"Technology always goes forward.
There are radical new technologies that surprise us all the time.
And we've got a long time in the future to go.
This is my conclusion:
Human evolution will be self-driven."

Lee Silver, PhD, 3/98

Objectives: Gene Therapy

See lecture objectives on web
Read pages 311-327 (chapter 13) in text

- Germline vs. somatic gene therapy
- Gene therapy vectors (advantages and disadvantages):
  - Retrovirus
  - Adenovirus
  - Adeno-associated virus (AAV)
  - Non-viral vectors
- *in vivo* vs *ex vivo* gene therapy
- Current status of human gene therapy experimentation
- Stem cell therapy
- Pharmaceuticals produced by recombinant DNA technology

Early Human Gene Therapy Experiments

- Marty Cline human experiments-- 1980
- NeoR/TIL marking studies-- 1989
- ADA/peripheral blood T cells-- 1990
- LDL receptor/ex vivo hepatocytes-- 1992
- HLA-B7 Melanoma-- 1992
- ADA/bone marrow, cystic fibrosis, multiple cancer protocols, HIV

ZAP! LIGHTNING TURNS WOMAN INTO A MAN

Electrical charge destroys her genes

ZAP

Electrical bolt destroys her chromosomes

Lightning turns woman into man

Trauma

Plummeting

Continues

ADA Deficiency

- Rare Immunodeficiency (fatal in childhood)
- Advantages as model for gene therapy:
  - Regulated expression not necessary
  - Low level expression sufficient
  - Site of synthesis not critical
  - Potential for *in vivo* selection
  - Bone marrow suitable target
- Problems:
  - Difficulty achieving high level, stable expression
  - Other effective therapy:
    - PEG-ADA therapy, allogeneic/haploidentical BMT
- First human experiments performed 1991 (2 patients)
  - ?successful; simultaneous PEG-ADA therapy

Gene Therapy in the News

- October 1999-- 1st reported death due to gene therapy
- November 1999-- Failure of scientists to report gene therapy trial deaths to FDA/RAC
- April 2000-- 1st definite success of human gene therapy (SCID-X1) Cavazzana-Calvo, et al. *Science* 288:669.
- Factor IX gene therapy (hemophilia B) ? promising
  - In vivo AAV: Kay et al. *Nat.Gen.* 24:257, 2000.
  - Ex vivo fibroblast: Roth et al. *NEJM* 344:1735, 2001.

Heard at the Genetics Clinic:

"Can you take out the bad gene?"

"Can you fix that gene?"

"Can you remove the extra chromosome?"

"By the time my daughter gets the disease, will be there be gene therapy to treat it, or at least to her babies"

"Are doctors working on gene therapy for this?"

Concerns about Genetic Engineering

The Council for Responsible Genetics

"in utero gene therapy efforts will result in eugenic practices"

Mothers for Natural Law

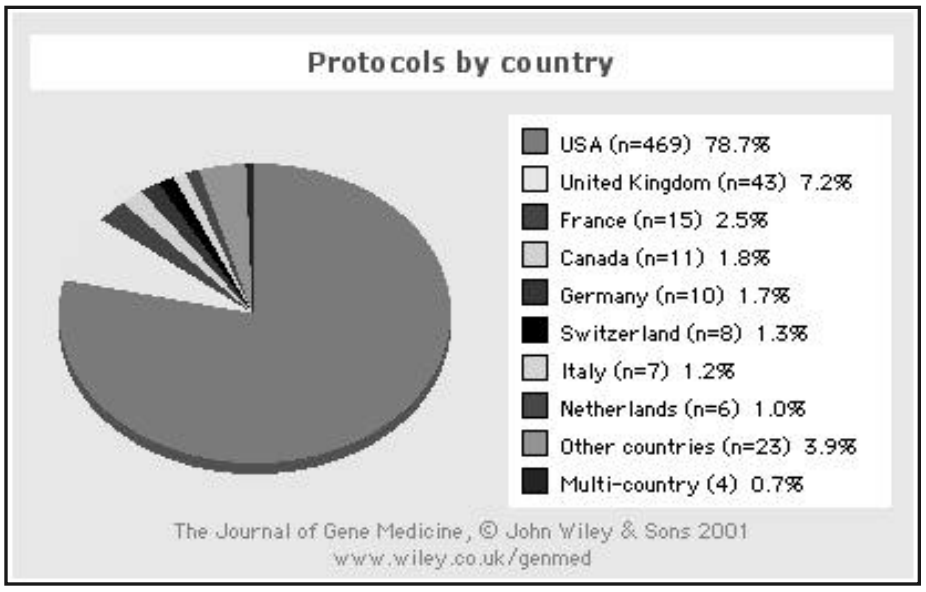
"fundamental weaknesses of genetic concepts and health hazards"

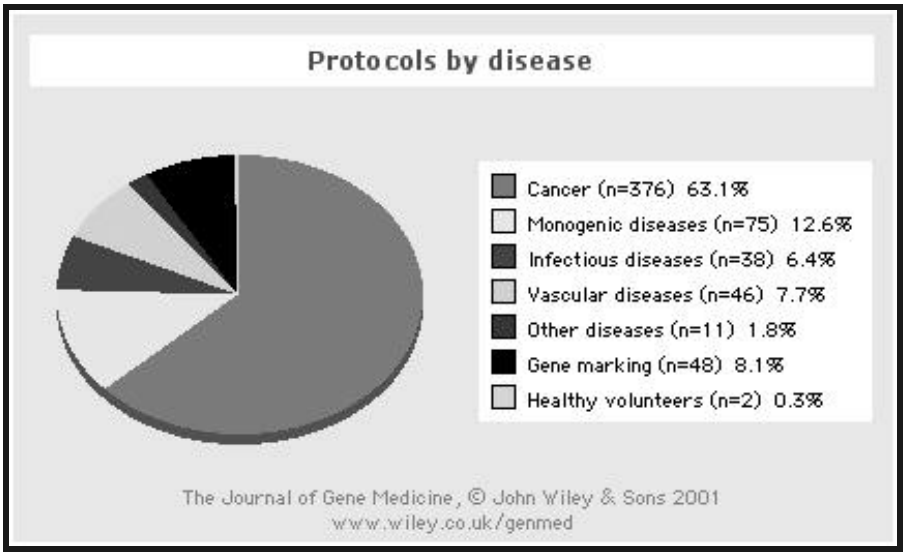
Washington Biotechnology Action Council

"Genetic engineering is a big business...major decisions are made in the boardrooms of corporations and by a handful of scientists and gene-splicing entrepreneurs…critical information is hidden from the public"

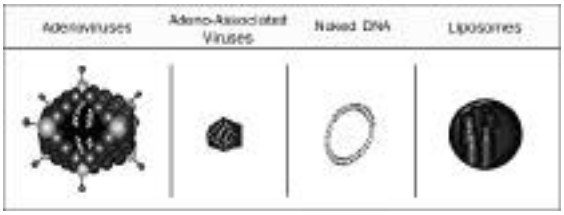
Physicians and Scientists for Responsible Application of Science and Technology

"We demand a global moratorium on the release of genetically engineered organisms and on the use of genetically engineered foods…..there are reasons to expect potentially serious hazards..."





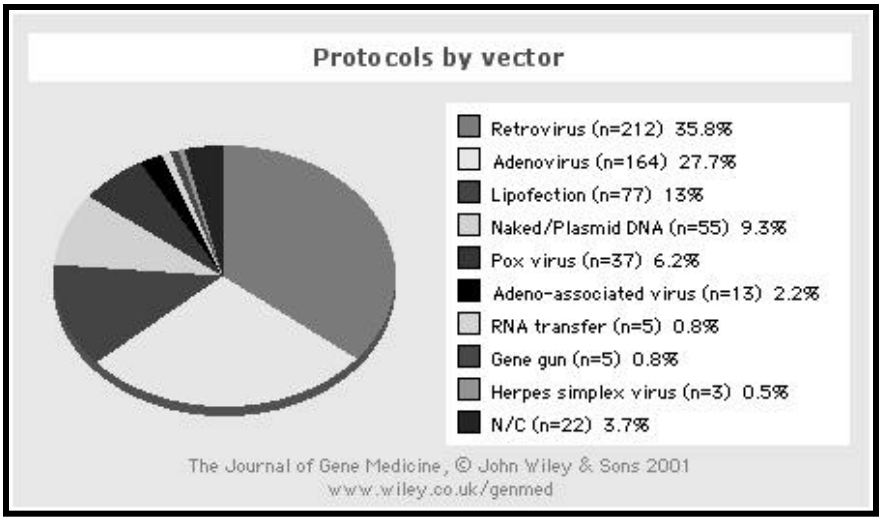
Gene Transfer Methods



- Retroviral vectors
  - Lentiviruses
- Adenovirus
- Adeno-associated virus (AAV)
- Other viral vectors
  - Vaccinia – Hepatitis virus
  - Herpes virus – Polio virus
  - Papilloma virus – Sindbis and other RNA viruses
- Non viral methods
  - Ligand-DNA conjugates – Adenovirus- ligand-DNA
  - Lipofection – Direct DNA injection
  - CaPO4 precipitation – Ribozymes
  - chimeric oligo/gene correction

Problems

- Delivery of DNA
- Achieving high level expression
- Maintaining stable expression
- Tissue-specific expression
- *in vivo* regulation



Retroviral Vectors

- Replace viral genes with therapeutic gene
  - Limited size (<8 kb)
- Limited cell targets
  - Require dividing cells
  - Specific cellular receptors
- High efficiency (1 virus/cell)
- Stable integration into genome
  - Potential for insertional mutagenesis

Adenovirus Vectors

- Respiratory diseases in man
  - type 2 and 5
- ~36 kb, linear, double stranded DNA
  - Early genes (E1-E4)
  - Late genes (L1-L5)
- Replication deficient viruses-
  - Delete E1a and part of E1b
  - grow on Ad transformed cell line (293), which contains E1 region and complements in *trans*
  - Infect target cell, but no replication
- Infects broad range of cells
  - liver
  - lung
  - muscle
  - CNS
  - endothelial cells
  - others

In addition to being safe and cost-effective, the most important properties of an efficacious gene transfer system will be;

1) target cell selective.

2) transcriptionally competent for the desired length of time.

3) available in a highly concentrated active form.

4) immunologically neutral.

Gene Therapy Vectors

Vector	Advantages	Disadvantages
Retrovirus	High efficiency transduction of appropriate target cells. Long-term expression-integration into chromosomal DNA).	Potential for insertional mutagenesis. Requires dividing cells. Limited size of DNA insert.
Adenovirus	High transduction efficiency. Broad range of target cells. Does not require cell division. Low risk of insertional mutagenesis.	Transient expression. Immunogenicity. Direct cytopathic effects of virus.
Adeno-associated virus (AAV)	Does not require cell division. ? Site specific integration.	Potential for insertional mutagenesis if integration not site-specific. Limited size of DNA insert.
Non-viral vectors	No infectious risk. Completely synthetic. No limitation on insert size.	Low efficiency. Limited target cell range. Transient expression.

Human Genetic Modifications

Somatic or Germline

Therapy or Enhancement

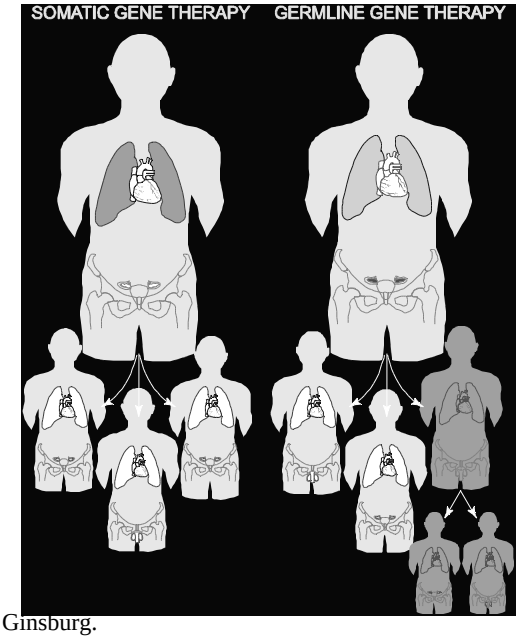
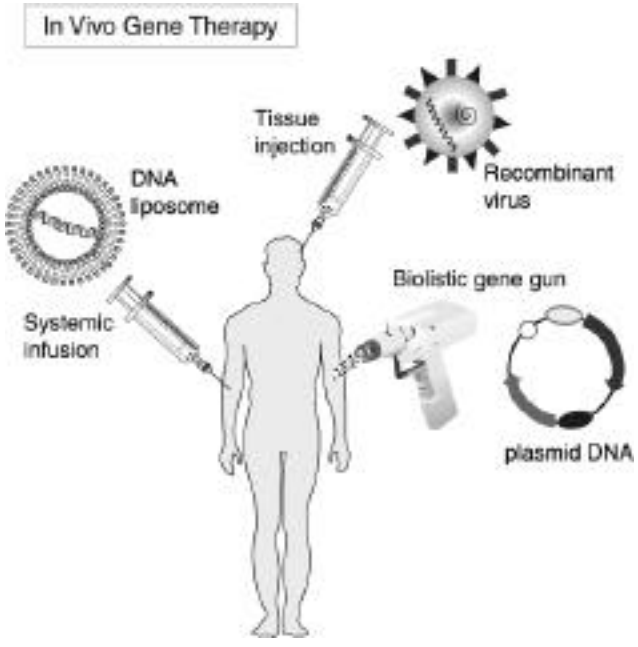


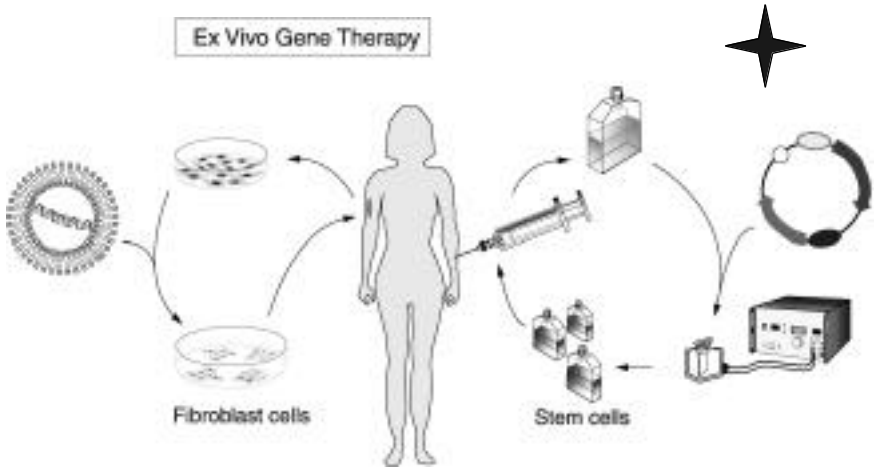
Figure 13.1
TD Gelehrter, FS Collins, D Ginsburg.
Principles of Medical Genetics. 1997.

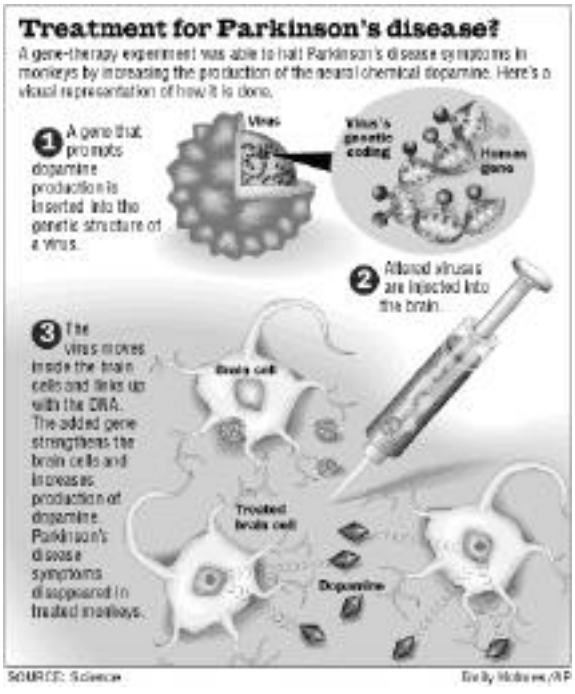
Somatic Gene Therapy

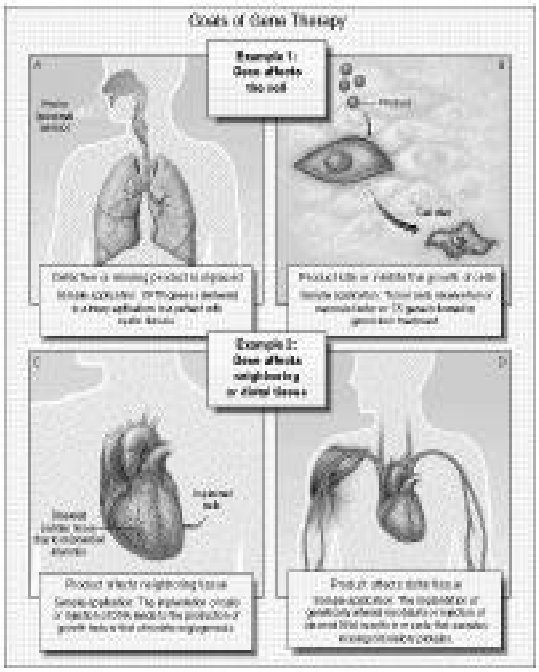
Treatment of human diseases by gene transfer

- transfer of DNA to somatic cells
  - ex vivo or in vivo
- no effect on germline
- usually targeted to specific organ/tissue




Ex Vivo Gene Therapy
Fibroblast cells
Stem cells

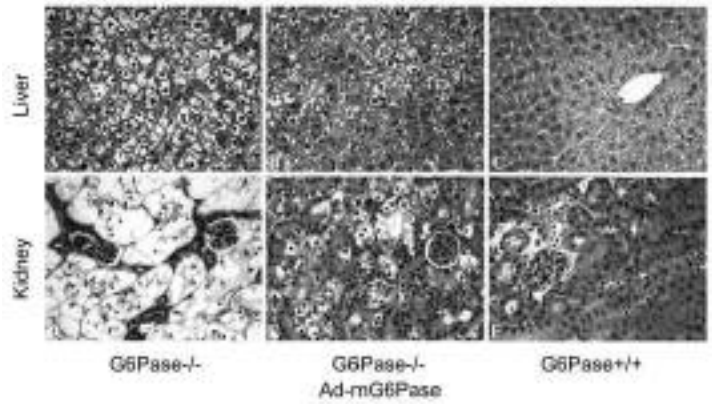

Treatment for Parkinson's disease?
A gene-therapy experiment was able to halt Parkinson's disease symptoms in monkeys by increasing the production of the neurochemical dopamine. Here's a visual representation of how it is done.
1 A gene that promotes dopamine production is inserted into the genetic structure of a virus.
Virus
Virus's genetic coding
Human gene
2 Altered viruses are injected into the brain.
3 The virus moves inside the brain cells and links up with the DNA. The added gene strengthens the brain cells and increases production of dopamine. Parkinson's disease symptoms disappeared in treated monkeys.
Brain cell
Treated brain cell
Dopamine
SOURCE: Science



Blau &Springer. ***NEJM*** 333:1204, 2000.

Glycogen storage disease type 1 (GSD-1)

Cause:
AR deficiency of G6Pase and glucose-6-phosphatase (G6Pase) system glucose-6-phosphate transporter (G6PT) cause GSD-1a and GSD-1b, respectively.
Features:
growth retardation, hypoglycemia, hepatomegaly, kidney enlargement, hyperlipidemia, hyperuricemia, and lactic acidemia. GSD-1b also have chronic neutropenia, functional deficiencies of neutrophils and monocytes, recurrent bacterial infections, ulcerations of the oral and intestinal mucosa



Disease	Target cells	Transfected gene(s)
Hemophilia A Hemophilia B	liver, muscle, bone marrow cells, fibroblasts	Factor VIII Factor IX
Familial hypercholesterolaemia	liver	LDL receptor
Severe combined immunodeficiency	bone marrow cells, T cells	Adenosine deaminase (ADA)
Hemoglobinopathies	red blood precursor cells	a-globin, b-globin
Cystic fibrosis	lung airway cells	CFTR
Gaucher disease	bone marrow macrophages	cellsglucocerebrosidase
Cancer	tumor cells	p53, Rb, interleukins growth-inhibitory genes apoptosis genes

Good justification for using this ex vivo gene therapy approach for hemophilia A (factor VIII):

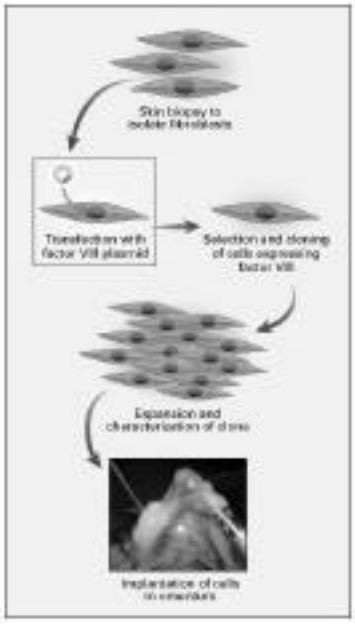
- Factor VIII production is not regulated in response to bleeding

- Only need to raise levels a little bit, not to 100%, as low levels of the Factor VIII can be beneficial to the patient

- Broad therapeutic index of factor VIII minimizes risk of overdose

- Delivery of factor VIII into the bloodstream does not require cell-specific expression

-



NEJM
(2001)
341:1735-1742

TABLE 1. CHARACTERISTICS OF THE SIX PATIENTS.*

CHARACTERISTIC	VALUE
Age (yr)	
Mean	46
Range	20–72
Weight (kg)	
Mean	70
Range	50–91
Pretreatment factor VIII activity <0.8% of normal (no. of patients)	6
Viral exposure (no. of patients)†	
Human immunodeficiency virus	4
Hepatitis A virus	5
Hepatitis B virus	5
Hepatitis C virus	6

*All six patients were men.

†Viral exposure was determined at the time of enrollment by testing for the presence of antibodies to the viruses listed.

TABLE 2. TOTAL FACTOR VIII PRODUCTION BY IMPLANTED AUTOLOGOUS FIBROBLASTS.*

PATIENT NO.	FACTOR VIII PRODUCTION BY HARVESTED CELLS†	NO. OF CELLS IMPLANTED	TOTAL FACTOR VIII PRODUCTION BY IMPLANTED CELLS‡
	IU/10⁶ cells/day		IU/kg/day
1	0.8	100×10^6	1.3
2	4.9	100×10^6	5.4
3	1.9	100×10^6	3.8
4	1.8	400×10^6	10.4
5	1.6	400×10^6	8.4
6	6.7	400×10^6	36.0

*The conditioned medium of each fibroblast clone was replaced with fresh medium 24 hours before it was assayed for factor VIII expression levels by a human factor VIII enzyme-linked immunosorbent assay.

†The production of factor VIII at the time of cell harvest, before implantation, is shown.

‡The total factor VIII production of each implanted clone is shown, normalized for the weight of each patient.

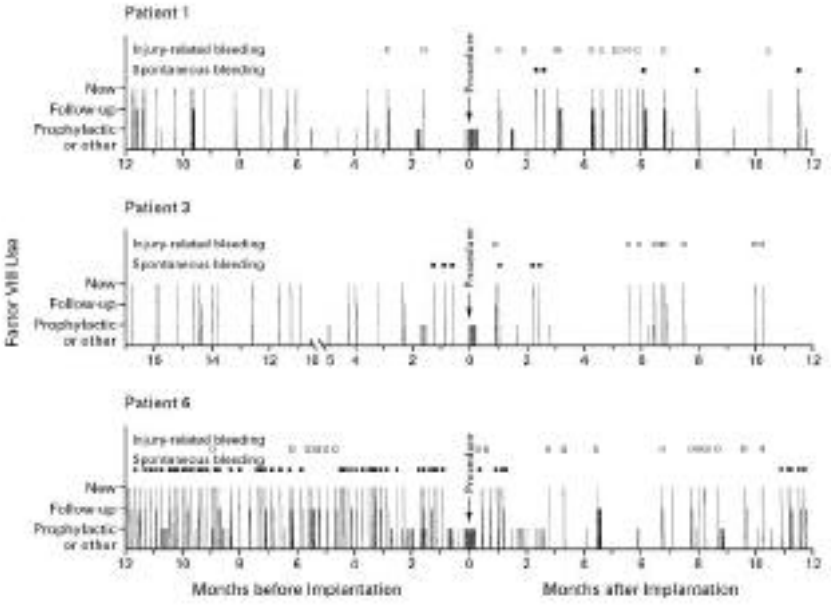
TABLE 3. LEVELS OF FACTOR VIII ACTIVITY.*

TIME POINT	PATIENT					
	1	2	3	4	5	6
	% of normal level					
Initial evaluation	<0.8	<0.8	<0.8	<0.4	0	0.5
Day before cell implantation	<0.8 (2 to <4 days)	<0.8	<0.4	<0.4	<0.4 (2 to <4 days)	4.0 (1 to <2 days)
Week 2	<0.8	<0.8	<0.4	<0.8	3.0 (1 to <2 days)	10† (<1 day)
Week 3	<0.8	<0.4	<0.8	<0.4	2.0 (2 to <4 days)	2.0†
Week 4	<0.8	<0.4	10.0 (<1 day)	0.8†	1.0†	8.0†
Week 6	<0.8	0.8 (2 to <4 days)	<0.4	0.6†	18.0 (<1 day)	1.0†
Week 8	<0.8	<0.4	<0.4	<0.5	1.0†	5.0 (1 to <2 days)
Week 12	<0.8	<0.4	<0.4	<0.4	1.0†	4.0†
Week 18	<0.4	<0.4	2.0†	<0.5	0.5†	1.0†
Month 6	<0.4	0.7	1.0†	<0.5	1.0†	2.0†
Month 9	—	—	—	—	1.0 (1 to <2 days)	<0.5
Month 12	0.5	<0.5	<0.5	0.5†	<0.5	<0.5

*Some measurements of factor VIII activity levels were most likely influenced by previous infusions of exogenous factor VIII. The interval between measurement and the most recent infusion of factor VIII is given in parentheses. Otherwise, the factor VIII activity level was determined at least five days after a previous factor VIII infusion. Factor VIII activity levels were measured at scheduled visits: weeks 2 and 3 (±1 day); weeks 4 and 6 (±2 days); weeks 8 and 12 (±4 days); and week 18 and months 6, 9, and 12 (±2 weeks). Dashes indicate that measurement of factor VIII activity was not performed.

†The factor VIII activity level is considered elevated above levels measured before implantation.

)01.



Cautions Related to Somatic Cell Gene Therapy

- Early trials often limited to desperate situations (fatal childhood illnesses, cancer); patients/parents will "try anything"
- Media "hype" may lead to false hopes and fears
- Future long term benefits and unanticipated risks difficult to judge from animal experiments, especially in healthy individuals
- Truly informed consent may be difficult to obtain given the lack of general genetic knowledge in the public and gravity of some situations

9/17/1999
Jesse Gelsinger, 18 yo
High school graduate with OTC deficiency, died participating in a gene therapy experiment at the University of Pennsylvania in Philadelphia,

Somatic Cell Gene Therapy

- Ethical considerations similar to those related to use of any novel therapeutics
- Benefits should outweigh risks
- Allocation of resources should be fair
- Patients should understand benefits, risks, potential outcomes with and without treatment, limitations, and alternative therapies

Germline gene therapy

- transfer of DNA into germline
- transmitted to subsequent generations
- routinely applied in animals (transgenic/ES)
- Moral/ethical/legal issues in humans

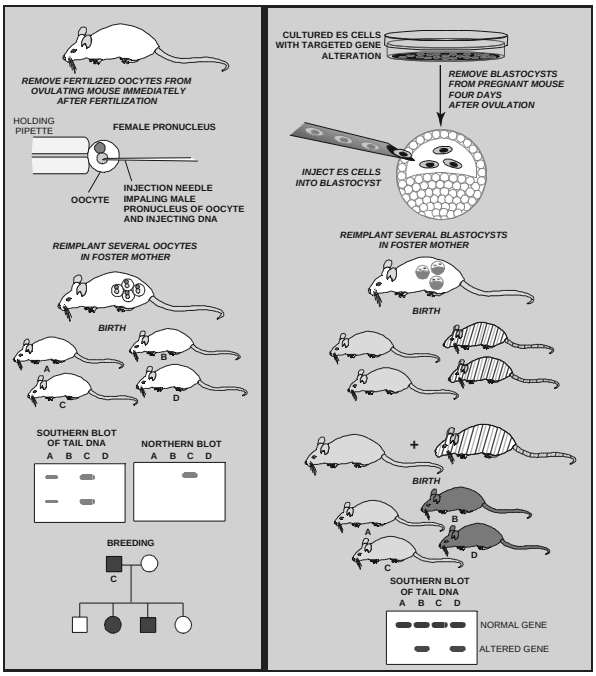


Figure 5.27
TD Gelehrter, FS Collins, D Ginsburg. **Principles of Medical Genetics.** 1997.

Arguments For Germline Gene Therapy

- Medical utility - the potential of a true “cure”
- Medical necessity - may be only way to cure some diseases
- Prophylactic efficacy - better to prevent a disease rather than to treat pathology
- Parental autonomy - parents can make choices about what is best for their children
- Easier, more effective, and less risky than somatic gene therapy
- Eradication of disease in future generations
- Foster scientific knowledge
- Part of being human - supporting human improvement

"I'm absolutely for it [germline gene therapy] on the most fundamental of grounds. And that's the grounds of human nature… Germline gene therapy will be done because of human nature. None of us wants to pass on to our children lethal genes if we can prevent it."

W. French Anderson 3/98
Director of Gene Therapy Laboratories, USC

Arguments Against Germline Gene Therapy

- Slippery slope - leads to misuse and abuse "eugenics"
- Lack of informed consent - fetus/embryo cannot consent
- Unknown/unforeseeable risks to individual, their offspring
- Violates genetic integrity of future generations
- Less "risky" alternatives exist
- Too costly - poor/misguided use of scarce resources
- Should not be attempted until more success in somatic gene therapy
- Will widen the gap between the haves and have-nots
- Devalues sense of "humanness"
- Is playing god

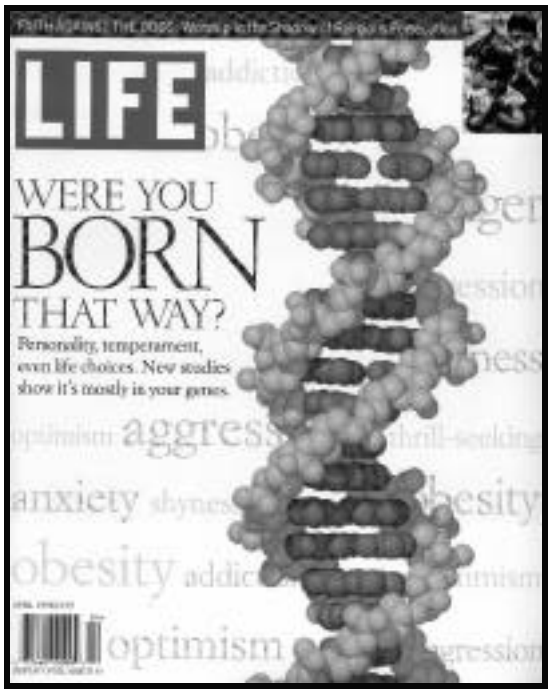
Caution for Somatic Cell Genetic Enhancement?

- Cost of development difficult to justify
- Equal allocation of resources unlikely; utilization by some may adversely impact others
- Media "hype" may lead to false hopes, optimism and fears
- Future long term benefits and risks in "healthy" individuals may be even more difficult to judge
- Truly informed consent may be difficult to obtain in "competitive" societies
- Parents may not be able to give informed consent for children

How is it different that enhancement therapies done today?

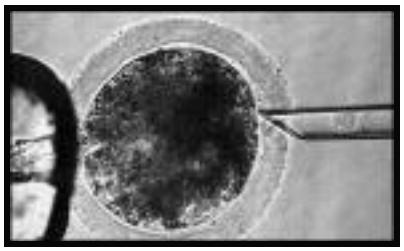
"It is the prospect of genetic engineering that helps us appreciate what it means to be human:
It means to be mortal,
to be imperfect,
and to be flawed.
It also means to wish to be better"

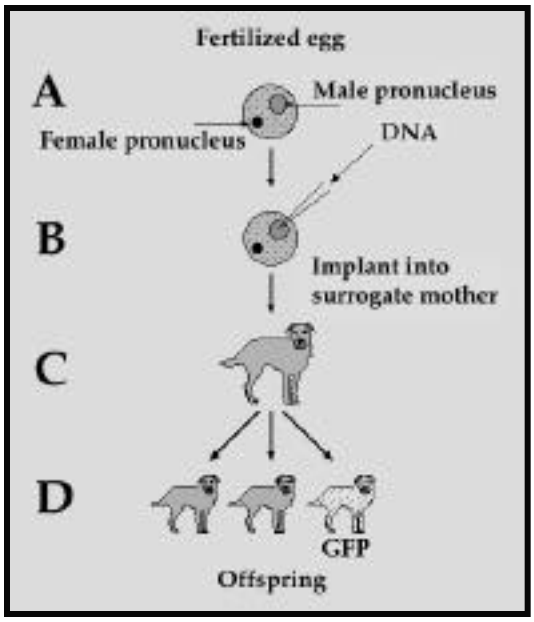
Allen R. Dyer, 1997
"The Ethics of Human Genetic Intervention: A Postmodern Perspective"



Germline Genetic Enhancement

- Most problematic area to consider and clearly the area which raises the most public concern
- Additional ethical issues need to be addressed related to:
  - Impact on individual
  - Impact on society
  - Impact on future societies
  - Costs and benefits
  - Allocation of resources
  - Prevention of misuses, abuses
  - Informed consent issues



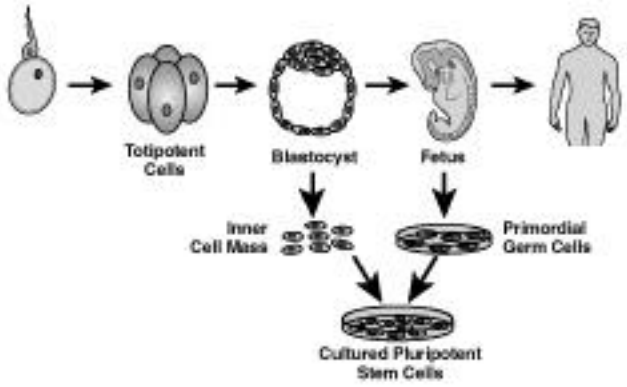


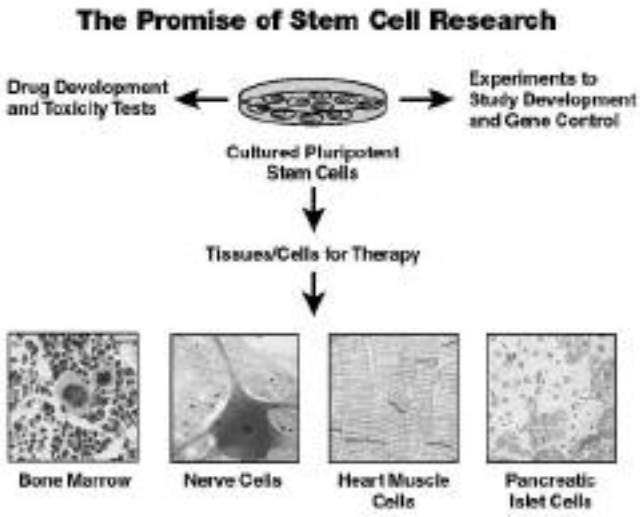
Alternatives to Gene Therapy

- Conventional transplantation
  - Bone marrow
  - Other tissues (islets)
- Implantable bioreactors
- Infusion of recombinant proteins
  - Continuous pump
  - Implantable devices
- Other novel pharmaceuticals
- Prenatal diagnosis and prevention

Pharmaceuticals Produced by Recombinant DNA Technology

Recombinant Product	Disease Target
• Human insulin	• Diabetes
• Growth hormone	• Growth hormone deficiency
• Recombinant factor VIII	• Hemophilia A
• Tissue plasminogen activator	• MI and stroke
• Erythropoietin	• Anemia
• G-CSF	• Neutropenia following chemotherapy
• Hepatitis B vaccine	• Prevention of hepatitis B
• α interferon	• Hairy cell leukemia,chronic hepatitis
• β interferon	• Multiple sclerosis
• γ interferon	• Infections in chronic granulomatous disease patients

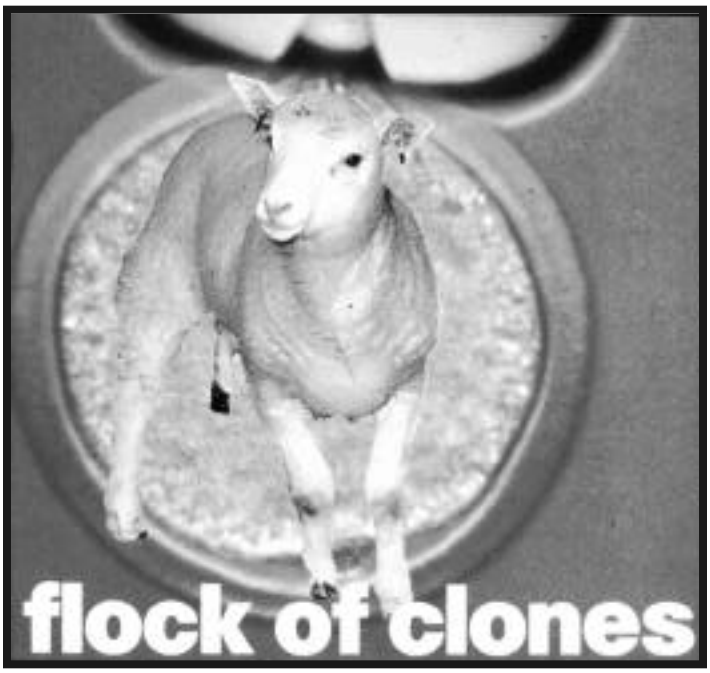


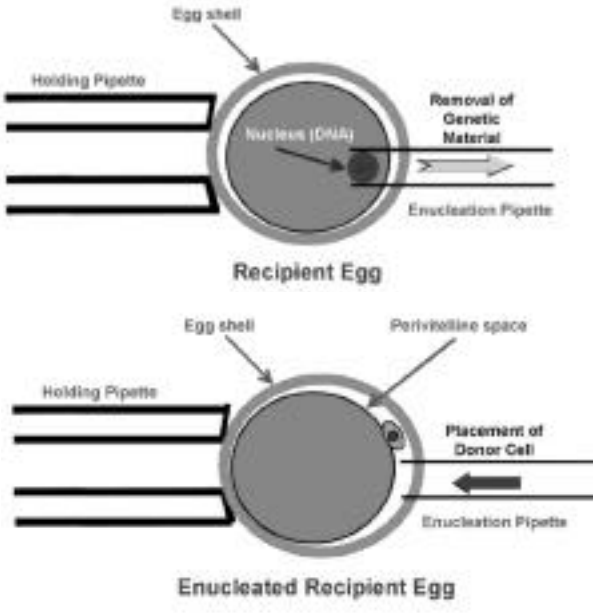


History of Genetic Discoveries

- 1997 Dolly is cloned from a non-reproductive cell of an adult sheep, marking the first of several successful cloning experiments involving mammals

Dr. James, Dr. Campbell and Dr. Wilmut
cloned Dolly the sheep


flock of clones


Egg shell
Holding Pipette
Nucleus (DNA)
Removal of Genetic Material
Enucleation Pipette
Recipient Egg
Egg shell
Perivitelline space
Holding Pipette
Placement of Donor Cell
Enucleation Pipette
Enucleated Recipient Egg

12/22/01
Genetic Savings & Clone
announced the birth of
"CC - the world's first cloned cat"

Texas A&M University
Operation CopyCat, (part of the Missyplicity Project),

How much do we value science and technology advances?

How do we view quality of human life in past, present, and future generations?

How can we comfortably merge our desire for scientific advances with our respect for human life and diversity within our own value system and ethical frameworks?

Key Concerns and Related Ethical Concepts

- Safety (Nonmaleficence)
- Efficacy (Beneficence)
- Informed Consent (Autonomy)
- Allocation of Resources (Justice and Equity)
- Respect for Human Dignity

Review: Gene Therapy

- Germline vs. somatic gene therapy
- Gene therapy vectors (advantages and disadvantages):
  - Retrovirus
  - Adenovirus
  - Adeno-associated virus (AAV)
  - Non-viral vectors
- *in vivo* vs *ex vivo* gene therapy
- Current status of human gene therapy experimentation
- Pharmaceuticals produced by recombinant DNA technology

".. if we could make better human beings by knowing how to add genes, why shouldn't we do it?"

- James Watson, 3/98