Cloning and Stem Cell Research

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Human Gene Cloning

- **Human DNA restriction enzyme cleaves DNA**
- **DNA ligase seals human gene and plasmid**
- **Recombinant DNA**
- **Host cell takes up recombined plasmid**
- **Cloning**
  - **Cloned insulin gene**
  - **Insulin**
Cell Cloning
One cell is placed into the dish or well by itself. The cell divides and forms a population of identical cells (cell clones.)
Fertilization vs. Cloning (somatic cell nuclear transfer, SCNT)
Cloning (Somatic Cell Nuclear Transfer, SCNT)

Remove udder cell from white-face sheep
Remove DNA from unfertilized egg
Fuse cells
Early embryo with donor DNA
Cloned embryo
Clone of white-face sheep
Implant in surrogate
Dolly

Remove skin cell from patient
Remove DNA from unfertilized egg
Fuse cells
Early embryo with donor DNA
Cloned embryo
Infant clone of patient
Implant in surrogate
Embryonic stem cells

"Reproductive cloning"  "Therapeutic cloning"
"Reproductive cloning"  "Therapeutic cloning"
Figure 1 Stages of Development of the Human Embryo

Figure 2 Isolation and Culture of Human ESCs from Blastocysts

Figure 4 Somatic Cell Nuclear Transfer (SCNT).

[From: Stem Cells and the Future of Regenerative Medicine, Report of the National Academy of Sciences and the Institute of Medicine, National Academy Press, Washington, DC, Sept. 2001; Pg. 10, 11, 26]
**Cloning (SCNT) produces a human embryo**

“The method used to initiate the reproductive cloning procedure is called either nuclear transplantation or somatic cell nuclear transfer.”

*Scientific and Medical Aspects of Human Reproductive Cloning*, Report of the National Academy of Sciences and the Institute of Medicine, National Academy Press, Washington, DC, Jan 2002

“I know that you are grappling with this [question of whether a cloned embryo created in the lab is the same thing as an embryo produced by egg and sperm, and whether we should call it an “embryo”], but anything that you construct at this point in time that has the properties of those structures to me is an embryo, and we should not be changing vocabulary at this point in time. It doesn’t change some of the ethical issues involved.”

**Dr. John Gearhart**, Johns Hopkins University, 25 April 2002; before the U.S. President’s Council on Bioethics.

**“Therapeutic” Cloning creates an embryo for destruction**

“Moreover, because therapeutic cloning requires the creation and disaggregation *ex utero* of blastocyst stage embryos, this technique raises complex ethical questions.”

“Unlike much stem cell research, which can use spare embryos remaining from infertility procedures, CRNT [cell replacement through nuclear transfer, a.k.a. therapeutic cloning] requires the deliberate creation and disaggregation of a human embryo.”

Theoretical Concept of “Therapeutic Cloning”

1. Enucleated egg
2. Nuclear transfer
3. One-cell cloned embryo
4. Blastocyst stage cloned embryo
5. Remove inner cell mass
6. Culture embryonic stem cells
7. Produce desired healthy cells
8. Nerves
9. Muscle
10. Transplant
11. Patient
Therapeutic Cloning is a Failure

- **Transplanted cells from cloned mouse embryo rejected**
  
  “Our results raise the provocative possibility that even genetically matched cells derived by therapeutic cloning may still face barriers to effective transplantation for some disorders.”

  *RYL. Tsai, R Kittappa, and RDG McKay; “Plasticity, niches, and the use of stem cells”;
  Developmental Cell 2, 707-712; June 2002
  “Jaenisch addressed the possibility that ES clones derived by nuclear transfer technique could be used to correct genetic defects… However, the donor cells, although derived from the animals with the same genetic background, are rejected by the hosts.”

- **Clones may need to be gestated to “harvest” already-differentiated tissues**
  
Transplant rejection still likely using cells from cloned embryos

- “Robert Lanza, chief scientist at Advanced Cell Technology in Worcester, Mass., an ardent advocate for both embryonic stem cell studies and therapeutic cloning, agreed that in the course of the political debate, the need for cloning to overcome immune system rejection has been overstated. ‘It’s not all or nothing. You can move ahead.’”
  
San Francisco Chronicle, Monday, March 18, 2002   Page E – 1

- “There is no question in my mind that the possibility exists that if you are doing an egg donor, and nuclear transfer into an egg, that there possibly exists that that cell -- that the embryonic stem cells derived from that could be rejected. Absolutely.”
  
  Dr. John Gearhart, Johns Hopkins, 25 April 2002 meeting of the President’s Council on Bioethics

- “I should say that when you put the nucleus in from a somatic cell, the mitochondria still come from the host.” He concluded, “And in mouse studies it is clear that those genetic differences can lead to a mild but certainly effective transplant rejection and so immunosuppression, mild though it is, will be required for that.”

  Dr. Irving Weissman, Stanford, 13 February 2002 meeting of the President’s Council on Bioethics
“Therapeutic cloning”—unlikely chance of clinical success

- “[T]he poor availability of human oocytes, the low efficiency of the nuclear transfer procedure, and the long population-doubling time of human ES cells make it difficult to envision this [therapeutic cloning] becoming a routine clinical procedure…”
- “However, it is unlikely that large numbers of mature human oocytes would be available for the production of ES cells, particularly if hundreds are required to produce each ES line. The technical capability for nuclear transfer would also need to be widely available and this is unlikely. In addition, epigenetic remnants of the somatic cell used as the nuclear donor can cause major functional problems in development, which must remain a concern for ES cells derived by nuclear transfer. …it would appear unlikely that these strategies will be used extensively for producing ES cells compatible for transplantation.”
  Alan O. Trounson, “The derivation and potential use of human embryonic stem cells”, Reproduction, Fertility, and Development 13, 523-532; 2001
- Thomas Okarma, CEO, Geron Corporation says: “The odds favoring success are vanishingly small, and the costs are daunting.” “It would take thousands of [human] eggs on an assembly line to produce a custom therapy for a single person. The process is a nonstarter, commercially.”
  Denise Gellene, “Clone Profit? Unlikely”, Los Angeles Times, 10 May 2002
Cloning of Human Embryos—South Korea

Woo Suk Hwang, lead author of the study, admitted at a news conference that the technique developed in his lab "cannot be separated from reproductive cloning…"

Development of “therapeutic” cloning techniques can lead to “reproductive” cloning:

“It is true that the techniques developed in CRNT [cell replacement through nuclear transfer, a.k.a. therapeutic cloning] research can prepare the way scientifically and technically for efforts at reproductive cloning.”

“If undertaken, the development of SCNT for such therapeutic purposes, in which embryos are not transferred for pregnancy, is likely to produce knowledge that could be used to achieve reproductive SCNT.”
American Society for Reproductive Medicine Ethics Committee; “Human somatic cell nuclear transfer (cloning)”; Fertility and Sterility 74, 873-876; November 2000
“Cloning Unnecessary and Obsolete”
--leading embryonic stem cell expert

- **Alan Trounson**, Australian embryonic stem cell expert and a leader in the field worldwide, says that stem cell research has advanced so rapidly in the past few months that **therapeutic cloning is now unnecessary**. “My view is there are at least three or four other alternatives that are more attractive already,” he said. Trounson abandoned his call for therapeutic cloning, saying scientific breakthroughs mean there is now no need for the controversial technique.

Professor Trounson said therapeutic cloning faced logistical problems, and that other techniques were showing great promise and offered better options. “I can't see why, then, you would argue for therapeutic cloning in the long term because it is so difficult to get eggs and you've got this issue of (destroying) embryos as well.”

“Stem-cell cloning not needed, says scientist”, The Age (Melbourne), pg. 2, July 29, 2002;
“Stem-cell research outpaces cloning”, The Australian, pg. 3, July 29, 2002;
“Therapeutic cloning no longer necessary: expert”, AAP Newsfeed, July 29, 2002
“Therapeutic” cloning places women at risk

Because both cloning and embryonic stem cell production are extremely inefficient, a tremendous number of eggs will be required.

For example, to treat only the 17 million Diabetes patients in the U.S.:

Will require *minimum* of 850 million-1.7 billion human eggs
(Optimistically 50-100 human eggs/patient, estimated cost US$100,000-200,000)


[South Korean human cloning -- one cell line from 242 eggs—would be >4 billion eggs for diabetes]

--Collecting 10 eggs/donor:
Will require *minimum* 85-170 million women - childbearing age donors

Health risks—High-dose hormone therapy and surgery to obtain eggs risks the donor’s health and future reproductive success
Commercial exploitation—of poor women globally
Regenerative Medicine with Stem Cells

Bone marrow stem cells

Damaged heart muscle
Stem Cells

Human Developmental Continuum

- Single-cell Embryo
- 3-day Embryo
- 5-7 day Embryo
- 4-week Embryo
- 6-week Embryo

Embryonic Stem (ES) cells
- Totipotent

Embryonic Germ (EG) cells
- (primordial germ cells)

Fetal Tissue
- Stem cells
- Pluripotent or Multipotent

Cord Blood Stem cells
- Pluripotent

"Adult" Stem cells
- Pluripotent or Multipotent

Embryonal Carcinoma (EC) cells
- Pluripotent

Teratocarcinoma (germ cell tumor)
Derivation of Embryonic Stem Cells

Purported Advantages:
1) Proliferate indefinitely
2) Form any tissue

Method patented
U.S. patent held by Univ. Wisconsin

Human Blastocyst → Remove Trophoblast → Inner Cell Mass → Dissociate and Plate → Embryonic Stem Cells
Promises, Premises, and Published Data…

Claims unsubstantiated for embryonic stem cells

Current or potential embryonic stem cell problems:

- Difficult to establish and maintain
- Difficulty in obtaining pure cultures in the dish
- Potential for tumor formation and tissue destruction
  *Wakitani S et al.; “Embryonic stem cells injected into the mouse knee joint form teratomas and subsequently destroy the joint”; Rheumatology 42, 162-165; January 2003
- Questions regarding functional differentiation
  *Hansson M et al., “Artifactual insulin release from differentiated embryonic stem cells”, Diabetes 53, 2603-2609, October 2004
  *Sipione S et al., “Insulin expressing cells from differentiated embryonic stem cells are not beta cells”, Diabetologia 47, 499-508, 2004 (published online 14 Feb 2004)
  *Rajagopal J et al.; “Insulin staining of ES cell progeny from insulin uptake”; Science 299, 363; 17 Jan 2003
  *Zhang YM et al.; “Stem cell-derived cardiomyocytes demonstrate arrhythmic potential”; Circulation 106, 1294-1299; 3 September 2002
- Problem of immune rejection
- Genomic instability
  *Cowan CA et al., “Derivation of embryonic stem-cell lines from human blastocysts”, New England Journal of Medicine 350, 13; published online 3 March 2004
  *Draper JS et al., “Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells”, Nature Biotechnology 22, 53-54; January 2004
  *Humpherys S et al.; “Epigenetic instability in ES cells and cloned mice”; Science 293, 95-97; 6 July 2001
- Few and modest successes in animals, no clinical treatments
- Ethically contentious
Evidence that Some Adult Stem Cells show Pluripotent Capacity

Bone Marrow Stem Cells can form all 3 germ layers, and regenerate damaged heart.

Human Cord Blood stem cells show pluripotent potential and extensive proliferation

Human Bone Marrow Adult Stem Cells with pluripotent potential
D’Ippolito G et al., “Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential”, J. Cell Science 117, 2971-2981, 15 July 2004 (published online 1 June 2004)

Peripheral blood stem cells express Oct-4 gene and can form cells from all 3 primary germ layers, including endothelial cells, neuronal cells, and liver cells.
Zhao Y et al.; “A human peripheral blood monocyte-derived subset acts as pluripotent stem cells”; Proceedings of the National Academy of Sciences USA 100, 2426-2431; 4 March 2003

Adult stem cells from bone marrow can form new neurons in the human brain.
Mezey E et al.; “Transplanted bone marrow generates new neurons in human brains”; Proceedings of the National Academy of Sciences USA 100, 1364-1369; 4 Feb 2003

Adult stem cells from bone marrow can form all body tissues
Jiang Y et al.; “Pluripotency of mesenchymal stem cells derived from adult marrow”; Nature 418, 41-49; 4 July 2002

A single adult mouse bone marrow stem cell can form functional marrow, blood cells, liver, lung, gastrointestinal tract, skin, heart and skeletal muscle.
Krause DS et al.; “Multi-Organ, Multi-Lineage Engraftment by a Single Bone Marrow-Derived Stem Cell”; Cell 105, 369-377; 4 May 2001
Adult Stem Cells

Bone Marrow
- Marrow
- Bone
- Cartilage
- Tendon
- Muscle
- Fat
- Liver
- Brain/Nerve
- Blood cells
- Heart
- All Tissues

Peripheral Blood
- Bone Marrow
- Blood cells
- Nerves

Skeletal Muscle
- Skeletal muscle
- Smooth muscle
- Bone
- Cartilage
- Fat
- Heart

Brain
- Brain
- Nerves
- Blood cells
- Muscle
- All Tissues
- Cornea
- Retina
- Pancreas
- Liver
- Heart
- Lung
- Spermatogonia
- Amniotic Fluid
- Umbilical Cord Matrix

Stem Cells from Fat
- Bone
- Cartilage
- Muscle
- Nerves

Hair Follicle
- Skin
- Brain
- Smooth Muscle
- Fat

Gastrointestinal
- Esophagus
- Small Intestine
- Stomach
- Large Intestine/Colon

Placenta
- Bone
- Nerve
- Cartilage
- Muscle
- Tendon

CORD BLOOD
- Bone Marrow
- Blood vessel

Various Tissues
Adult stem cells effective in tissue repair

**Stroke**—Adult stem cells from brain, bone marrow, and umbilical cord blood provide therapeutic benefit after stroke. First clinical trials under way.

*Shyu W-C et al., “Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells”, *Circulation* 110, 1847-1854, 2004
*Willing AE et al., “Mobilized peripheral blood stem cells administered intravenously produce functional recovery in stroke”, *Cell Transplantation* 12, 449-454; 2003
*Arvidsson A et al.; “Neuronal replacement from endogenous precursors in the adult brain after stroke”; *Nature Medicine* 8, 963-970; Sept 2002
*Li Y et al.; “Human marrow stromal cell therapy for stroke in rat”; *Neurology* 59, 514-523; August 2002
*Chen J et al.; “Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats”; *Stroke* 32, 2682-2688; November 2001

**Spinal Cord Injury**—Adult stem cells capable of re-growth and reconnection in spinal cord. Clinical trials started in Portugal.

*Hofstetter CP et al., “Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery”, *Proc Natl Acad Sci USA* 99, 2199-2204; 19 February 2002
*Sasaki M et al., "Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons," *Glia* 35, 26-34; July 2001
Adult stem cells effective in tissue repair

Diabetes—Pancreatic, liver, intestinal, spleen or bone marrow cells can form insulin-secreting islets. FDA approval of first clinical trial.

*Oh S-H et al., “Adult bone marrow-derived cells transdifferentiating into insulin-producing cells for the treatment of type I diabetes,” Laboratory Investigation published online 22 March 2004
*Kodama S et al., “Islet regeneration during the reversal of autoimmune diabetes in NOD mice”, Science 302, 1223-1227; 14 Nov 2003
*Steptoe RJ et al.; “Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes”; Journal of Clinical Investigation 111, 1357-1363; May 2003
*Suzuki A et al.; “Glucagon-like peptide 1 (1-37) converts intestinal epithelial cells into insulin-producing cells”; Proc Natl Acad Sci USA 100, 5034-5039; 29 April 2003
*Ianus A et al.; In vivo derivation of glucose competent pancreatic endocrine cells from bone marrow without evidence of cell fusion; Journal of Clinical Investigation 111, 843-850; March 2003
*Abraham et al.; “Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells”; Endocrinology 143, 3152-3161; Aug 2002
*Yang L et al.; “In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells”; Proceedings of the National Academy of Sciences USA, 99, 8078-8083; 11 June 2002
Adult stem cells effective in tissue repair

Heart Damage—Bone marrow, muscle, and heart stem cells repair heart.

*Dawn B et al., “Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function”, *Proceedings of the National Academy of Sciences USA* 102, 3766-3771, 8 March 2005

*Yoon Y-s et al., “Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction”, *Journal of Clinical Investigation* 115, 326-338, February 2005

**Wollert KC et al., “Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial”, *Lancet* 364, 141-148, 10 July 2004


**Perin EC et al.; “Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure”; *Circulation* 107, r75-r83; published online May 2003

**Stamm C et al.; “Autologous bone-marrow stem-cell transplantation for myocardial regeneration”; *The Lancet* 361, 45-46; 4 January 2003

**Tse H-F et al.; “Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation”; *The Lancet* 361, 47-49; 4 January 2003

**Strauer BE et al.; “Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans”; *Circulation* 106, 1913-1918; 8 October 2002


Adult stem cells effective in tissue repair

Parkinson’s Disease—Neural stem cells can form all neuronal types, migrate throughout brain to repair damage, and prevent loss of neurons associated with Parkinson’s disease.
* Liker MA et al.; “Human neural stem cell transplantation in the MPTP-lesioned mouse”; *Brain Research* 971, 168-177; May 2003
* Åkerud P et al.; “Persephin-overexpressing neural stem cells regulate the function of nigral dopaminergic neurons and prevent their degeneration in a model of Parkinson’s disease”; *Molecular and Cellular Neuroscience* 21, 205-222; Nov 2002
* Ourednik J et al.; “Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons”; *Nature Biotechnology* 20, 1103-1110; Nov 2002

Using the patient’s own adult neural stem cells, a group at Los Angeles Cedars-Sinai Medical Center report a reversal of symptoms in the first Parkinson’s patient treated.
Lévesque M and Neuman T, “Autologous transplantation of adult human neural stem cells and differentiated dopaminergic neurons for Parkinson disease: 1-year postoperative clinical and functional metabolic result”, American Association of Neurological Surgeons annual meeting, Abstract #702; 8 April 2002

Injecting growth signals into the brain stimulates the patients’ own adult neural stem cells, provided a 61% improvement.
* Gill SS et al.; “Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease”; *Nature Medicine* 9, 589-595; May 2003 (published online 31 March 2003)
Current Clinical Uses of Adult Stem Cells

- **Cancers**—Lymphomas, multiple myeloma, leukemias, breast cancer, neuroblastoma, renal cell carcinoma, ovarian cancer
- **Autoimmune diseases**—multiple sclerosis, systemic lupus, rheumatoid arthritis, scleroderma, scleromyxedema, Crohn’s disease
- **Anemias** (incl. sickle cell anemia)
- **Immunodeficiencies**—including human gene therapy
- **Bone/cartilage deformities**—children with osteogenesis imperfecta
- **Corneal scarring**—generation of new corneas to restore sight
- **Stroke**—neural cell implants in clinical trials
- **Repairing cardiac tissue after heart attack**—bone marrow or muscle stem cells from patient
- **Parkinson’s**—retinal stem cells, patient’s own neural stem cells, injected growth factors
- **Growth of new blood vessels**—e.g., preventing gangrene
- **Gastrointestinal epithelia**—regenerate damaged ulcerous tissue
- **Skin**—grafts grown from hair follicle stem cells, after plucking a few hairs from patient
- **Wound healing**—bone marrow stem cells stimulated skin healing
- **Spinal cord injury**—clinical trials currently in Portugal, Italy, S. Korea
Diseases Treated in Human Patients
Regeneration Mechanism?
(evidence for all of these)

Dedifferentiation-Redifferentiation

Cell fusion with already-differentiated cell

Transdifferentiation

Stimulate Differentiation of Tissue Cells

“[Robert] Lanza noted ‘there is ample scientific evidence that adult stem cells can be used to repair damaged heart or brain tissue… if it works, it works, regardless of the mechanism,’ he said.”

“Study casts doubt on adult stem cells”, Steve Mitchell, UPI; 12 October 2003
Adult Stem Cells

Most promising source for treatments
Able to generate virtually all adult tissues
Can multiply almost indefinitely, providing numbers sufficient for clinical treatments
Proven success in laboratory culture
Proven success in animal models of disease
Proven success in current clinical treatments
Ability to “home in” on damage
Avoid problems with tumor formation
Avoid problems with transplant rejection
Avoid ethical quandary

www.stemcellresearch.org
GLOBAL VIEW

• France (7 yrs jail), Canada (5 yrs jail), Australia, Germany, Norway, Switzerland, et al.
  – All uses of human SCNT cloning banned

• United Nations February 2005–Declaration to prohibit all forms of human cloning
FEDERAL LEGISLATION

• **HHS Appropriations language** (since 1996)

SEC. 510.

(a) None of the funds made available in this Act may be used 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 under 45 CFR 46.208(a)(2) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).

(b) For purposes of this section, the term “human embryo or embryos” includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.