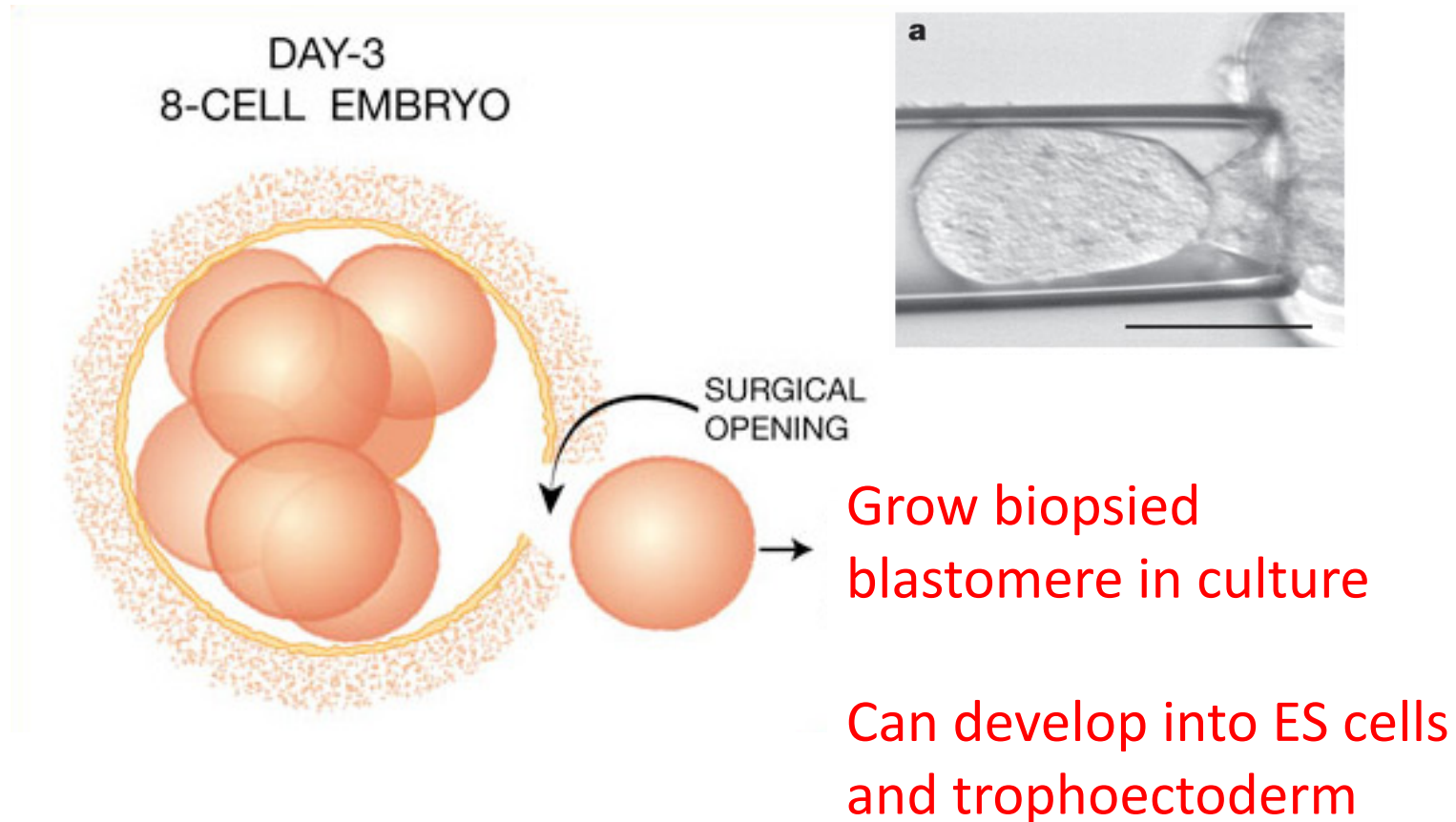
- Right to privacy extends to a woman's right to have an abortion
- Right must be balanced against the protection of prenatal life and mother's health

What does Roe v. Wade say about moral status and when personhood begins

- Original opinion:
 - The state could not restrict abortion in the first trimester
 - In second trimester, the state can issue regulations “that are reasonably related to maternal health”
 - In third trimester, the state and regulate or prohibit abortion except “where it is necessary, in appropriate medical judgment, for the preservation of the life or health of the mother”
- Planned Parenthood v. Casey (1992)
 - Supreme court rejected rigid trimester formula, rather it asserted ***viability as the point where the protection of the life of the fetus outweighs the rights of the woman*** and abortion can be banned “except where it is necessary, in appropriate medical judgment, for the preservation of the life or health of the mother”

Can we generate pluripotent stem cells without destroying an embryo?

ES cells can be derived from a single blastomere



Uses same technique as that for PGD

No babies have been born from biopsied embryos

Making embryonic stem cells without embryos

Embryonic stem cells theoretically could be used for medical treatments because they give rise to all types of specialized cells. Until now, the only way to extract these versatile cells was to destroy embryos. The breakthrough uses skin cells.

- 1 Deactivated viruses ferry four regulator genes into the nucleus of a skin cell

Deactivated viruses

Skin cell

- 2 Regulator genes land randomly on a chromosome inside the nucleus

Chromosome

- 3 The genes integrate into the skin cell's DNA and begin issuing instructions

Regulator gene

- 4 The new genes start regulating all the other genes in the skin cell, reversing the cell's developmental clock; as the cell replicates, the new cells gradually become identical to embryonic stem cells

Skin cell

Embryonic stem cell

Embryonic stem cell colony

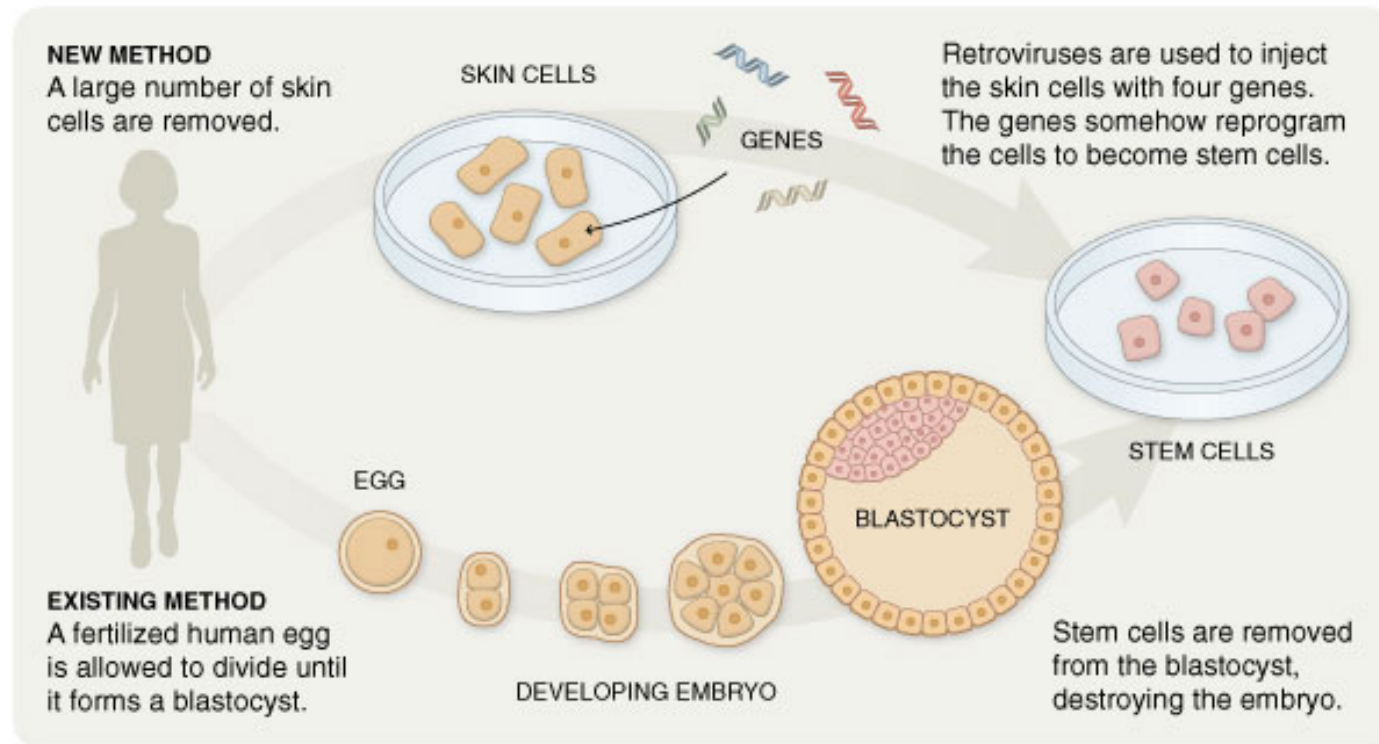
- 5 The cells can be signalled to develop into blood cells, muscle cells, and neurons.

Deactivated viruses have viral genes replaced with targeted human gene

Human gene(s) are inserted into the chromosome of the infected cell and directs the expression of the inserted gene

Reprogramming Human Skin Cells

Researchers have developed a technique for creating stem cells without the controversial use of human eggs or embryos. If the method can be perfected, it could quell the ethical debate troubling the field.

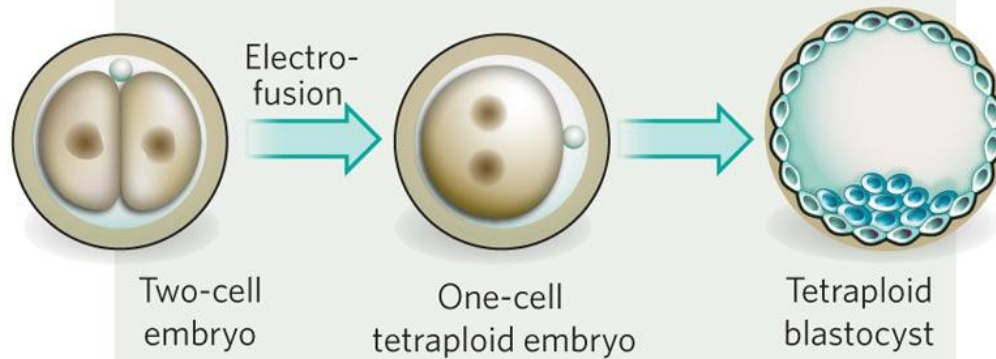


TIMELINE

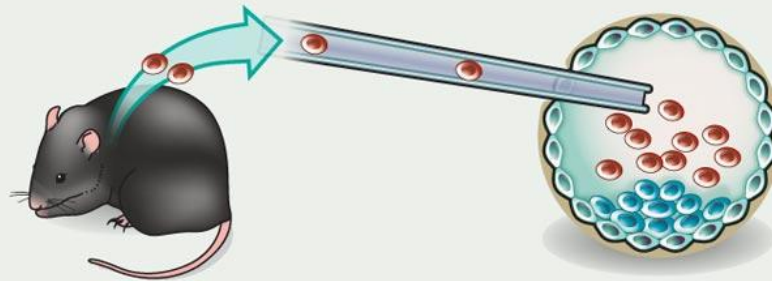
1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
July 1995 Congress bans federal financing of research on human embryos.	July 1996 Dolly is born. The lamb is the first clone of an adult mammal.		Nov. 1998 First isolation and cultivation of embryonic stem cells. The cells are derived from fertilized human eggs.			Aug. 2001 President Bush announces that federal money will pay for research on existing stem cell lines, but not new lines.			Nov. 2004 California voters approve a measure to spend \$3 billion over 10 years on embryonic stem cell research.			Nov. 2007 New Jersey voters reject a measure to borrow \$450 million for stem cell research.

MAKING AN iPS-CELL MOUSE

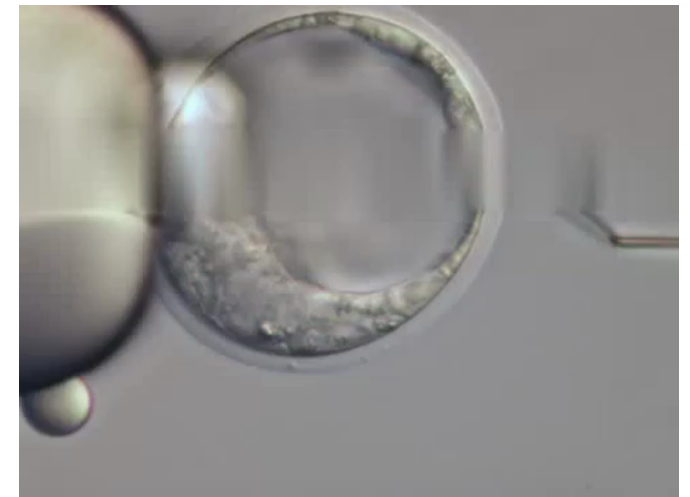
Two-cell embryo is fused to generate a tetraploid blastocyst



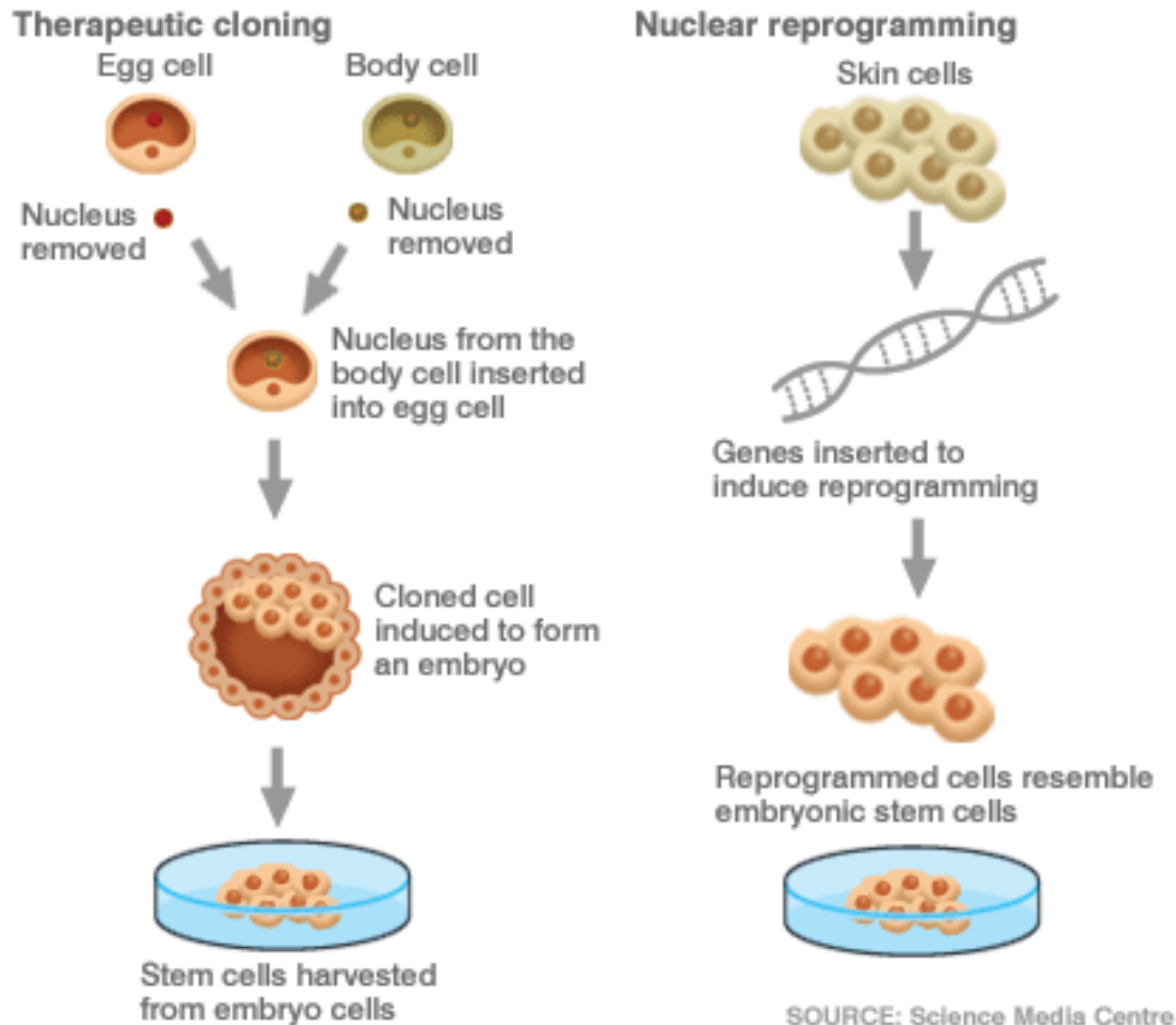
iPS cells are injected into the tetraploid blastocyst, which then steer development



Developing embryo is implanted in surrogate mother



SCNT vs. IPS cells



Use iPS
cells to
study
diseases!

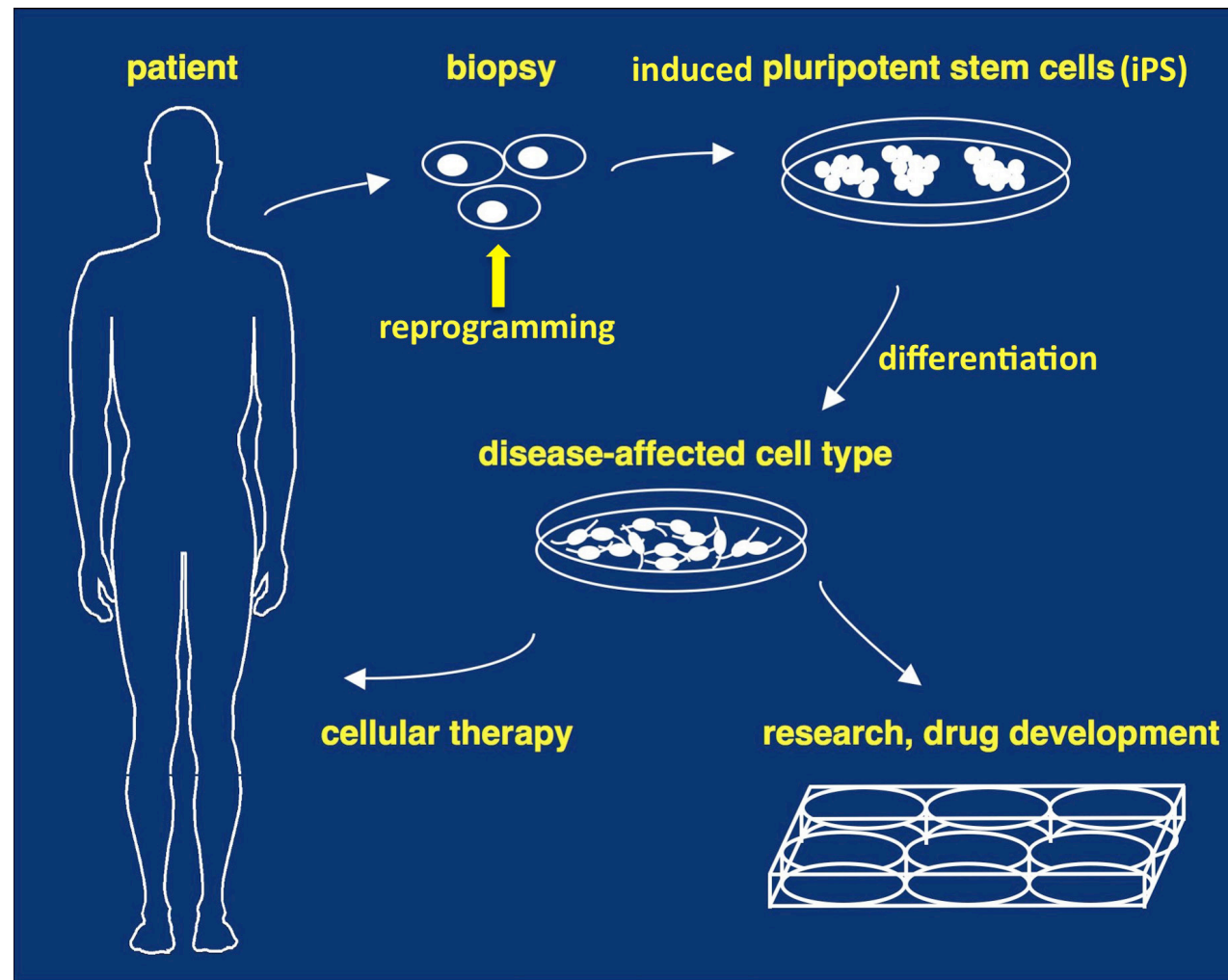


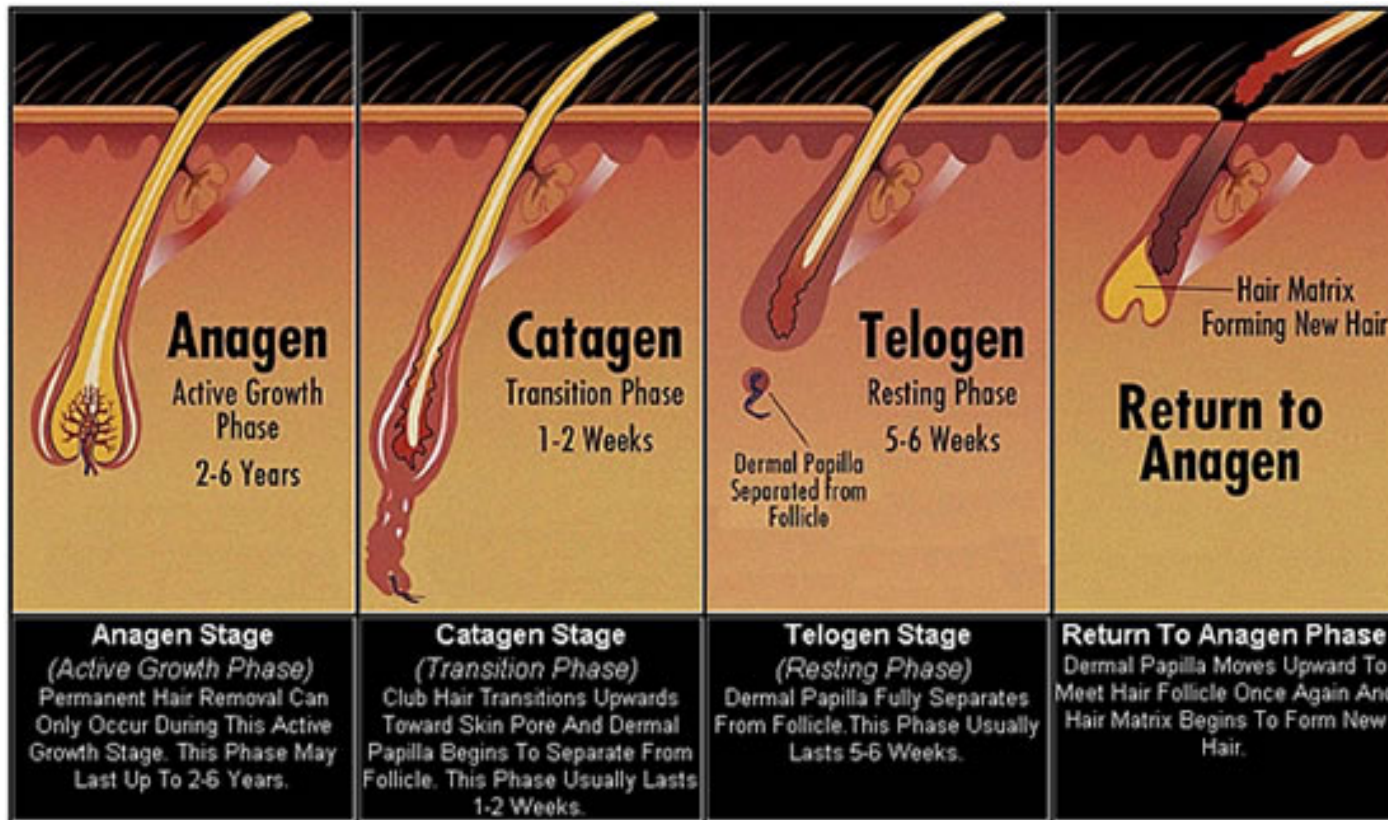
Table 1. iPS Cells Derived from Somatic Cells of Patients with Genetic Disease

Name	Disease	Molecular Defect	Donor Cell	Age	Sex
ADA	ADA-SCID	GGG > AGG, exon 7 and Del(GAAGA) exon 10, ADA gene	Fibroblast	3 M	Male
GD	Gaucher disease type III	AAC > AGC, exon 9, G-insertion, nucleotide 84 of cDNA, GBA gene	Fibroblast	20 Y	Male
DMD	Duchenne muscular dystrophy	Deletion of exon 45–52, dystrophin gene	Fibroblast	6 Y	Male
BMD	Becker muscular dystrophy	Unidentified mutation in dystrophin	Fibroblast	38 Y	Male
DS1, DS2	Down syndrome	Trisomy 21	Fibroblast	1 Y, 1 M	Male
PD	Parkinson disease	Multifactorial	Fibroblast	57 Y	Male
JDM	Juvenile diabetes mellitus	Multifactorial	Fibroblast	42 Y	Female
SBDS	Swachman-Bodian-Diamond syndrome	V2 + 2T > C and IV3 – 1G > A, SBDS gene	Bone marrow mesenchymal cells	4 M	Male
HD	Huntington disease	72 CAG repeats, huntingtin gene	Fibroblast	20 Y	Female
LNSc	Lesch-Nyhan syndrome (carrier)	Heterozygosity of HPRT1	Fibroblast	34 Y	Female

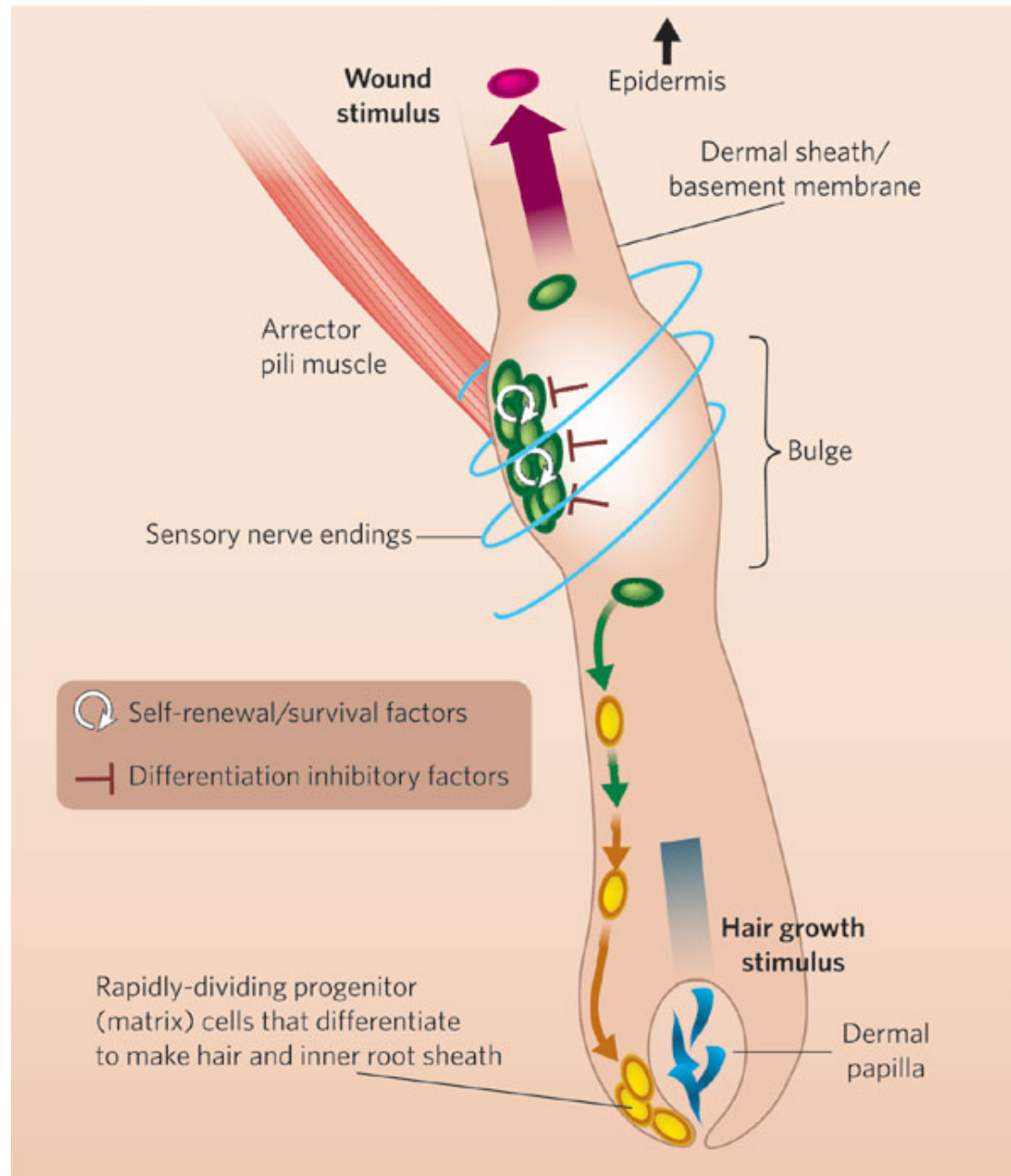
Adult stem cells are...

- Also present in children
- Have limited differentiation potential, usually restricted to a few cell types

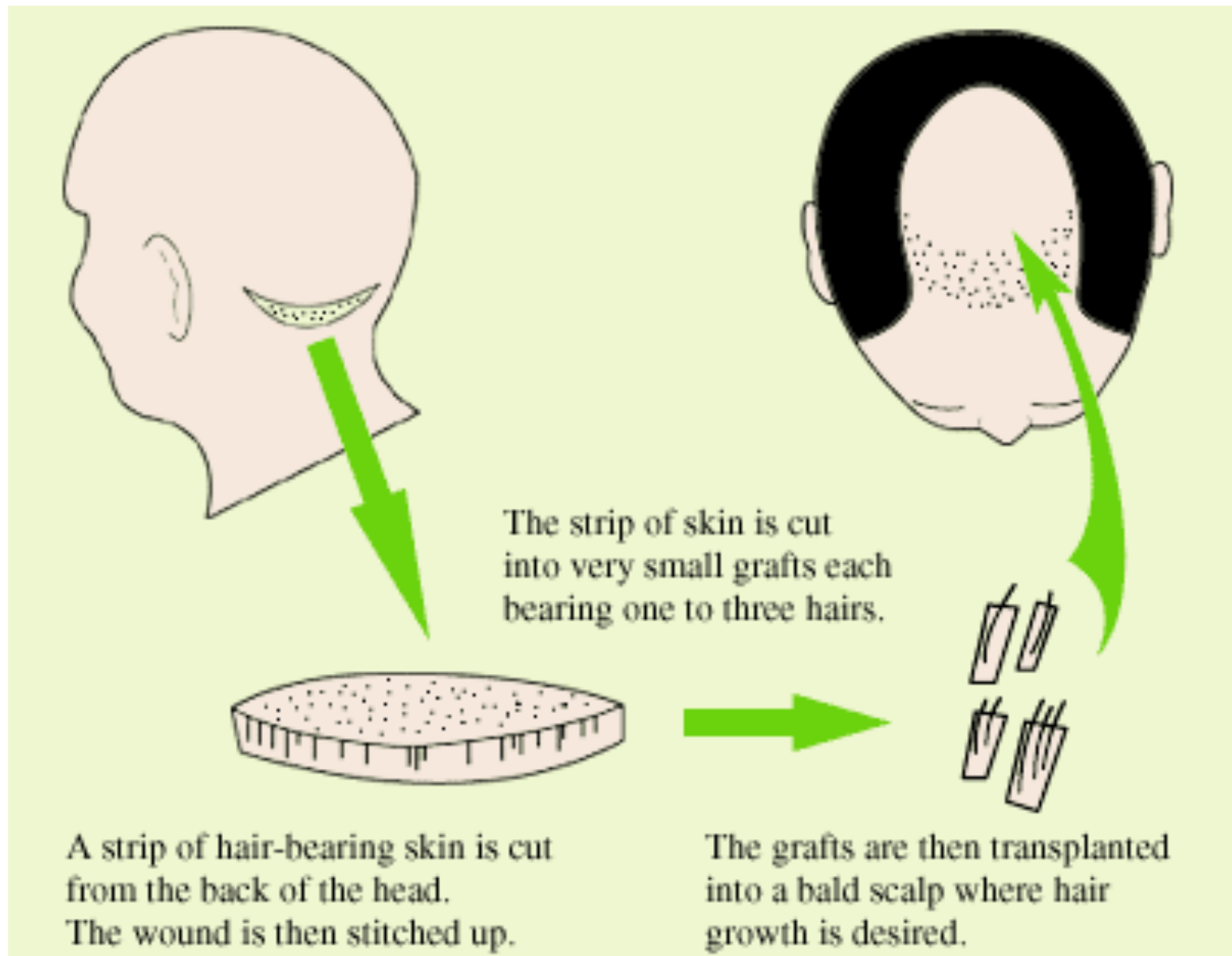
Stem Cells in the Hair Follicle



Sorry Rapunzel!



Hair Transplantation is Stem Cell Therapy!



Limitations of multipotent stem cells

- Limited developmental potential
- Difficult to find and isolate
- Difficult to grow in culture

Policy under Barack Obama



March 9, 2009

Executive order 13505

- The Secretary of Health and Human Services (Secretary), through the Director of NIH, may support and conduct **responsible, scientifically worthy** human stem cell research, including human embryonic stem cell research, **to the extent permitted by law.**
- The Presidential statement of August 9, 2001, limiting Federal funding for research involving human embryonic stem cells, shall have no further effect as a statement of governmental policy.
- Executive Order 13435 of June 20, 2007, which supplements the August 9, 2001, statement on human embryonic stem cell research, is revoked.

NIH Guidelines (July 2009)

ES cell research eligible for NIH funding if:

1. that were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose
2. that were donated by individuals who sought reproductive treatment (hereafter referred to as "donor(s)") and who gave voluntary written consent for the human embryos to be used for research purposes
3. No payments, cash or in kind, were offered for the donated embryos.
4. Decisions related to the creation of human embryos for reproductive purposes should have been made free from the influence of researchers proposing to derive or utilize hESCs in research.

Research NOT eligible for NIH funding:

1. NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations ban on funding of human embryo research (Section 509, Omnibus Appropriations Act, 2009, Pub. L. 111-8, 3/11/09), otherwise known as the Dickey Amendment.
2. Research using hESCs derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not eligible for NIH funding.

Dickey-Wicker Amendment (1995)

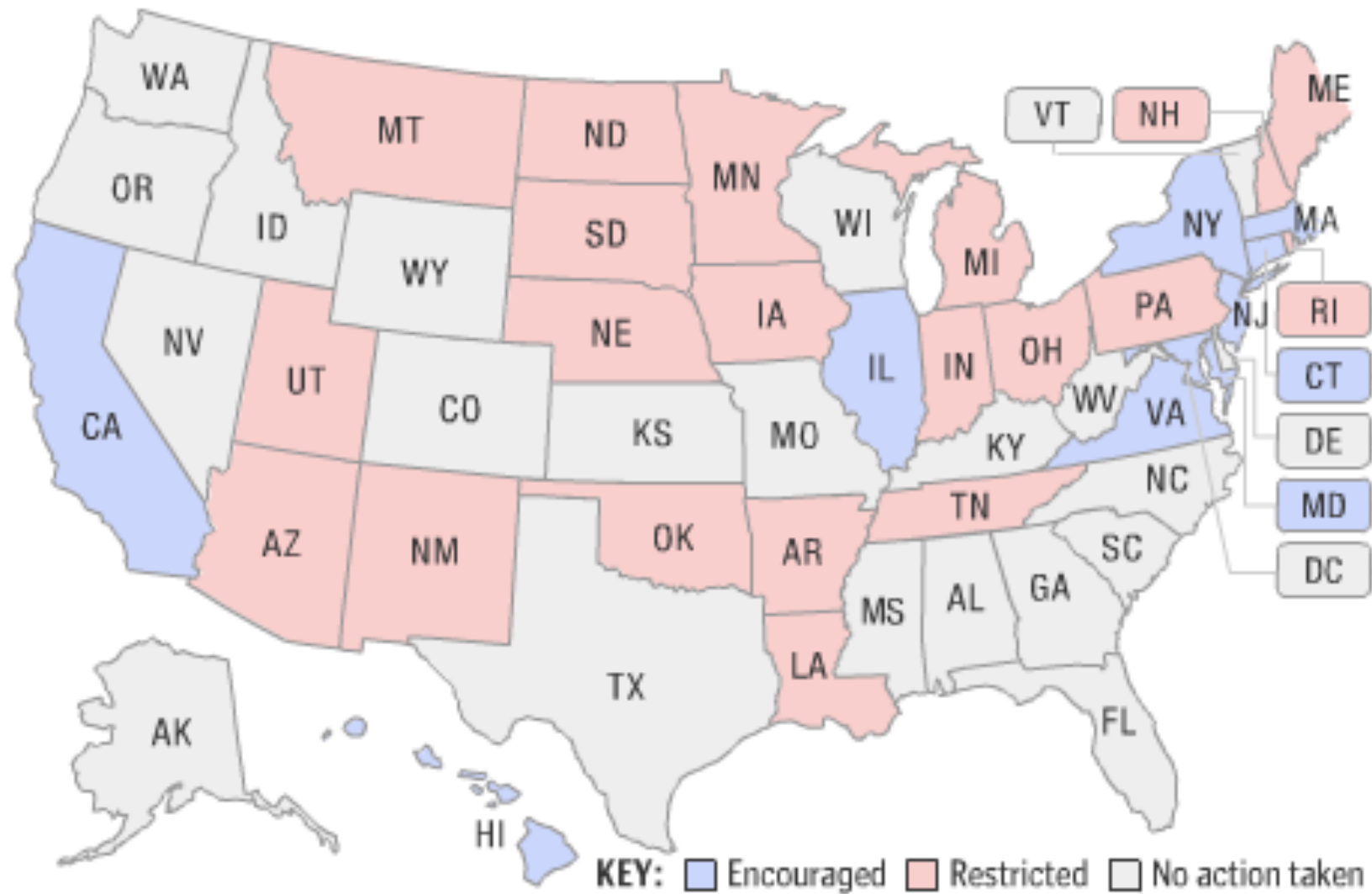
Prohibits Dept HHS appropriations for:

- (1) the creation of a human embryo or embryos for research purposes; or
- (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero

Sherley v. Sebelius

- 8/19/2009—complaint filed challenging the legality of NIH guidelines
- 8/23/2010—preliminary injunction from DC District court blocking implementation of NIH's 2009 guidelines, saying that it violates the Dickey-Wicker amendment
- 9/9/2010—preliminary injunction lifted pending decision from US court of appeals
- 4/29/2011—injunction vacated by US court of appeals. Using the Chevron doctrine, the court concluded that the Dickey-Wicker amendment is ambiguous and that NIH has acted reasonably in concluding that public funds could be used for human embryonic stem cell research. Also, the panel that the government would be harmed by the injunction more than the plaintiffs by not having one.
- 1/7/13—US Supreme court refuses to hear appeal

Stem cell research policy by state:



California Institute for Regenerative Medicine (CIRM)

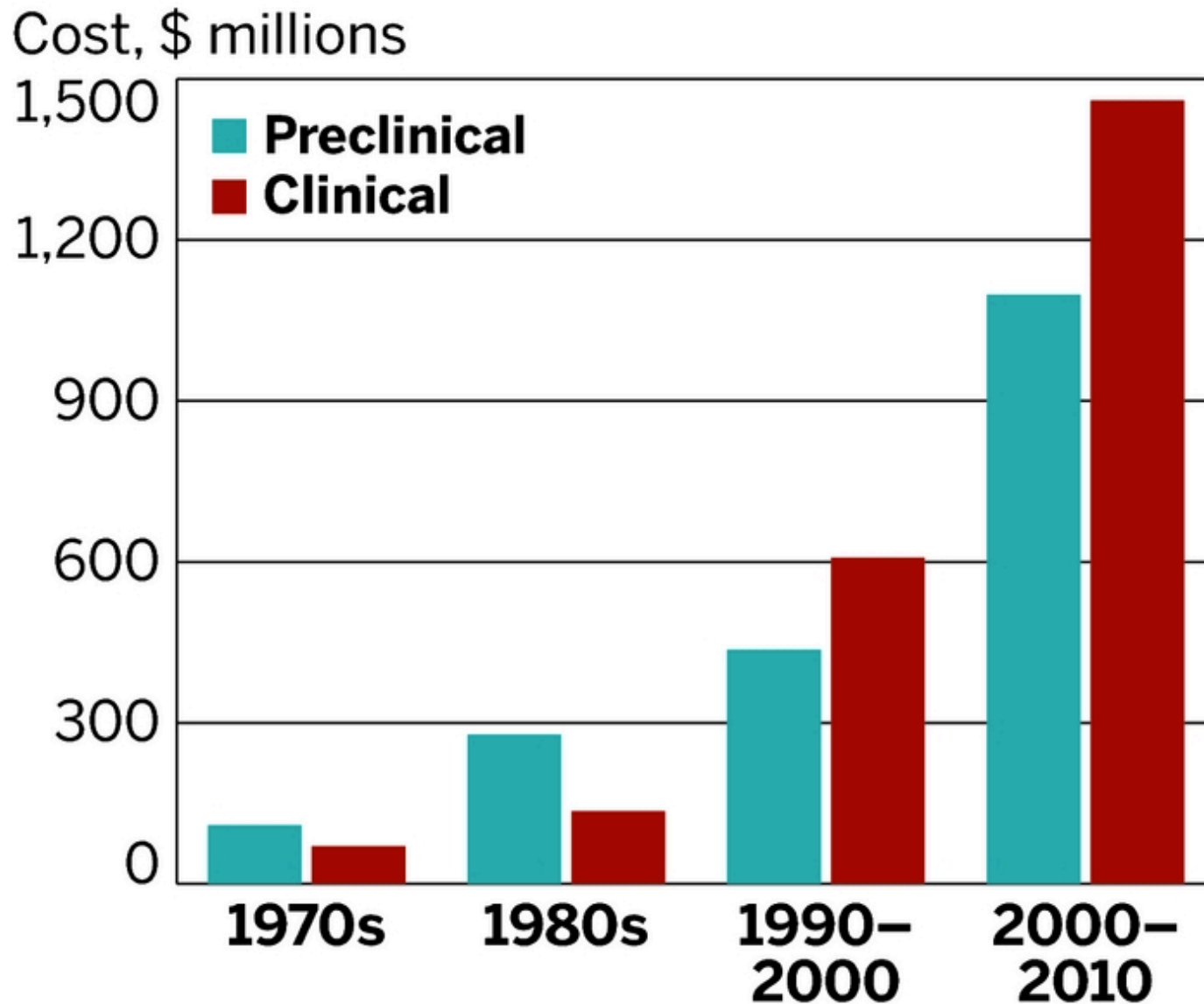
- Created in 2004 through the passage of prop 71 (59% of vote)
 - Allocates grant money for research purposes
 - Sets appropriate regulatory standards
- Prop 71:
 - Makes conducting stem cell research a constitutional right
 - Uses general obligation bonds to fund scientific research (normally for brick and mortar projects)
 - Takes on typical federal government role of funding scientific research
 - Represents a unique example where the public decided to fund scientific research
- Issues 3 billion in grants funded by bonds over 10 years
 - Money can be used for all stem cell research, with priority for human embryonic stem cell research
- First research grants were awarded in 2007
- Funding through prop 71 expected to end by 2017

The Promise of Regenerative Medicine



"The cure to your disease came from stem cell research. Sign here if you wish to refuse treatment for moral, religious or ideological reasons."

Cost of Developing a New Drug



New Drug Development Timeline

Pre-Clinical Testing, Research and Development

Range: 1-3 years
Average: 18 months

Initial Synthesis

Animal Testing

Short-Term

Long-Term

30-Day
Safety Review

Clinical Research and Development

Range: 2-10 years
Average: 5 years

Phase 1

Phase 2

Phase 3

FDA Time
Industry Time

NDA Review

Range: 2 months-7 years
Average: 24 months

NDA
Submitted

NDA
Approved

Post-Marketing Surveillance

Adverse
Reaction
Reporting

Surveys/
Sampling/
Testing

Inspections



Clinical Trials

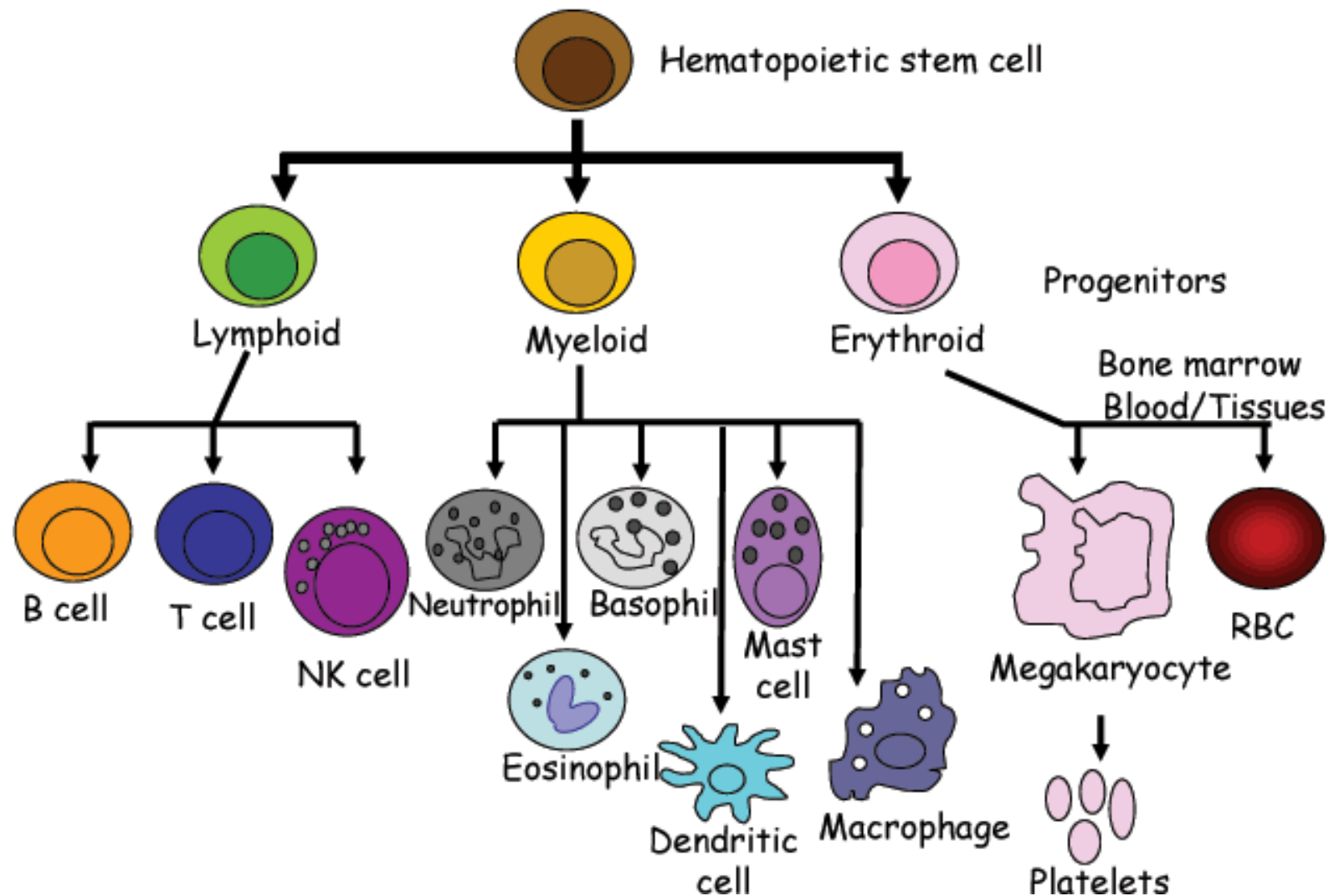
- Phase I: Safety
 - Usually includes healthy (paid) volunteers
- Phase II: Efficacy
 - Patients are involved
 - Usually where drug fails
- Phase III---Randomized controlled trial
 - Involves larger numbers of patients
 - Compares efficacy of drug against current “gold standard” treatment
 - Expensive

There are currently 2 clinical trials using ES cell derived therapy

Hurdles to using stem cells for disease treatment

- Reproducibly proliferate and generate sufficient tissue
- Reproducibly differentiate into the desired cell type
- Delivery to desired organ
- Survive in the recipient after transplant
- Integrate into the surrounding tissue
- Function properly
- No harm (esp. ESC)

Immune cell development: Hematopoiesis



Using Stem Cell Therapy to Treat Immune System Disorders

- Certain characteristics of the hematopoietic (blood) system make its diseases good candidates for stem cell therapy
 - Hematopoietic stem cells have been well studied
 - Long history of using the hematopoietic stem cell to treat disease (bone marrow transplant)
 - Easy to isolate, manipulate and replace
- 2 diseases:
 - SCID
 - Sickle cell anemia
- Both from research done in Donald Kohn's lab here at UCLA!

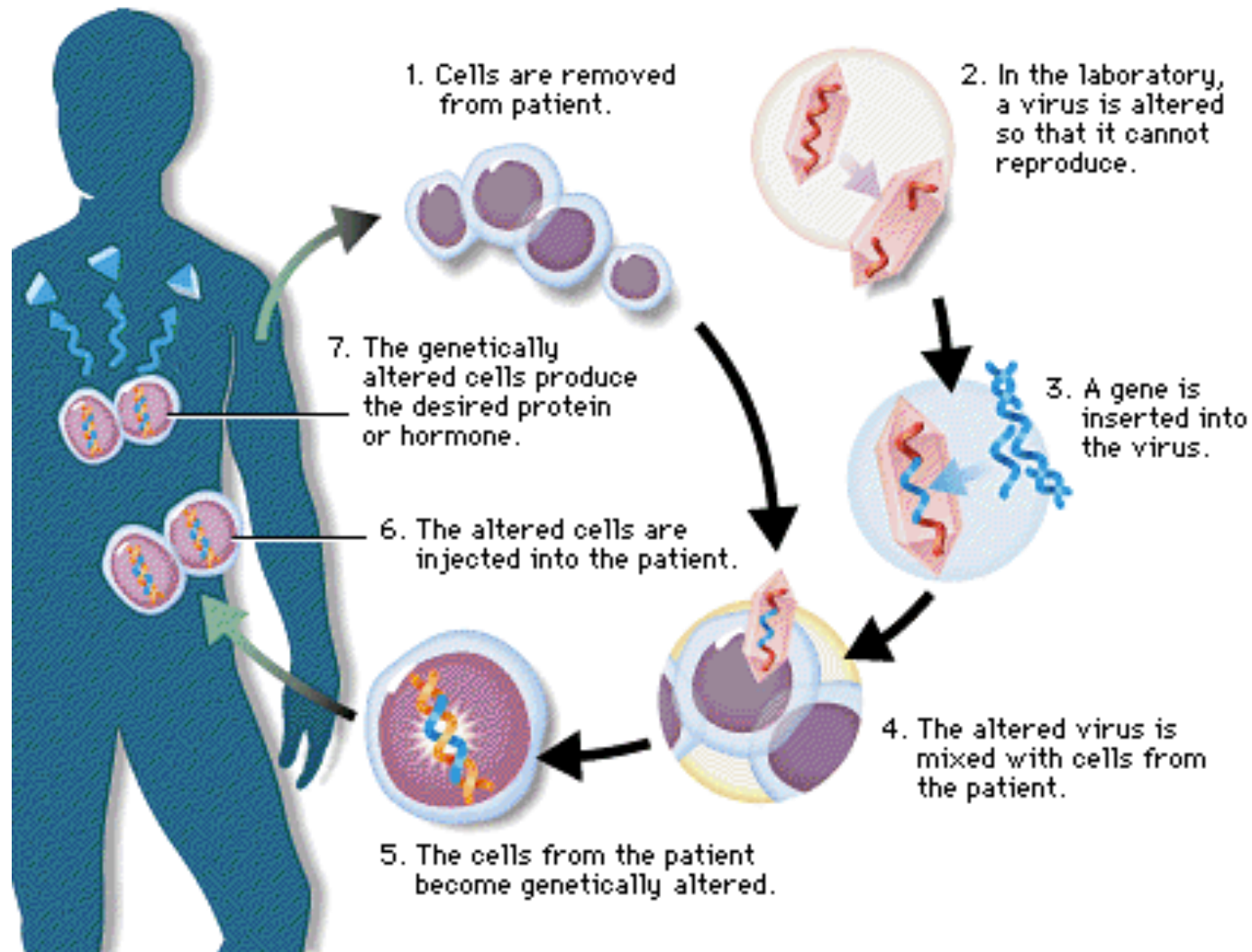
Severe Combined Immune Deficiency (SCID)

- Bubble boy disease
- Defect leads to lack of functional B or T cells of the immune system
- SCID babies generally die in the first year of life
- Many different mutations can lead to disease, caused by a loss of function of the protein

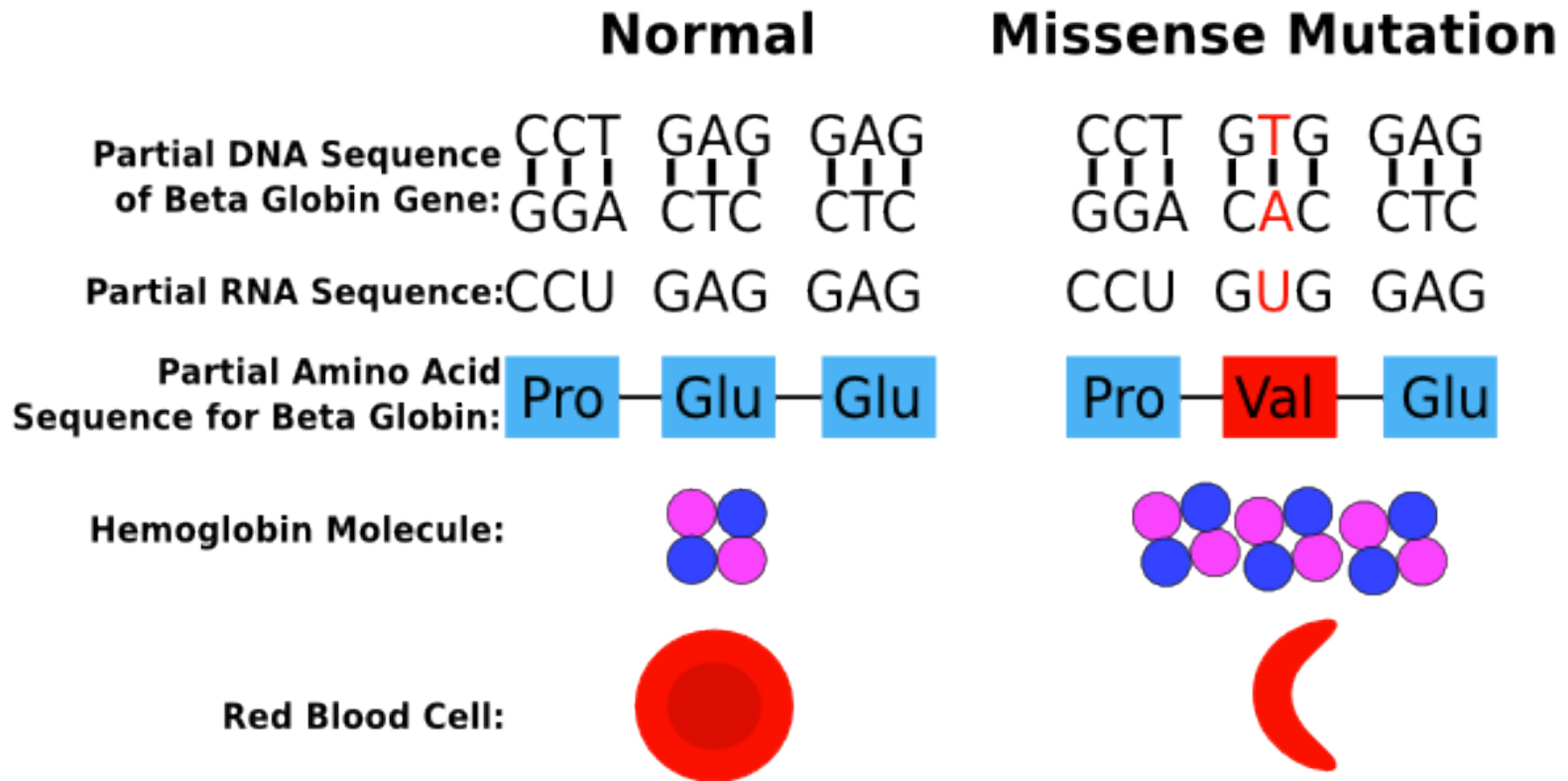


David Vetter—the “bubble boy”
1971-1984

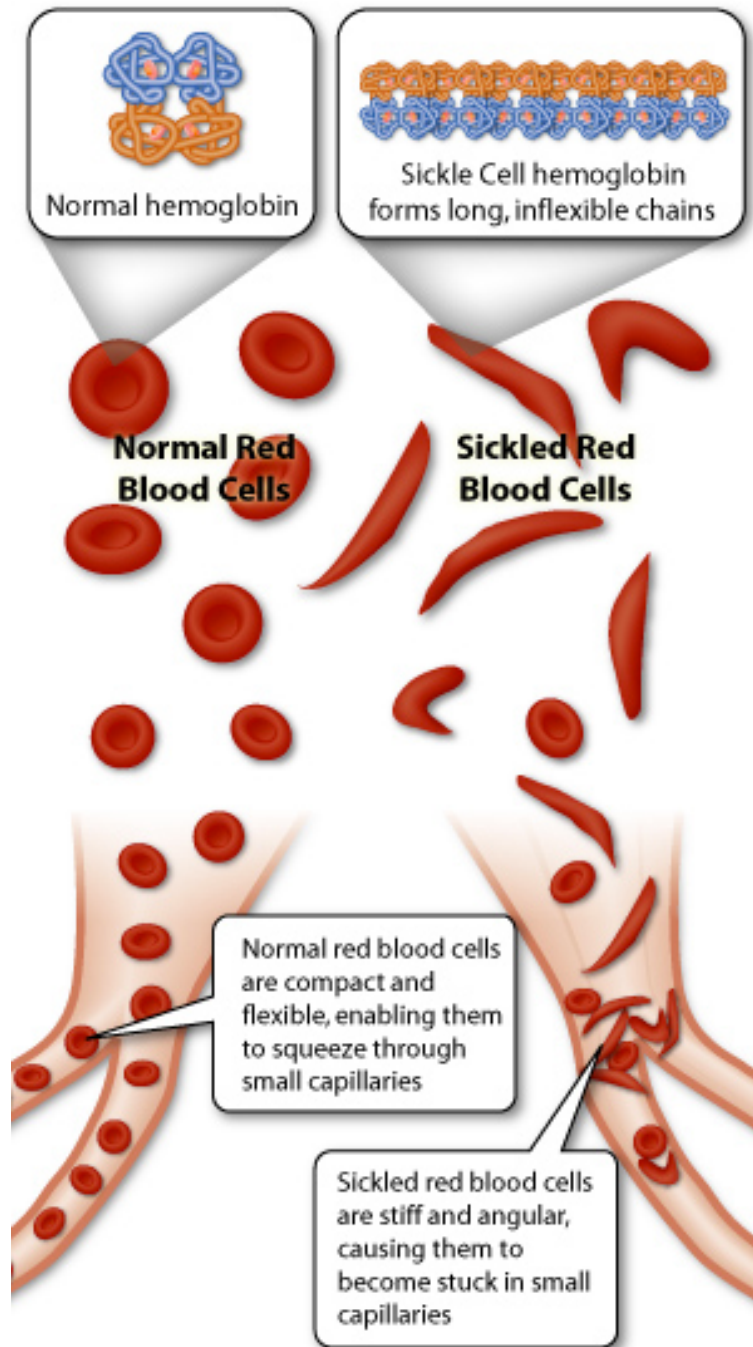
Gene Therapy Using Hematopoietic Stem Cells



Sickle Cell Anemia



Sickle cell anemia is more difficult to treat because it is not caused by the *lack* of a protein, but the incorrect function of a mutated protein



- Mutation causes the hemoglobin protein to form long stiff rods, leading the sickle shape
- Lifespan of a patient with sickle cell anemia is under 40 years
- Clinical trials currently under way

Correction of Sickle-Cell Disease Mutation in Human Blood Stem Cells

