

College of Physicians and Surgeons

An Introduction to Stem Cell Biology

Michael L. Shelanski, MD,PhD Professor of Pathology and Cell Biology Columbia University

Columbia University Medical Center

Figures adapted from ISSCR. Presentations of Drs. Martin Pera (Monash University),

Dr.Susan Kadereit, Children's Hospital, Boston and Dr. Catherine Verfaillie, University of Minnesota



Science 1999, 283: 534-537

Turning Brain into Blood: A Hematopoietic Fate Adopted by Adult Neural Stem Cells in Vivo

Christopher R. R. Bjornson,*†‡ Rodney L. Rietze,*§ Brent A. Reynolds, M. Cristina Magli, Angelo L. Vescovi‡

Stem cells are found in various organs where they participate in tissue homeostasis by replacing differentiated cells lost to physiological turnover or

Hematopoietic potential of stem cells isolated from murine skeletal muscle PNAS 1999, 96: 14482-14486

Kathyjo Ann Jackson, Tiejuan Mi, and Margaret A. Goodell*

Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX 77030

Edited by Irving L. Weissman, Stanford University School of Medicine, Stanford, CA, and approved October 6, 1999 (received for review September 1, 1999)

We have discovered that cells derived from the skeletal muscle of adult mice contain a remarkable capacity for hematopoietic differentiation. Cells propared from muscle by enzymatic direction. self-renew in response to physiological stimuli (14-17). Therefore, satellite cells could represent stem cells capable of commitment to more than one lineage given the right environmental.

Turning Blood into Brain: Cells Bearing Neuronal Antigens Generated in Vitro from Bone Marrow

Science 2000, 290:1779-1782

Mezey, E., Chandross, K.J., Harta, G., Maki, R.A., McKercher, S.R.

From Marrow to Brain: Expression of Neuronal Phenotypes in Adult Mice Science 2000, 290:1775-1779

Brazelton, T.R., Rossi, F.M., Keshet, G.I., Blau, H.M.

Purified hematopoietic stem cells can differentiate in hepatocytes *in vivo*

Eric Lagasse¹, Heather Connors¹, Muhsen Al-Dhalimy², Michael Reitsma¹, Monika Dohse¹, Linda Osborne¹, Xin Wang², Milton Finegold³, Irving L. Weissman⁴ & Markus Grompe²

¹StemCells, 525 Del Rey Avenue, Suite C, Sunnyvale, California 94085, USA ²Department of Molecular and Medical Genetics, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Road, L103, Portland, Oregon 97201, USA ³Department of Pathology, Texas Children's Hospital, Baylor College of Medicine, Houston, Texas 77030, USA ⁴Department of Pathology and Developmental Biology, Stanford University School of Medicine, Stanford, California 94305, USA Correspondence should be addressed to E. L.; email: elagasse@stemcell.net

The characterization of hepatic progenitor cells is of great scientific and clinical interest. Here we report that intravenous injection of adult bone marrow cells in the FAH^{-/-} mouse, an animal model of tyregipenia, type L rescued the mouse, and restored the biochemical function of its liver.

College of Physicians and Surgeons

Nat Med 2000, 11: 1229-1234

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

* Department of Medicine, New York Medical College, Valhalla, New York 10595, USA

† Hematopoiesis Section, Genetics and Molecular Biology Branch, NHGRI, and ‡ Laboratory of M USA Nature 2001, 410:701-705

Stem Cell FAQs

- Do you need to get one from an egg?
- Must you sacrifice an Embryo?
- What is an ES cell?
- What about adult stem cells or cord blood stem cells
- Why can't this work be done in animals?
- Are "cures" on the horizon?
- Will this lead to human cloning human spare parts factories?

Are we going to make a Frankenstein?



Columbia University

What is a stem cell?

A primitive cell which can either self renew (reproduce itself) or give rise to more specialised cell types

The stem cell is the ancestor at the top of the family tree of related cell types. One blood stem cell gives rise to red cells, white cells and platelets



Stem Cells Vary in their Developmental capacity

A **multipotent** cell can give rise to several types of mature cell

A **pluripotent** cell can give rise to all types of adult tissue cells plus extraembryonic tissue: cells which support embryonic development

A **totipotent** cell can give rise to a new individual given appropriate maternal support



Columbia University

The Fertilized Egg

The "Ultimate" Stem Cell – the Newly Fertilized Egg (one Cell) will give rise to all the cells and tissues of the adult animal.

Truly Totipotential but difficult to use.





College of Physicians and Surgeons

Properties of human ES cells

The defining feature of an ES cell is its ability to differentiate into a wide range of tissues

Columbia University Medical Center

Embryonic stem cells

Derived from five day old spare human embryos before specialised tissues of the body begin to form

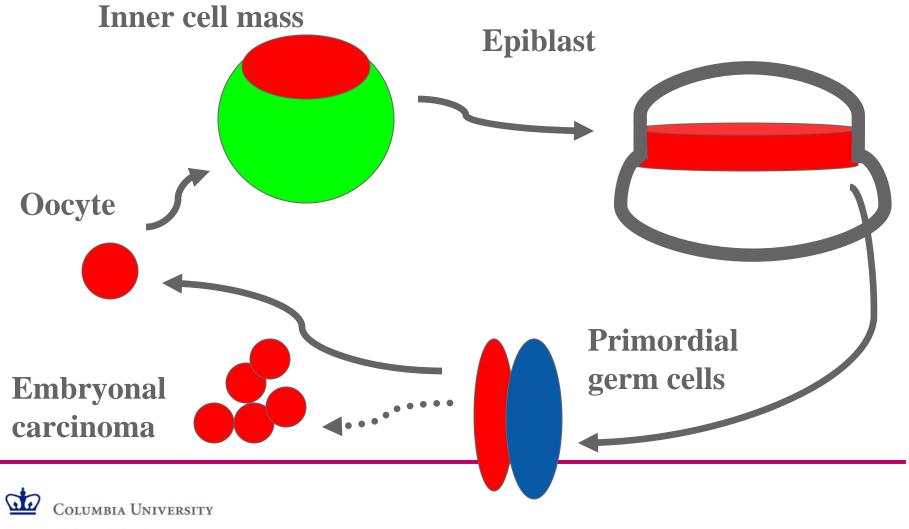
May be grown indefinitely in culture in the primitive embryonic state

Retain the property of pluripotency during extended growth in vitro



Columbia University

Pluripotent cell populations



College of Physicians and Surgeons

Martin F. Pera

Properties of pluripotent stem cells

Derived directly and at high frequency from pluripotent cell populations in vivo

Grow indefinitely in vitro (express telomerase)

Maintain normal karyotype

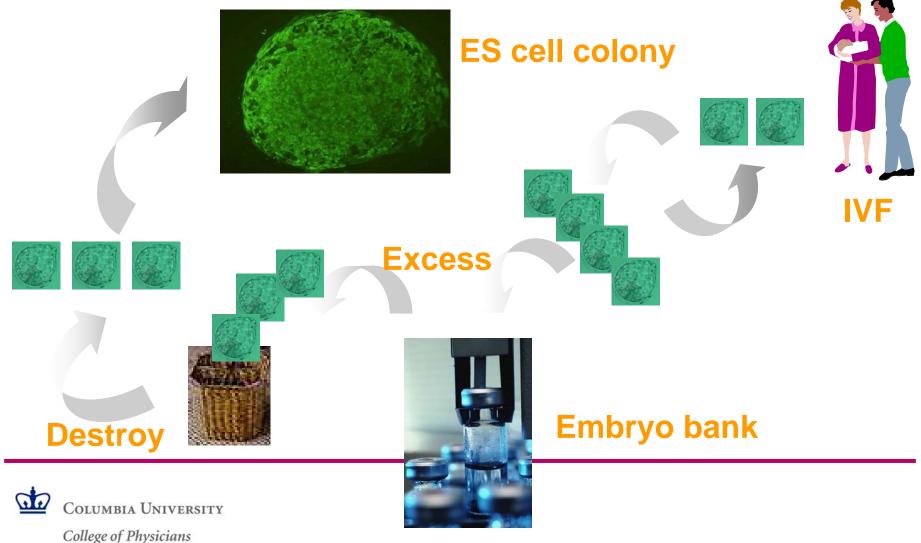
Cloned lines capable of differentiation into a wide range of somatic and extraembryonic tissues in vivo and in vitro-at high frequency and under a range of conditions

Capable of colonising all tissues including germ line after blastocyst injection to give chimeric offspring



Columbia University

Where do human embryonic stem cells come from?



and Surgeons

Martin F. Pera

Early Stages of Human Development











Blastocyst (pre-implantation)



Columbia University

Blastocyst stage of development

Body plan not yet apparent

Many cells will not form new human, but will give rise to tissue such as placenta which support pregnancy

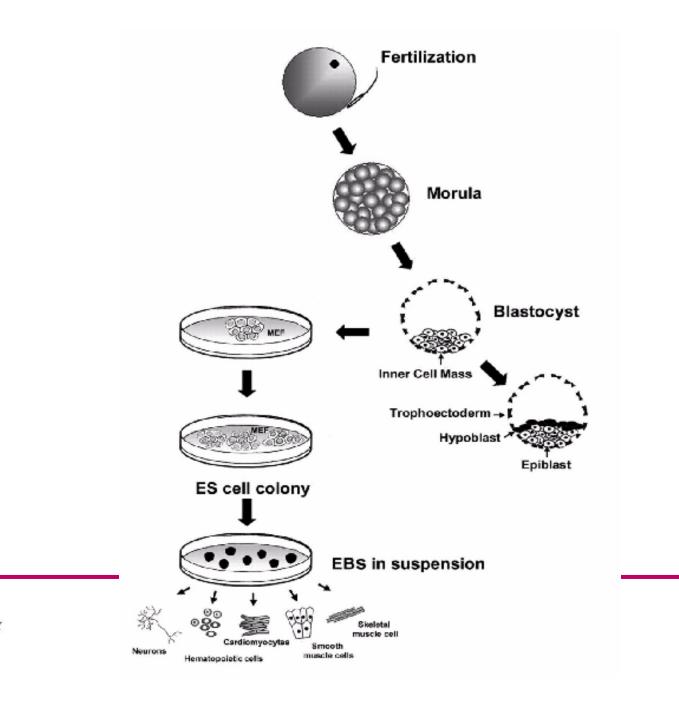
Embryo does not yet necessarily represent a unique individual (twins can form up to 14 days)

No precursors of nervous system present yet

Not possible to predict whether embryo will be able to develop to term

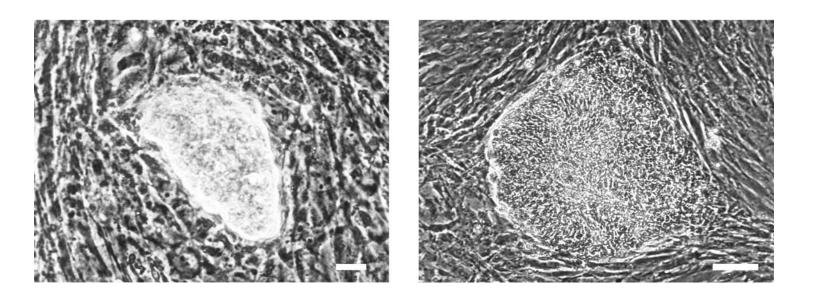


Columbia University





Establishment of ES cells



Inner cell mass

ES colony 10-15 days later



Columbia University

College of Physicians and Surgeons

Martin F. Pera

Characterisation of primate pluripotent stem cells

Immunological markers: TRA1-60 and related epitopes, SSEA-3, SSEA-4

Gene expression: transcripts for generic markers of pluripotent stem cells

Biological properties: Differentiation into derivatives of all three germ layers



Columbia University

Human Embryonic Stem Cells (hESC)

Derived from five day old spare human embryos created during IVF.

Retain the property of pluripotency during extended culture growth:

→ Unlimited supply for meaningful experiments.

→ Can give rise to clinically relevant cell numbers.



What Actually Happens When Stem Cells Do "Their Thing"

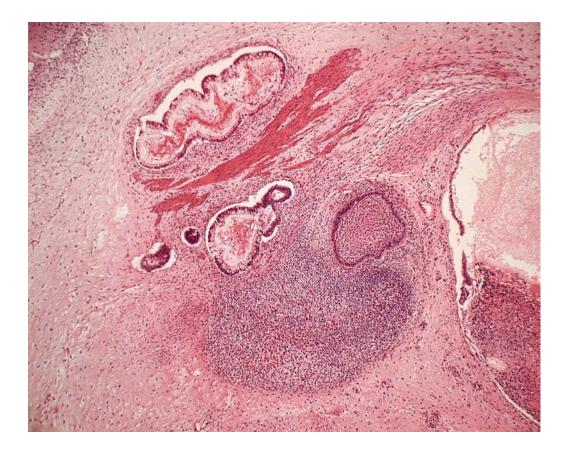
ALL SORTS OF THINGS

LET'S TAKE A LOOK



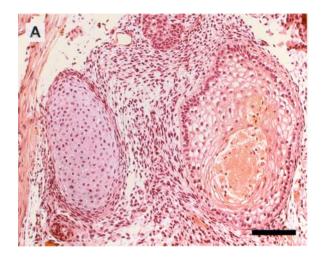
Columbia University

Teratoma formed by human ES cells





ES cells give rise to disorganised growths called teratomas



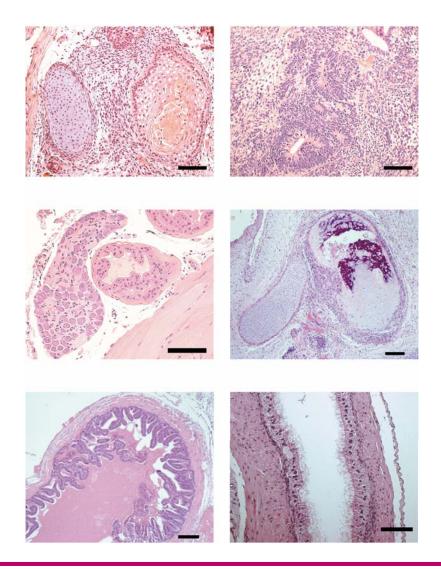
Teratomas do not display axis formation or segmentation. Unlike embryos, ES cells on their own are incapable of generating the body plan. This lack of organisation is also seen when ES cells differentiate in vitro



Columbia University

ES cell differentiation

Cartilage, bone, skin, nerves, gut and respiratory lining form when ES cells are injected into host animals



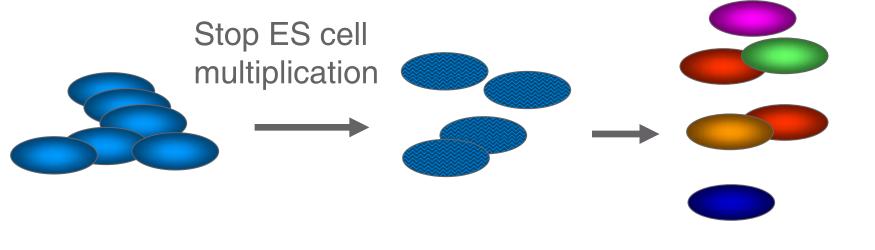


Columbia University

College of Physicians and Surgeons

Martin F. Pera

Spontaneous ES cell differentiation in vitro



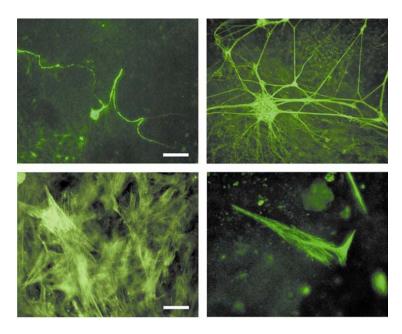
ES cell

Mixture of differentiated Cells with some pancreatic cells

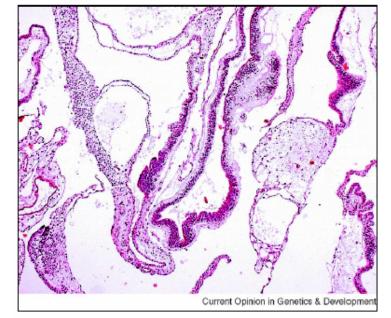




Spontaneous ES cell differentiation in vitro



Nerve and muscle cells are found in a complex mixture of many cell types



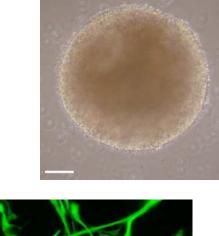
Cell differentiation in monolayer cultures of ES cells grown to high density. Aggregates of cells and cysts eventually form a multilayered structure when ES cells are left *in situ* and grown to high density from a monolayer culture. Multiple cell types and some organoid structures are seen in this section made by scraping the cell layer from the dish, embedding it, and staining with hematoxylin and eosin. The structures resemble those found in embryoid bodies formed by placing ES cell aggregates in suspension culture.

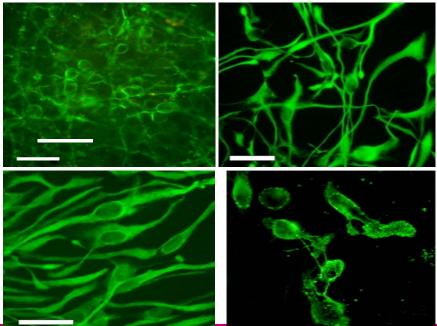


Columbia University

Neural precursors can be derived from ES cells





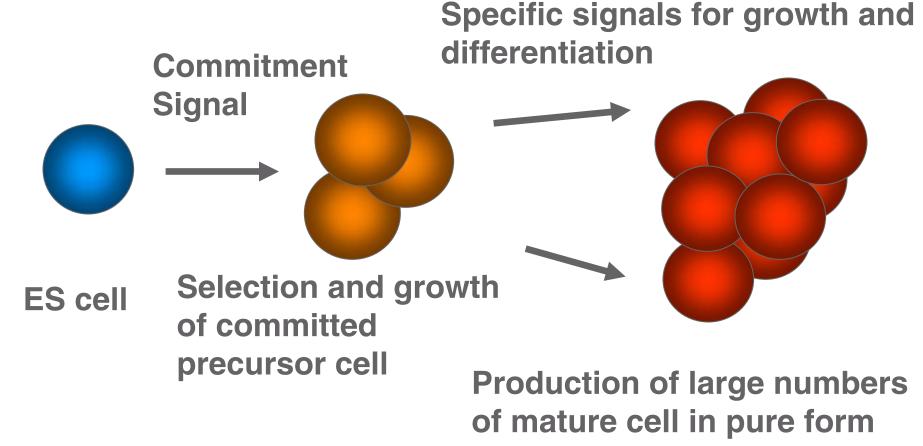




College of Physicians and Surgeons

Martin F. Pera

Routes to differentiation





How Do We Make A Stem Cell Into a Specific Cell Type?

Need to understand biology of differentiation

Much Data Can be Derived from Animal Experimentation

Use growth factors and "differentiation agents"

Key advantage is the ability to grow large quantities of "identical" cells



Studies of the mammalian embryo provide clues as to how embryonic stem cell differentiation might be controlled



Columbia University

College of Physicians and Surgeons

Martin F. Pera

The embryo and ES cells

Cell interactions between pluripotent cells and extraembryonic cells mediate patterning and fate decisions

Do the same cell populations exist in ES cell cultures?

Do the same molecules mediate fate decisions in ES cell culture?



Columbia University

Cell types derived from human ES cells in vitro Nerve, astrocyte, oligodendrocyte Hematopoietic stem cells Insulin producing cells Cardiomyocytes Hepatocytes Endothelial cells



Columbia University

```
Hematopoietic stem cells from
human ES cells (Kaufman et al.)
```

Culture of ES cells on marrow stromal support lines leads to formation of CD34⁺ hematopoietic precursor cells

Will form myeloid erythroid and megakaryocytic colonies.

Frequency 1-2%



Columbia University

Embryonic stem cells have important applications in biomedical research

Basic studies of early human development and its disorders-birth defects, childhood cancers

Functional genomics in human cells

Discovery of novel factors controlling tissue regeneration and repair

In vitro models for drug discovery and toxicology

Source of tissue for transplantation medicine



Columbia University

Successful treatment of animal models of disease with mouse ES derived cells

Severe immune deficiency

Diabetes

- Parkinson's disease
- Spinal injury
- Demyelination

Myocardial infarction



Columbia University

Challenges for transplantation therapy

Production of required cell type in sufficient numbers and pure form

What cell to transplant

Delivery

Problems of tissue rejection



Columbia University

The immune rejection issue in ES cell based therapy

How immunogenic are embryonic or fetal derived grafts?

Some transplantation sites will be immunologically privileged

Interesting data to suggest embryonic cells can induce tolerance in hosts Fandrich et al Nat. Med. 8: 171, 2002



Columbia University

Solutions to the rejection problem

- Large banks of ES cell lines
- Manipulation of histocompatibility genes in ES cells
- Replacement of hematopoietic/lymphoid tissue of patient with ES derived cells prior to transplant
- Manipulation of T cell response with antibodies or drugs
- Therapeutic cloning or related techniques



Columbia University

Therapeutic cloning

Combines cloning methods with embryonic stem cell technology to produce cells which are custom made for patient

A promising solution to problem of tissue rejection

Used to produce ES cells in mice and cure a severe immune disorder

More research may enable us to reprogram adult cells without going through embryo step



Columbia University

Adult stem cells

Proper tissue organisation and response to demands of growth or repair require that there be restrictions on developmental potential of adult stem cells

These limits are strictly imposed by powerful molecular restraints on gene expression and are heritable during many rounds of cell division

An adult stem cell may show relaxation of these restrictions in an altered environment, possibly accounting for plasticity. Even so, plasticity is observed usually at low frequency



Columbia University

Bone Marrow Stem Cells

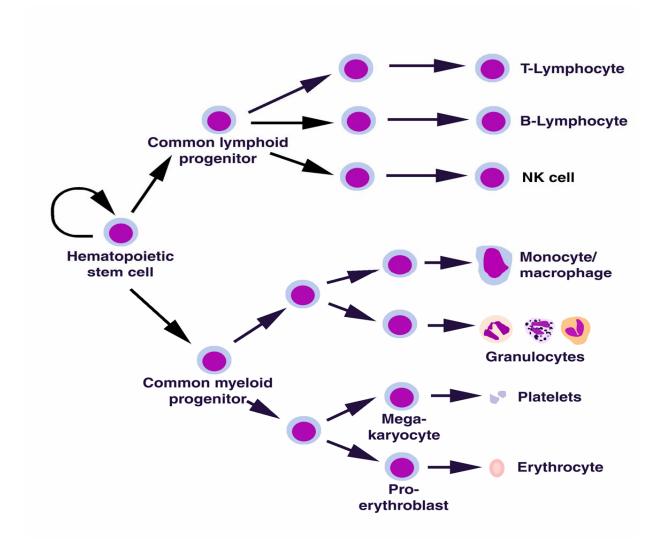
• isolated from human, mouse and rat.

• Appear only after 35 population doublings.

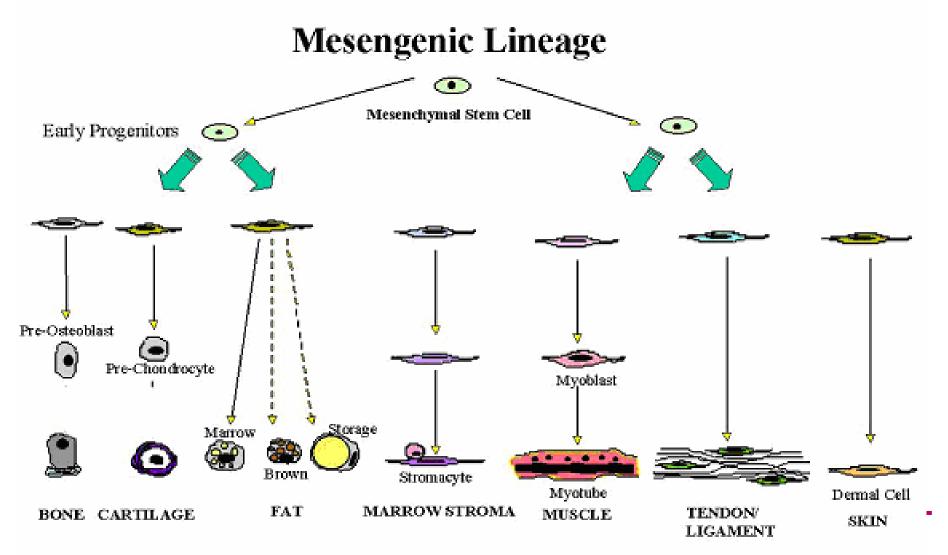
- Can be grown to 50-80 population doublings.
- Give rise to mesoderm, neuroectoderm and endoderm
- Can form chimeras with cells in all somatic tissues, when injected into blastocysts (multipotent).



Columbia University



Columbia University



COLUMBIA UNIVERSITY

Cord Blood Stem Cells

• rare cell, CD45 -/ HLA-

• Grow robustly in vitro without differentiating.

- Give rise to mesoderm, neuroectoderm and endoderm.
- In vivo: differentiation into neural cells, bone andcartilage, blood, myocardial cells and Purkinje fibers, hepatic cells.

Columbia University

Nuclear Transfer

To produce cells which are custom made for patient.

A promising solution to problem of tissue rejection, as cells express the patients genes.

Embryonic stem cell lines created from patients with certain diseases, to study disease development and to develop drugs.



Columbia University

Therapeutic cloning in transplantation: necessary or feasible?

How severe will immune rejection problem be?

Other solutions exist to the problem

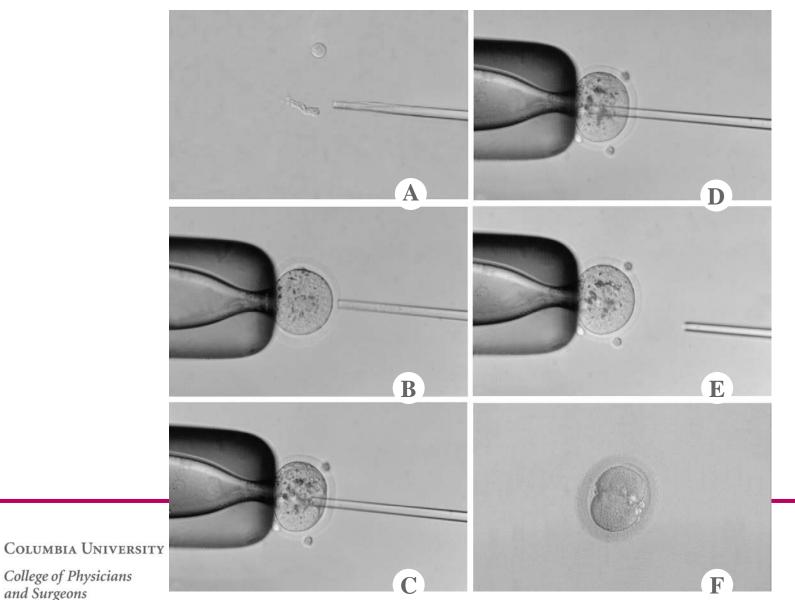
Is it practical? Where will the eggs come from? Can the procedure be turned around in the required time frame?

Is it safe? Is it easier to make normal cells from cloned ES than to make normal animals?



Columbia University

Nuclear Transfer





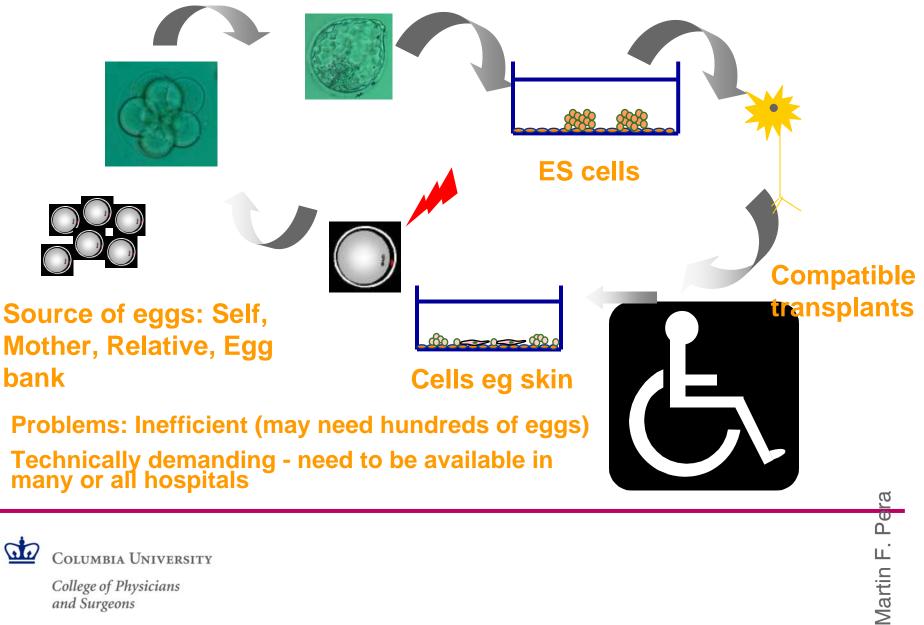
Problems with Nuclear Transfer (NT)

• Inefficient: 242 → 30 blastocysts → 1 cell line

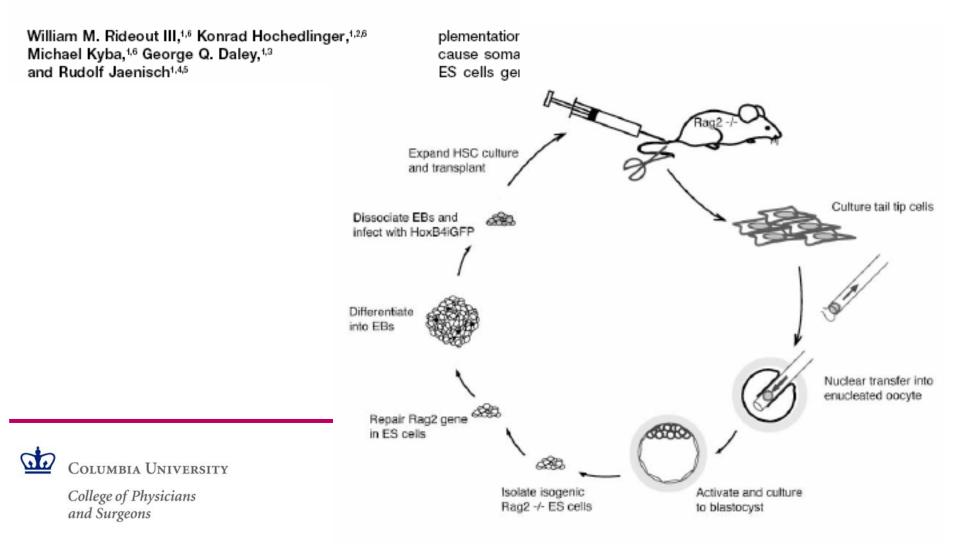
• Time to derive therapeutic cells from NT blastocysts will take several weeks to months.

Columbia University

Somatic Cell Nuclear Transfer



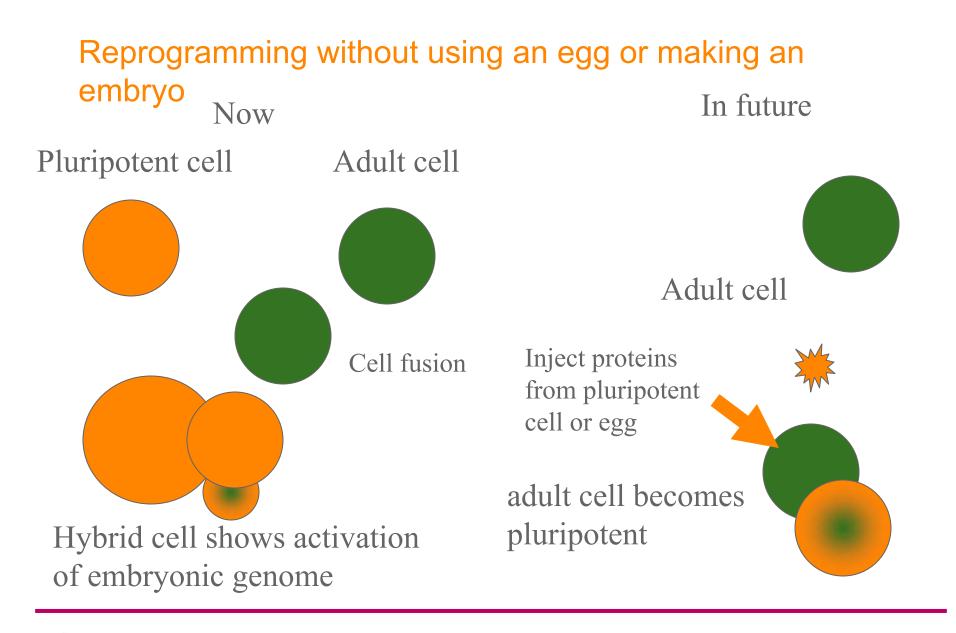
Correction of a Genetic Defect by Nuclear Transplantation and Combined Cell and Gene Therapy



ARE THERE WAYS AROUND USING EGGS AND EMBRYOS?

POSSIBLY







Columbia University

College of Physicians and Surgeons

Martin F. Pera

Do We Have the Ability to Repair Ourselves?

Endogenous Stem Cells

Need to learn to manipulate in the body

Indications from hematology that is possible



Cancer Stem Cells

- Not all the cells within a tumor can maintain tumor growth, most cancers are not clonal.
 - Several long-known oncogenic pathways are pivotal to the maintenance of normal stem cell self-renewal.
 - Techniques used in the stem cell field have identified selfrenewing cells.

•By identifying the stem cells in tumors, it could be shown that only the cancer stem cells propagated the tumor.



Columbia University

Cancer Stem Cells

• In breast cancer, brain tumors, certain forms of leukemia, and gastric tumor.

• Unknown whether the tissue stem cell degenerates, or if a more differentiated cell reacquires stem cell phenotype.

• Despite preventive mechanisms adult stem cells may accumulate mutations over the years.



Columbia University

Why we need new stem cell lines

Panels of cell lines required for tissue matching in transplantation

Safety hazards with current cells derived using animal tissue

Current bona fide lines few in number; improvements to techniques will enable production of second generation ES cells with better properties;

Need cell lines in the public domain without commercial restrictions on use



Columbia University

Are We Making a Frankenstein?

Not That Dramatic

But, Beware of hype

Remember the Law of Unintended Consequences

