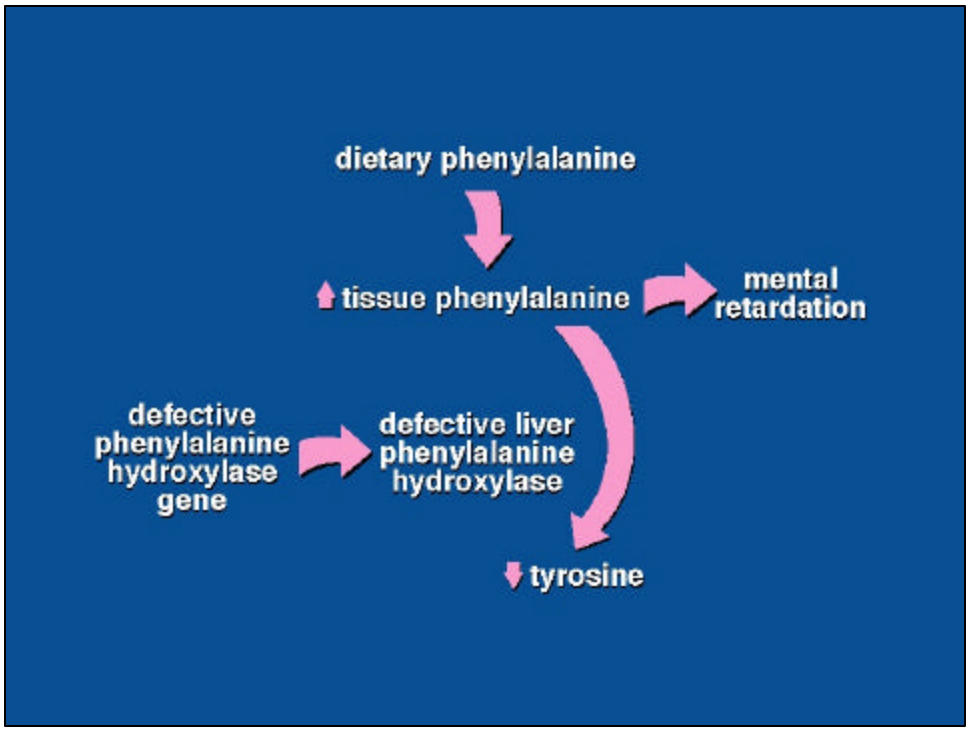
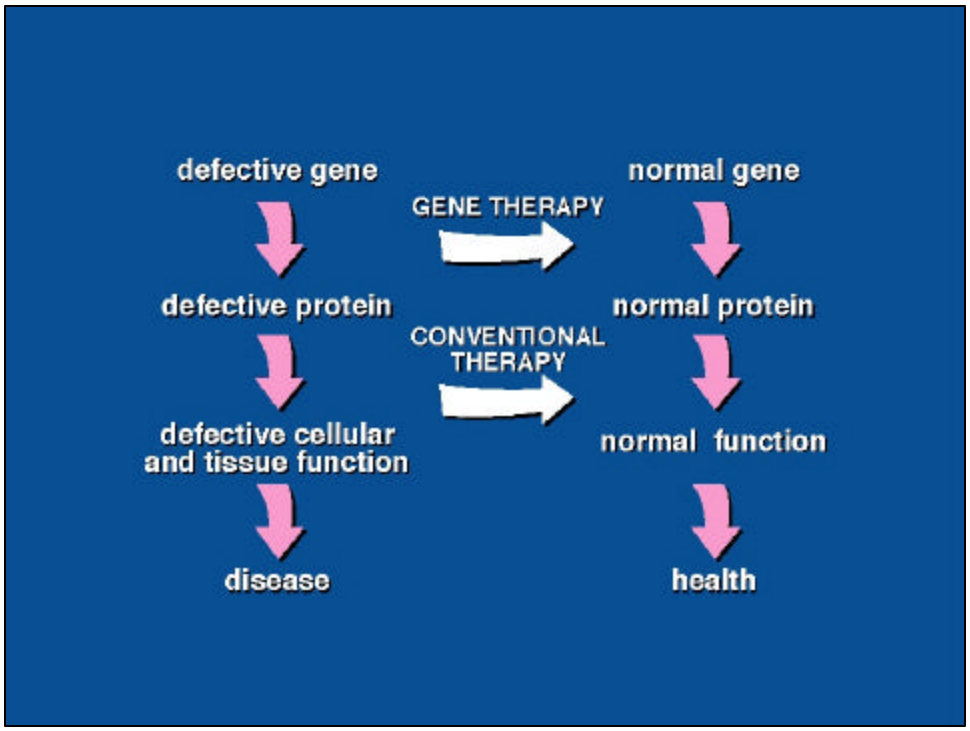
GENE THERAPY:
TREATMENT OR PREVENTION OF EVERYTHING

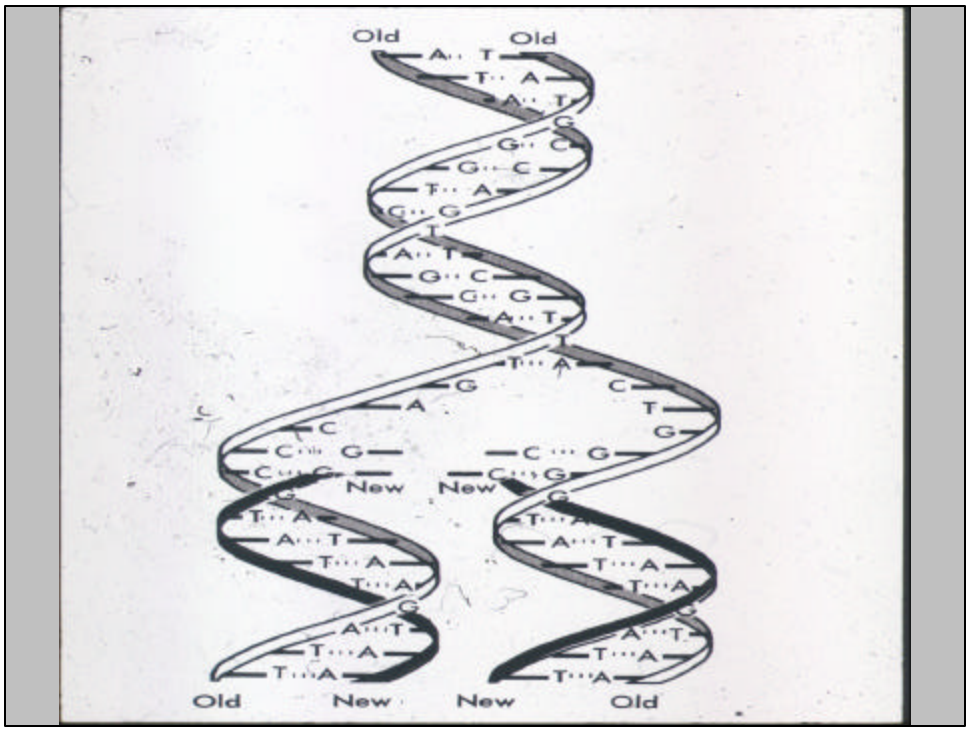
Or at least of many things

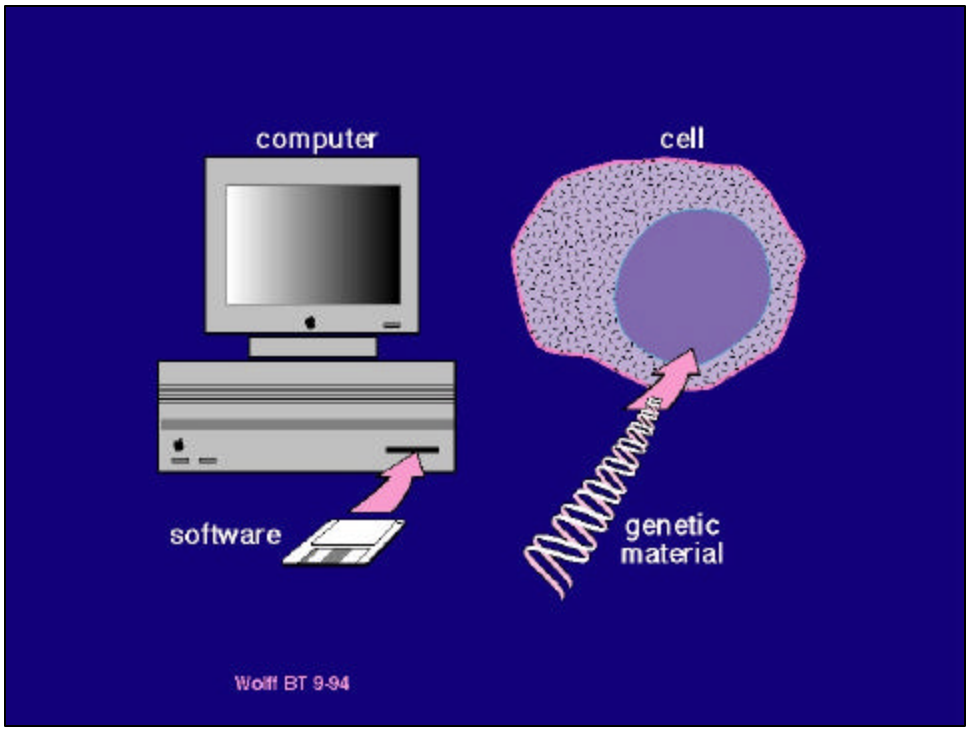
GENE → PROTEIN

BAD GENE → BAD PROTEIN Or NO PROTEIN

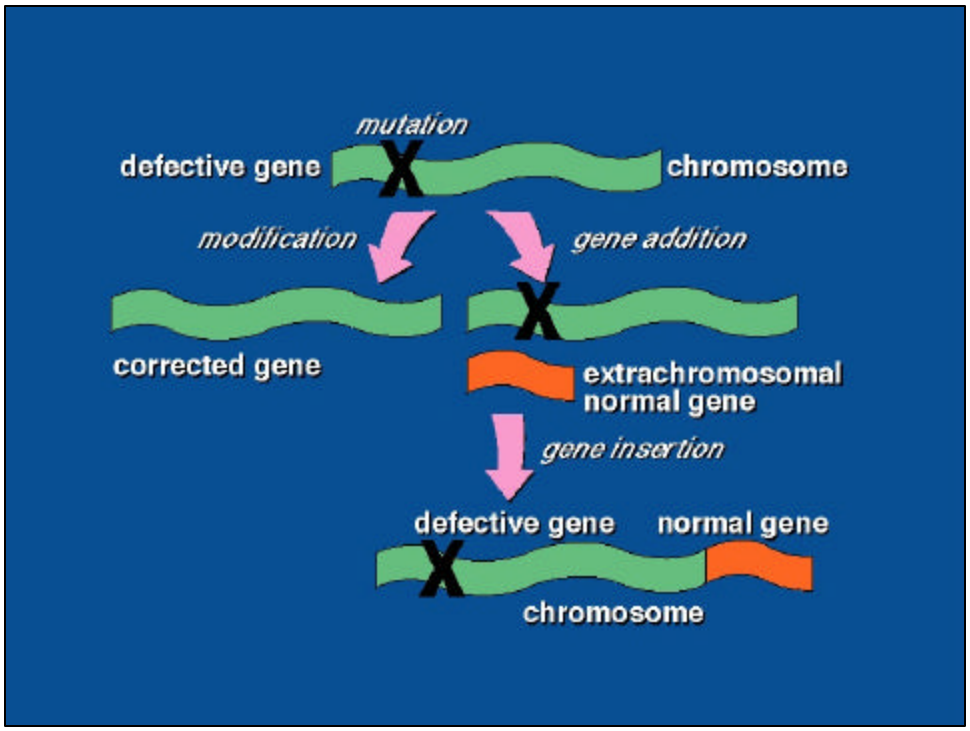



defective gene
GENE THERAPY
normal gene
defective protein
normal protein
CONVENTIONAL THERAPY
defective cellular and tissue function
normal function
disease
health


Old
Old
New
New
Old
New
New
Old



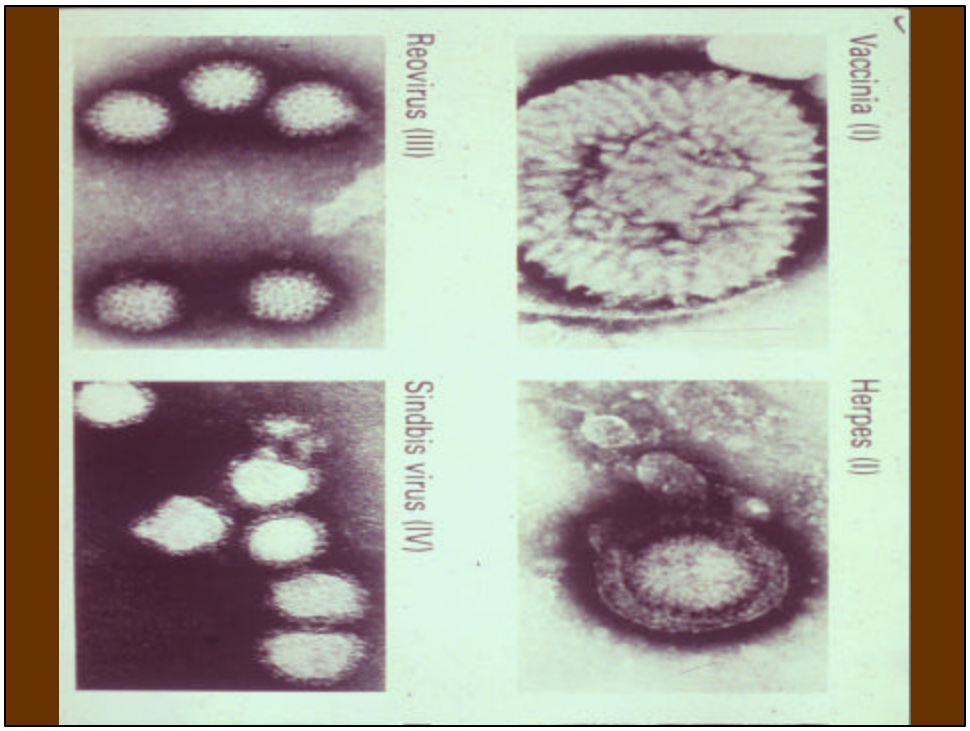
RECOMBINANT DNA TECHNOLOGY

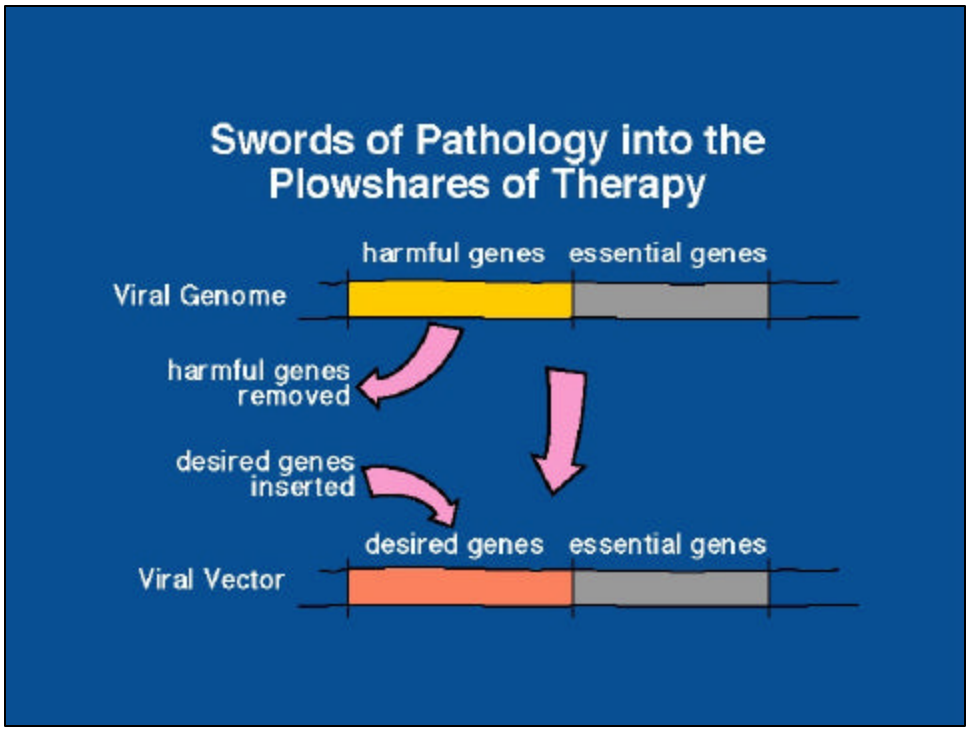


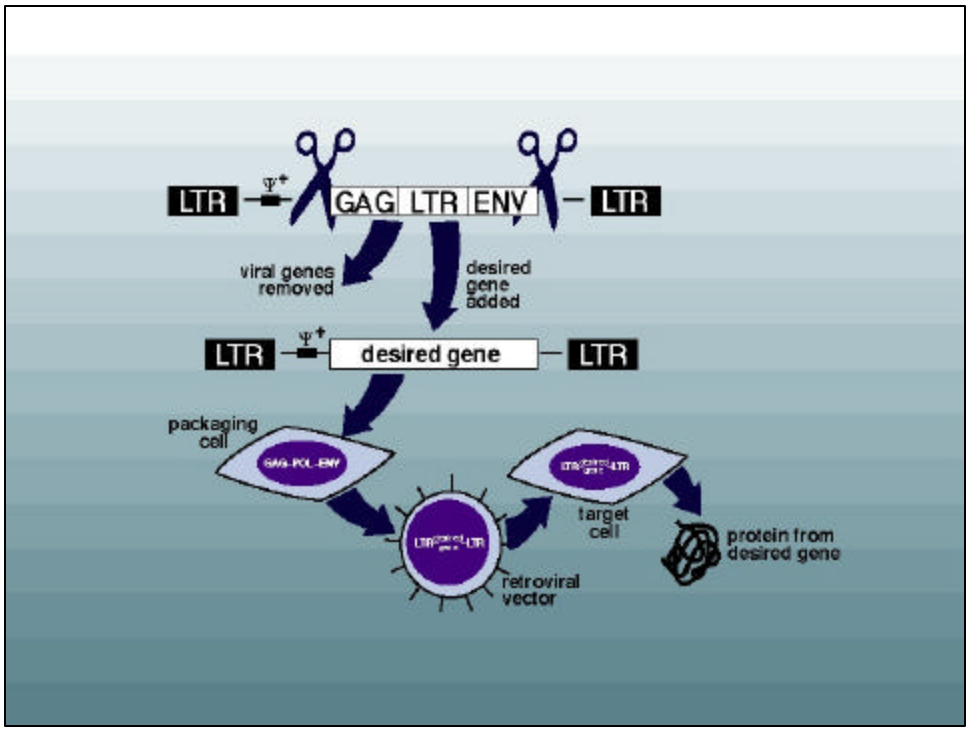
IN EVERY LARGE PROBLEM THERE IS A SMALL PROBLEM STRUGGLING TO GET OUT

AND

IN EVERY SMALL PROBLEM THERE IS A LARGE PROBLEM STRUGGLING TO GET OUT


Reovirus (III)
Vaccinia (I)
Sindbis virus (IV)
Herpes (I)

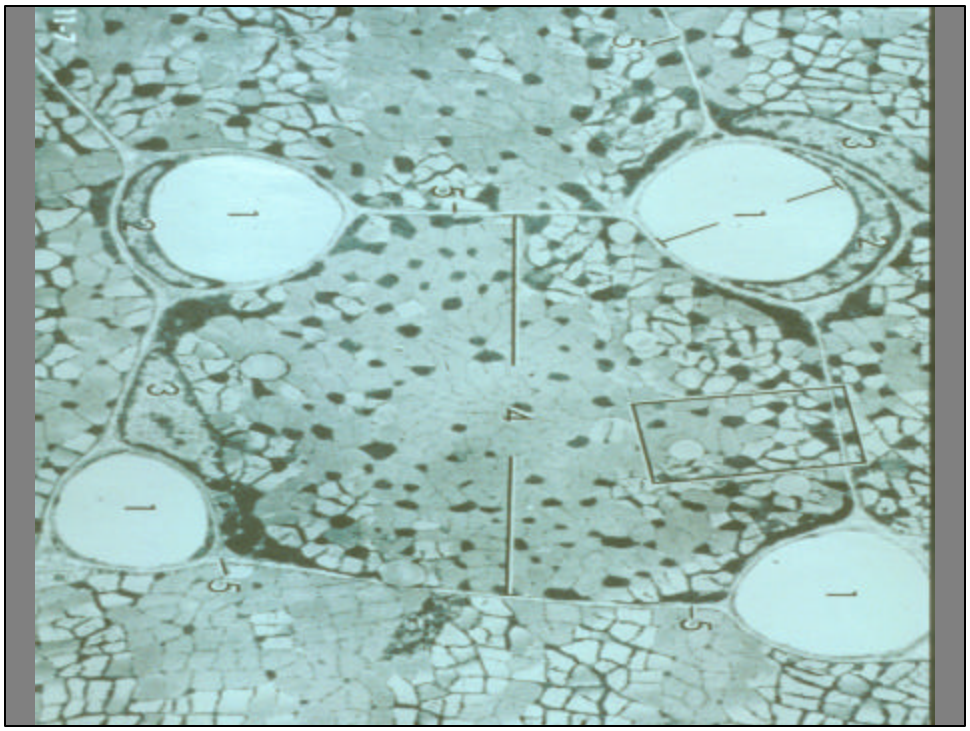

Swords of Pathology into the Plowshares of Therapy
harmful genes
essential genes
Viral Genome
harmful genes removed
desired genes inserted
desired genes
essential genes
Viral Vector

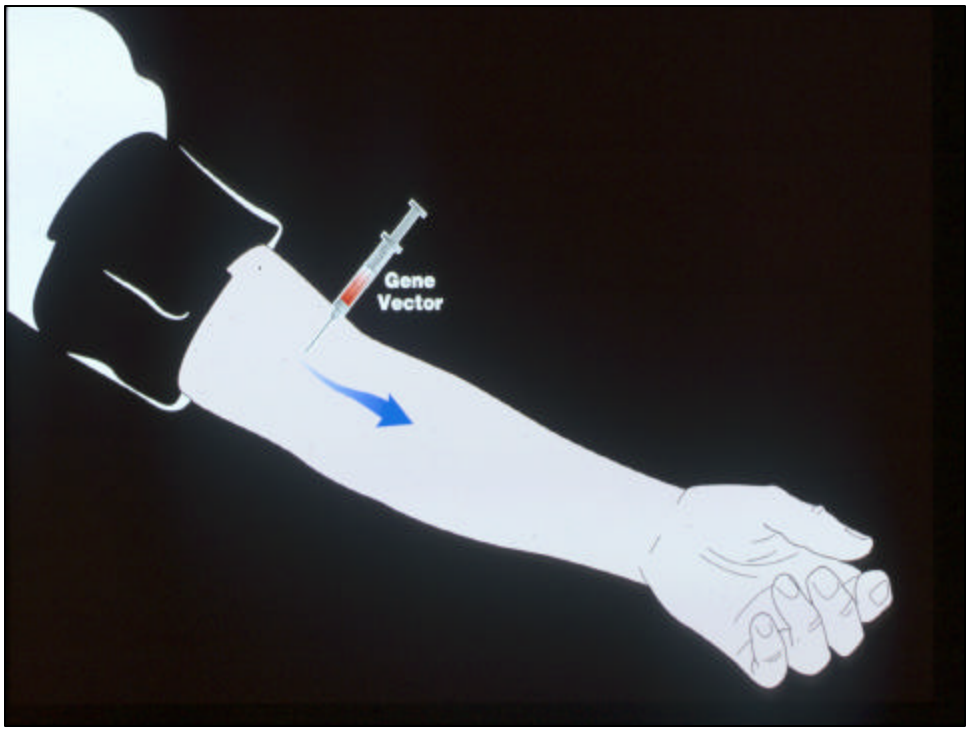


PHYSICAL-CHEMICAL APPROACHES FOR GENE TRANSFER AND GENE THERAPY

Physical-Chemical Transfection Methods for In Vitro Applications

- Calcium phosphate transfection
- DEAE dextran
- Liposomes (virosomes)
- Cationic lipids (lipofectin)
- Microparticle bombardment (gun)
- Microinjection
- electroporation



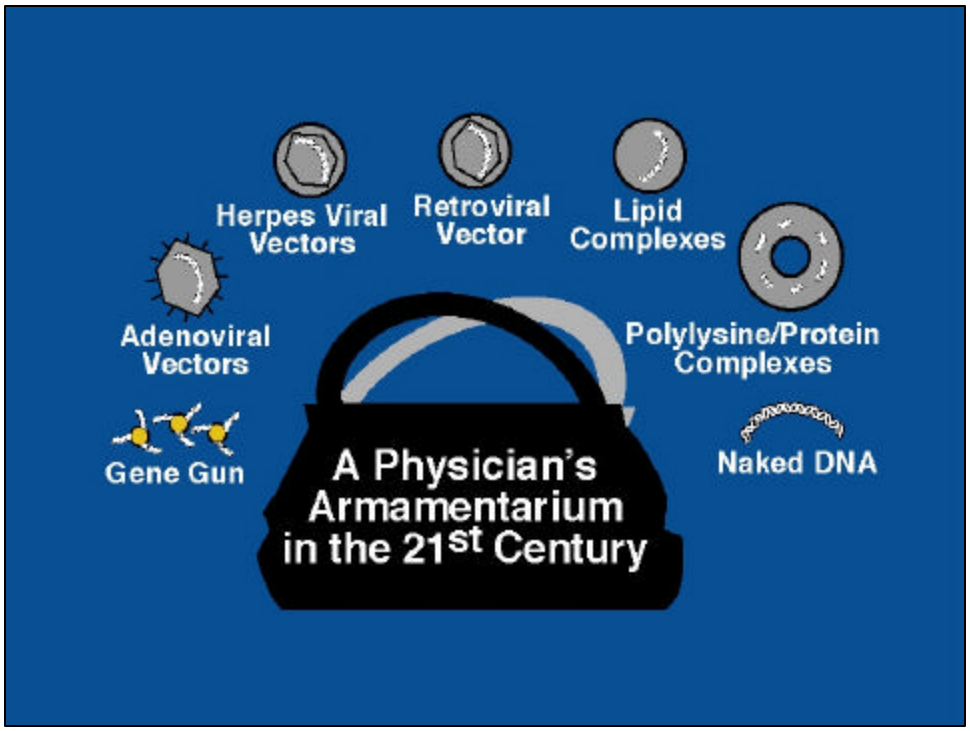

Gene
Vector

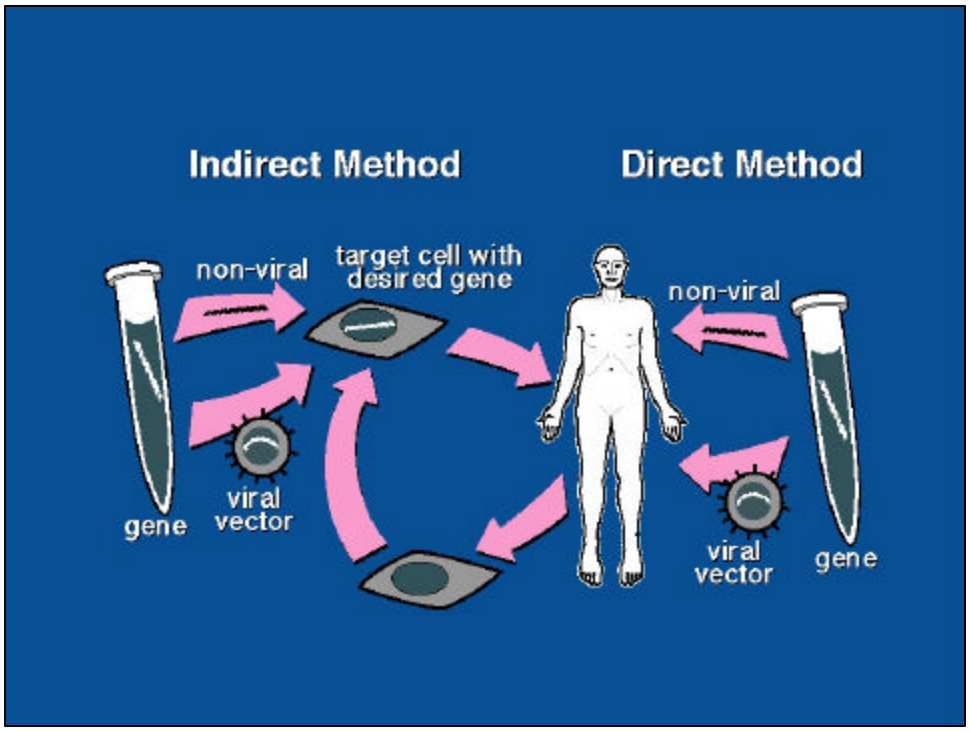
Physical Chemical Transfection Methods for In Vivo Applications

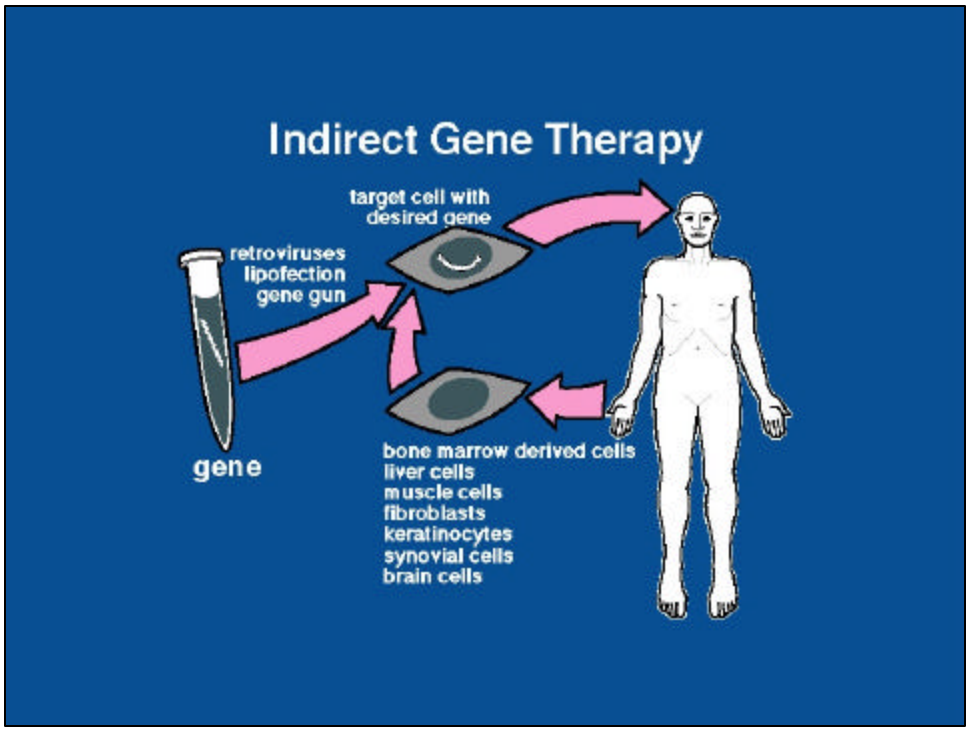
- Liposomes
- Cationic Lipids
- Microparticle Bombardment
- Polylysine Complexes
- Naked DNA

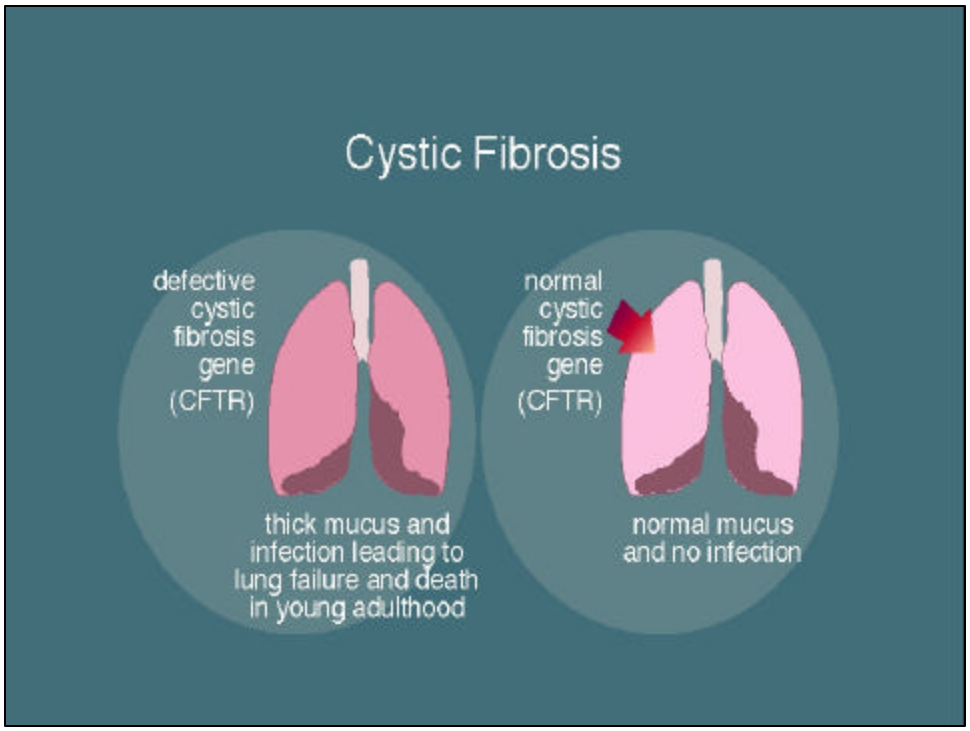
Advantages of Non-viral Gene Transfer

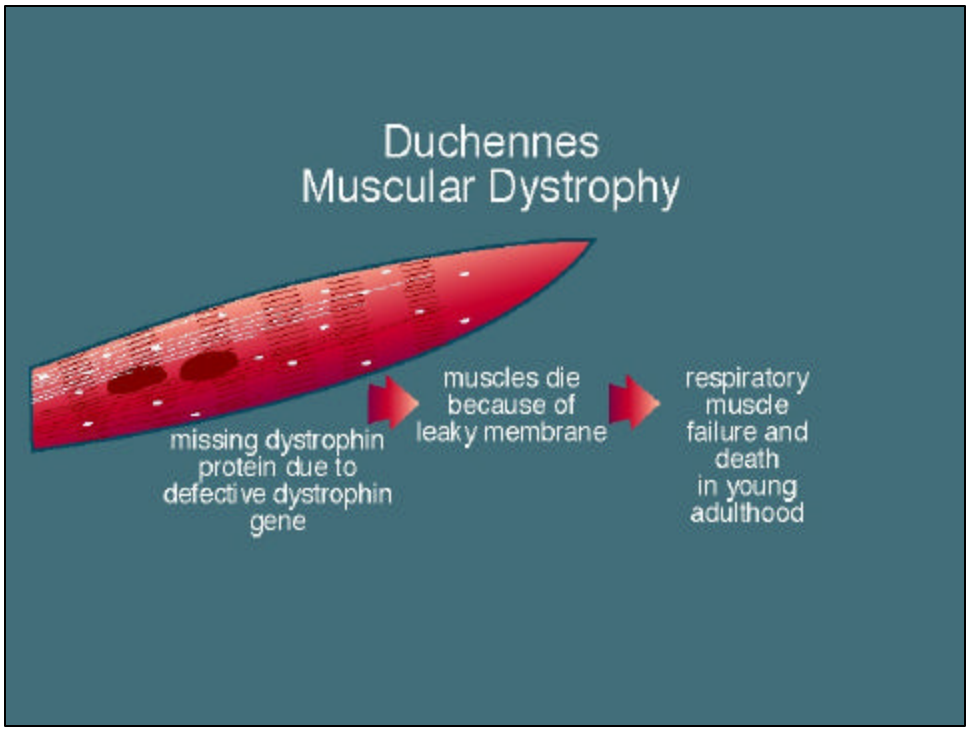
- Easier Productions
- Easier Scale-up
- Less Immunogenic
- Repeat Administrations Possible
- No Chance of Harmful Viral Infection

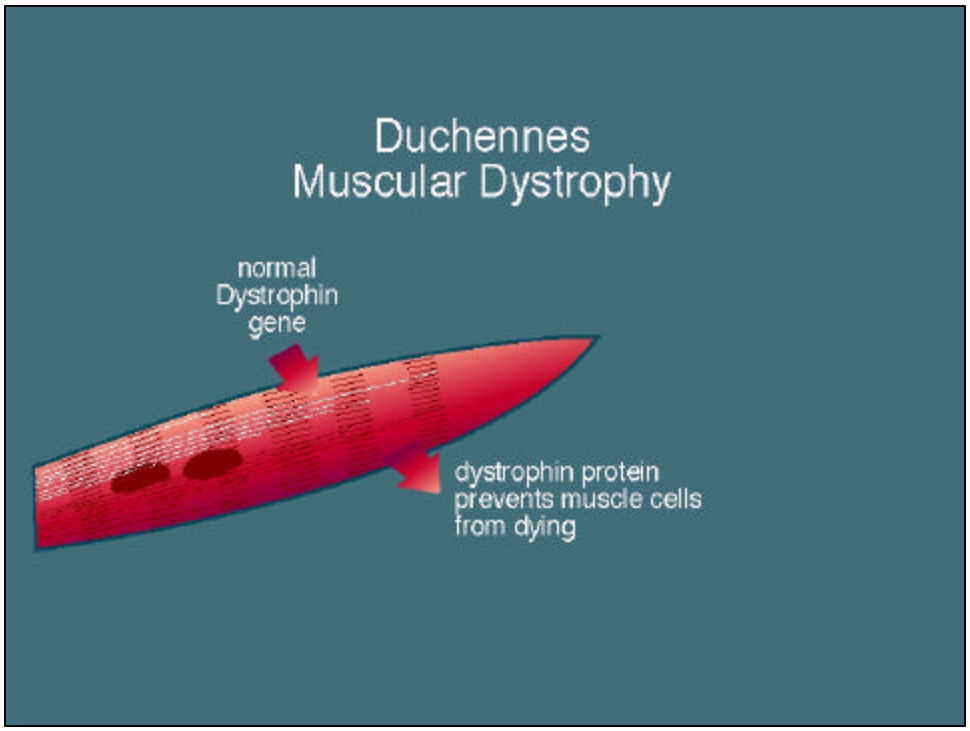

Herpes Viral Vectors
Retroviral Vector
Lipid Complexes
Adenoviral Vectors
Polylysine/Protein Complexes
Gene Gun
Naked DNA
A Physician's Armamentarium in the 21st Century


Indirect Method
Direct Method
non-viral
target cell with desired gene
non-viral
gene
viral vector
viral vector
gene


Indirect Gene Therapy
target cell with desired gene
retroviruses
lipofection
gene gun
gene
bone marrow derived cells
liver cells
muscle cells
fibroblasts
keratinocytes
synovial cells
brain cells

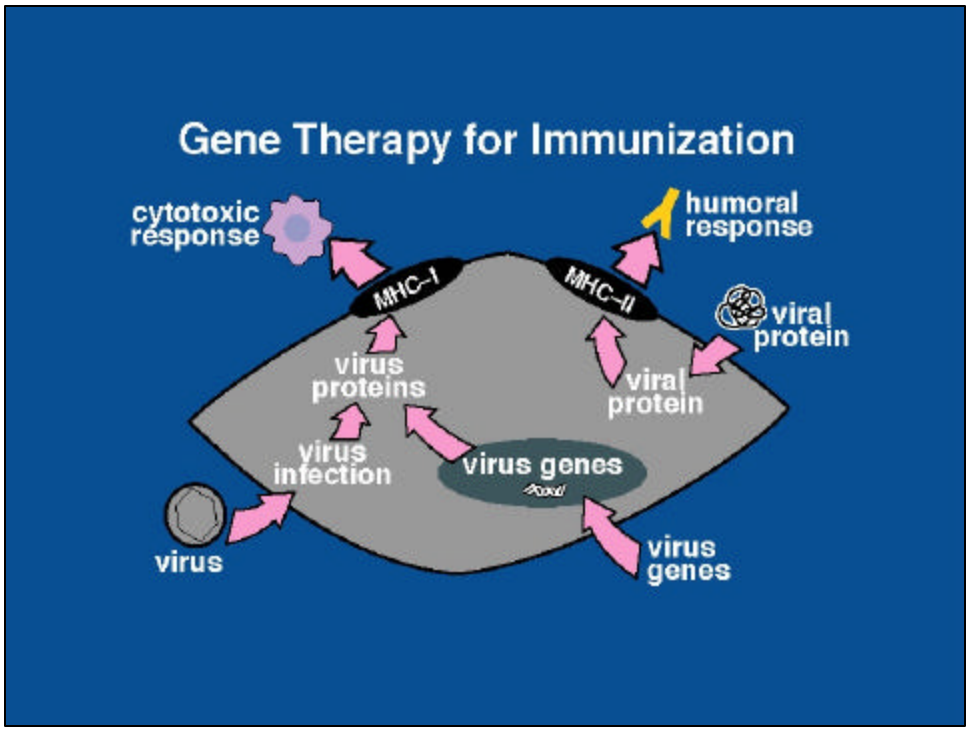

Cystic Fibrosis
defective cystic fibrosis gene (CFTR)
thick mucus and infection leading to lung failure and death in young adulthood
normal cystic fibrosis gene (CFTR)
normal mucus and no infection

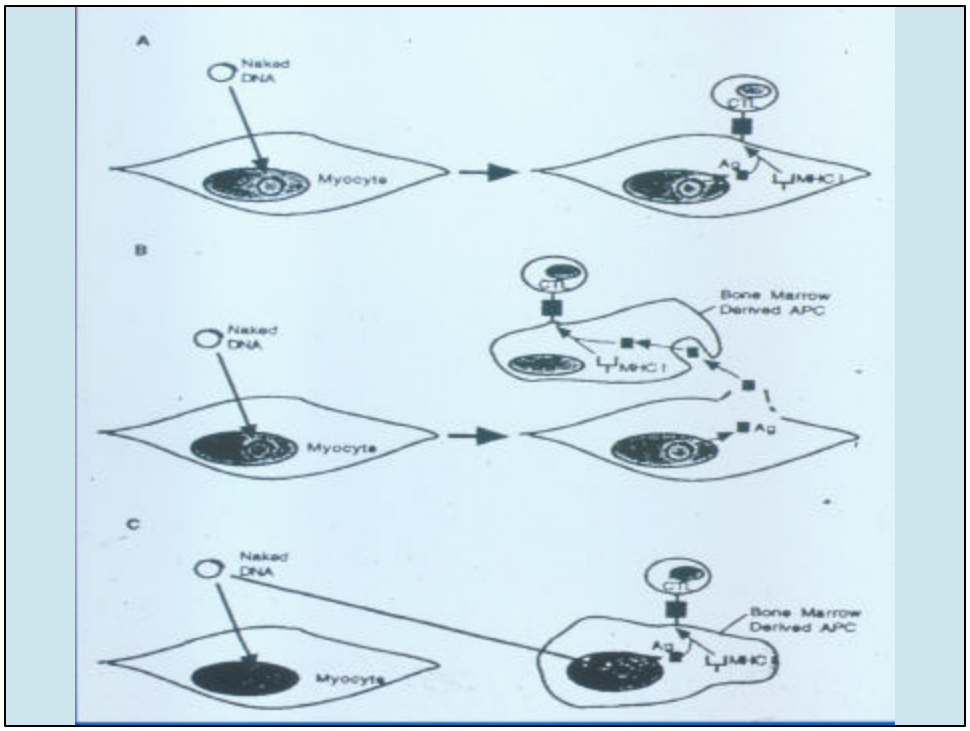

Duchennes
Muscular Dystrophy
missing dystrophin protein due to defective dystrophin gene
muscles die because of leaky membrane
respiratory muscle failure and death in young adulthood


Duchennes
Muscular Dystrophy
normal Dystrophin gene
dystrophin protein prevents muscle cells from dying

"These are the days of miracle and wonder…
Medicine is magical and magical is art
The Boy in the Bubble
And the baby with the baboon heart."

From The Boy in the Bubble by Paul Simon





THE NEW YORK TIMES **MEDICAL SCIENCE** TUESDAY, MARCH 26, 1991

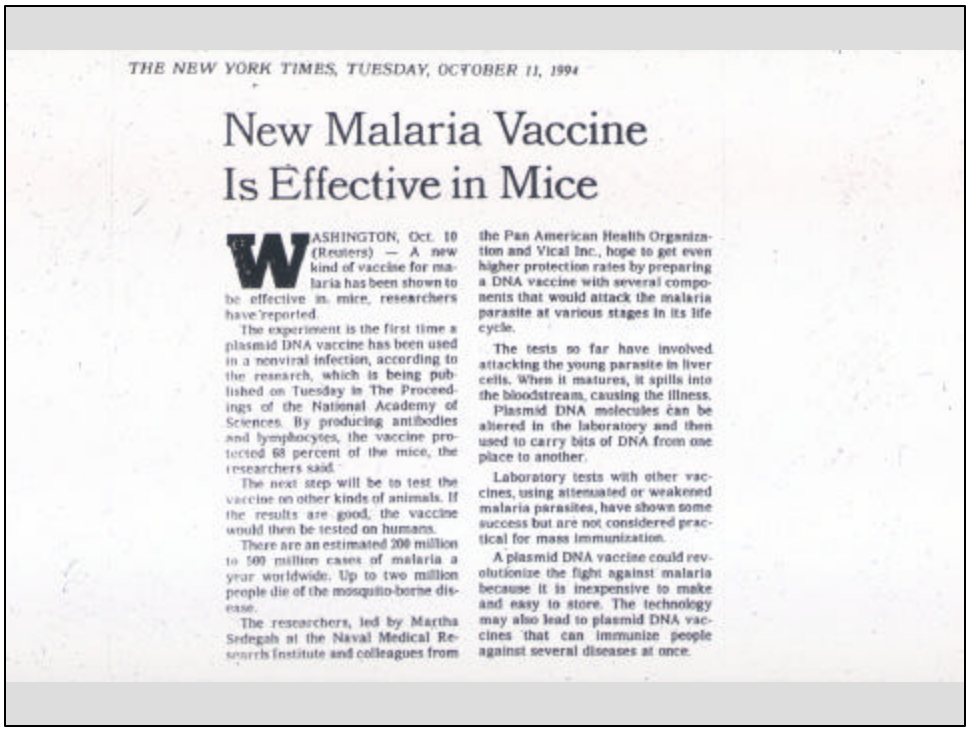
New Vaccine Method Using DNA Protects Mice Against a Flu Virus

Direct injection of genetic material may hold advantages.

By HARRIS MARANTZ HENIG

A potential weapon against genetic and immune disorders as well as cancer.

Immunization With DNA: Twofold Immunity



THE NEW YORK TIMES, TUESDAY, OCTOBER 11, 1994

New Malaria Vaccine Is Effective in Mice

WASHINGTON, Oct. 10 (Reuters) — A new kind of vaccine for malaria has been shown to be effective in mice, researchers have reported.

The experiment is the first time a plasmid DNA vaccine has been used in a nonviral infection, according to the research, which is being published on Tuesday in The Proceedings of the National Academy of Sciences. By producing antibodies and lymphocytes, the vaccine protected 68 percent of the mice, the researchers said.

The next step will be to test the vaccine on other kinds of animals. If the results are good, the vaccine would then be tested on humans.

There are an estimated 200 million to 500 million cases of malaria a year worldwide. Up to two million people die of the mosquito-borne disease.

The researchers, led by Martha Sedegah at the Naval Medical Research Institute and colleagues from the Pan American Health Organization and Vical Inc., hope to get even higher protection rates by preparing a DNA vaccine with several components that would attack the malaria parasite at various stages in its life cycle.

The tests so far have involved attacking the young parasite in liver cells. When it matures, it spills into the bloodstream, causing the illness.

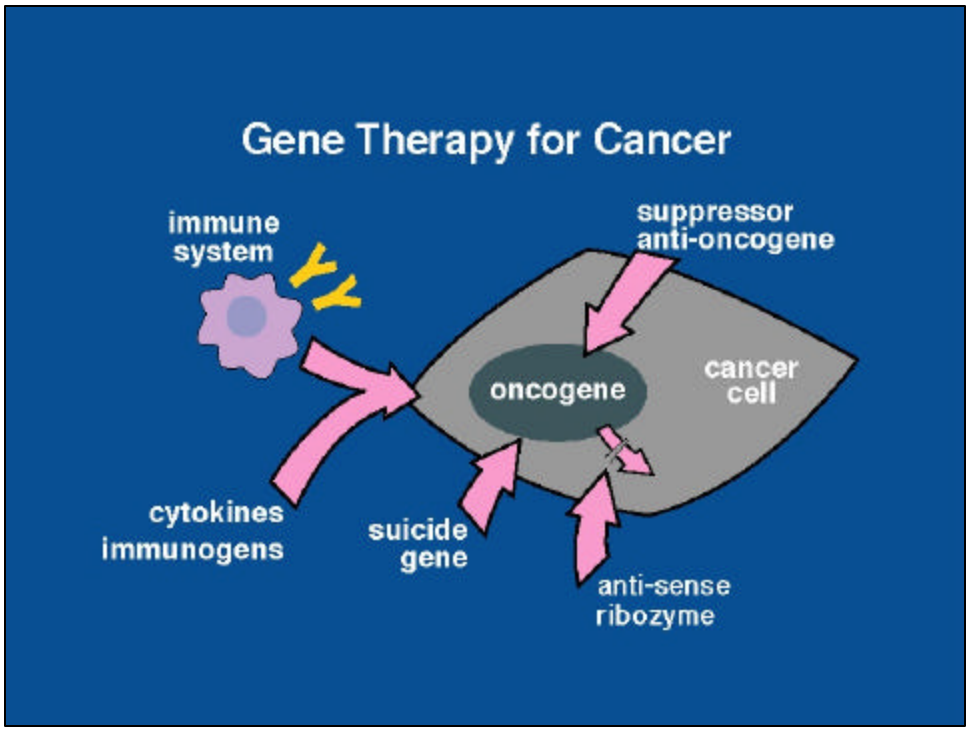
Plasmid DNA molecules can be altered in the laboratory and then used to carry bits of DNA from one place to another.

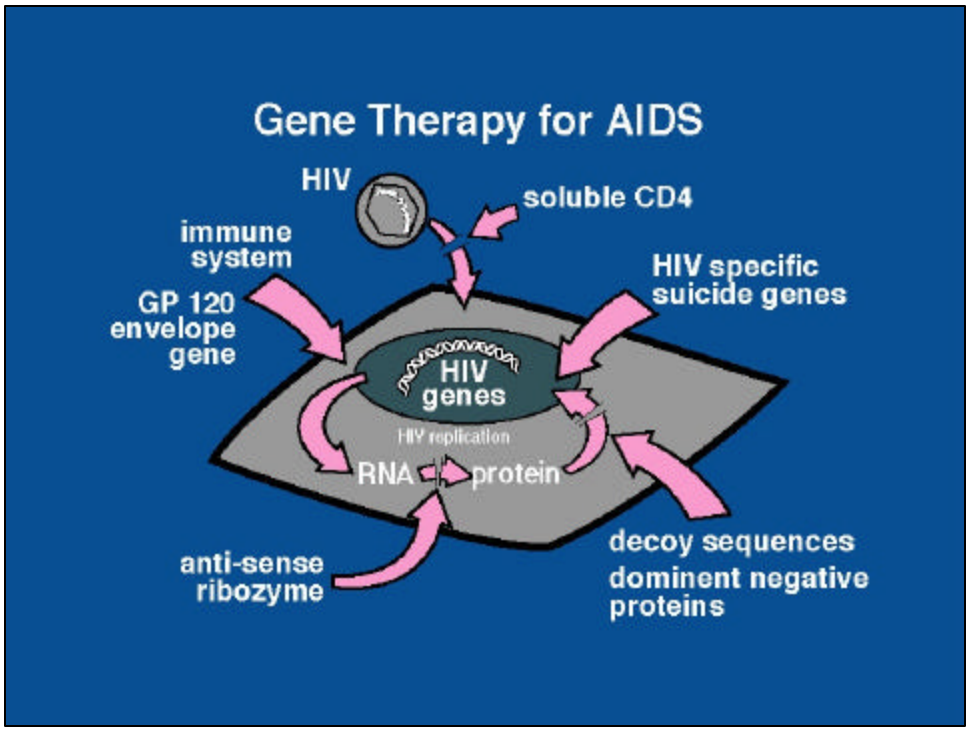
Laboratory tests with other vaccines, using attenuated or weakened malaria parasites, have shown some success but are not considered practical for mass immunization.

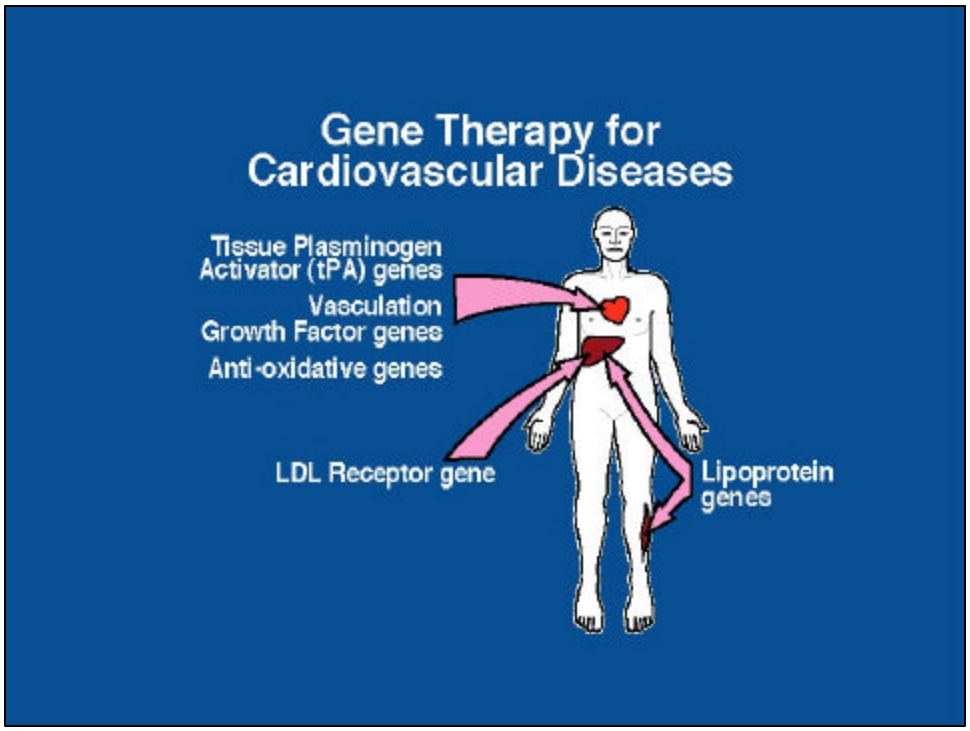
A plasmid DNA vaccine could revolutionize the fight against malaria because it is inexpensive to make and easy to store. The technology may also lead to plasmid DNA vaccines that can immunize people against several diseases at once.

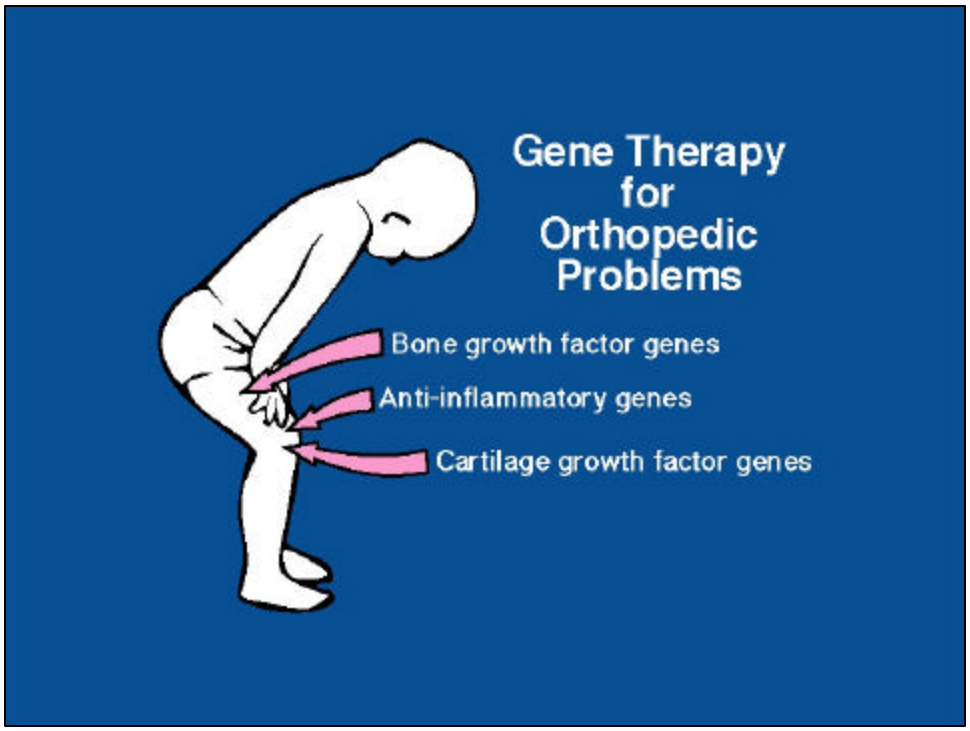
DNA Vaccines Under Development

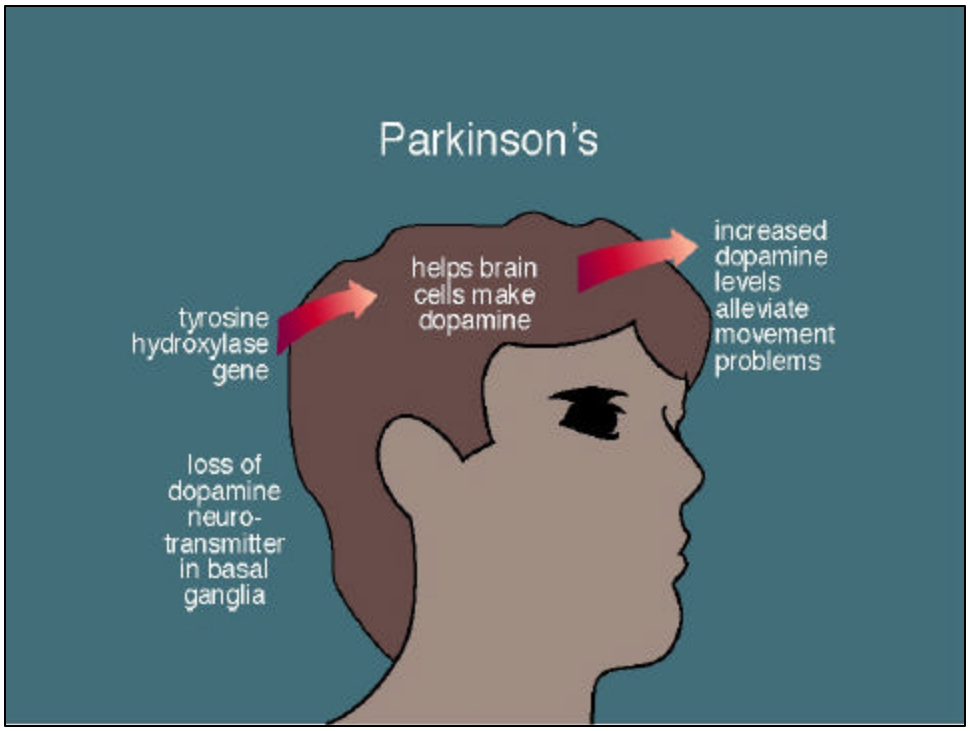
- Influenza
- Measles
- Rotavirus
- Hepatitis B
- HIV
- HSV
- Ebola
- Rabies
- Tuberculosis
- Malaria


Gene Therapy for Cancer
immune system
suppressor anti-oncogene
oncogene
cancer cell
cytokines immunogens
suicide gene
anti-sense ribozyme


Gene Therapy for AIDS
HIV
soluble CD4
immune system
GP 120 envelope gene
HIV specific suicide genes
HIV genes
HIV replication
RNA
protein
anti-sense ribozyme
decoy sequences
dominent negative proteins


Gene Therapy for Cardiovascular Diseases
Tissue Plasminogen Activator (tPA) genes
Vasculation Growth Factor genes
Anti-oxidative genes
LDL Receptor gene
Lipoprotein genes

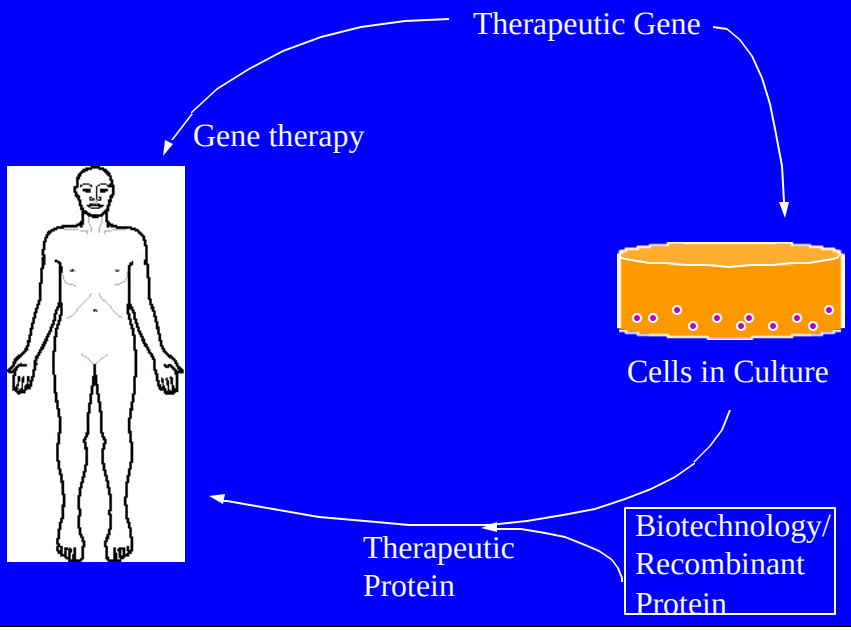

Gene Therapy for Orthopedic Problems
Bone growth factor genes
Anti-inflammatory genes
Cartilage growth factor genes


Parkinson's
tyrosine hydroxylase gene
helps brain cells make dopamine
increased dopamine levels alleviate movement problems
loss of dopamine neuro-transmitter in basal ganglia

Applications for Muscle Delivery

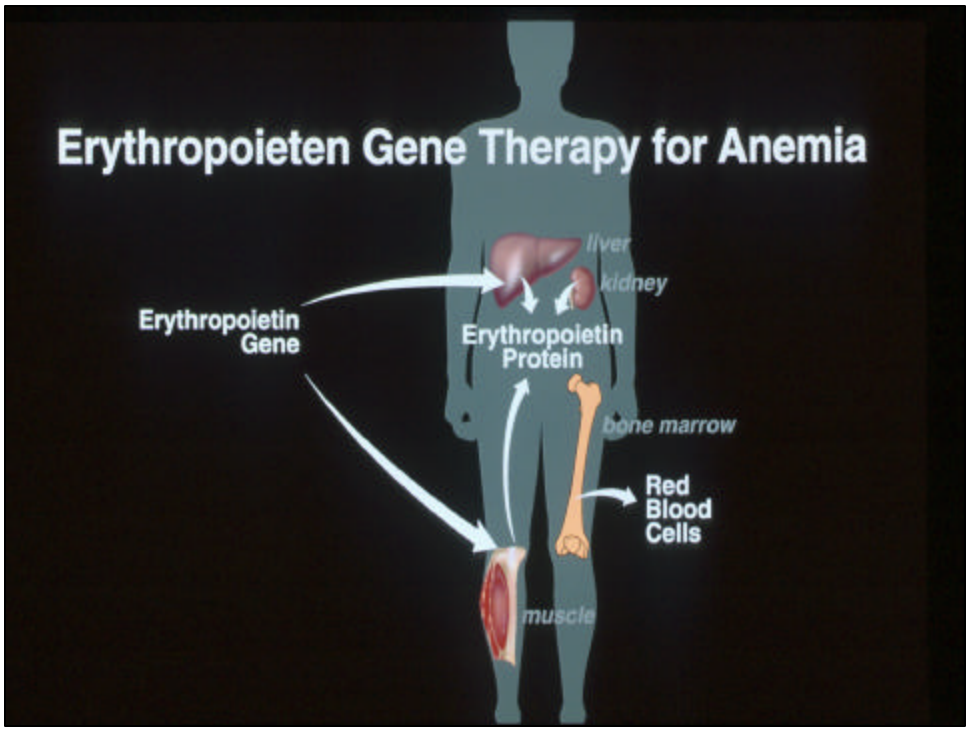
- Immunization
- Secreted Protein
- Clearing a Toxic Circulating Metabolite
- Duchenne Muscular Dystrophy

Secreted Therapeutic Protein

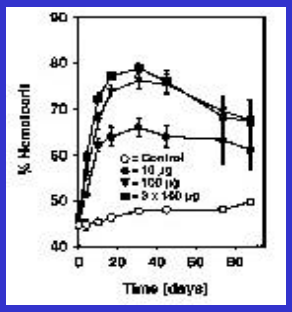


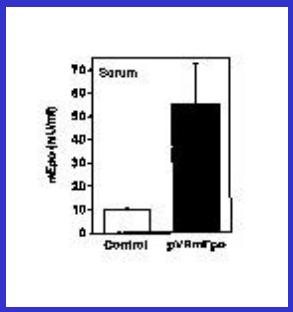
Secreted Proteins

- Erythropoietin
  - Stimulates production of red blood cells
  - Anemia of any cause
  - Anemia secondary to renal disease
- Blood Clotting Factors
  - Required for clotting blood
  - Hemophilia A-Factor VII
  - Hemophilia B-Factor IX
- Vascular Growth Factors
  - Causes new blood vessels to grow
  - Blocked vessels in limb
  - Blocked coronary arteries

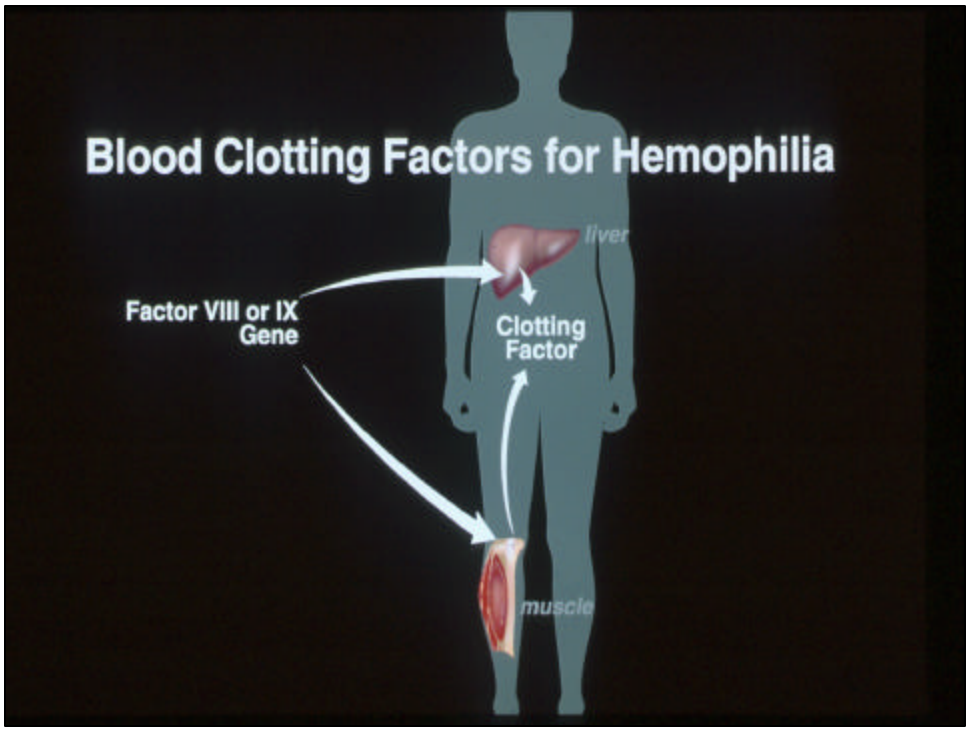


Long-term expression of erythropoietin in the systemic circulation of mice after intramuscular injection of a plasmid DNA vector

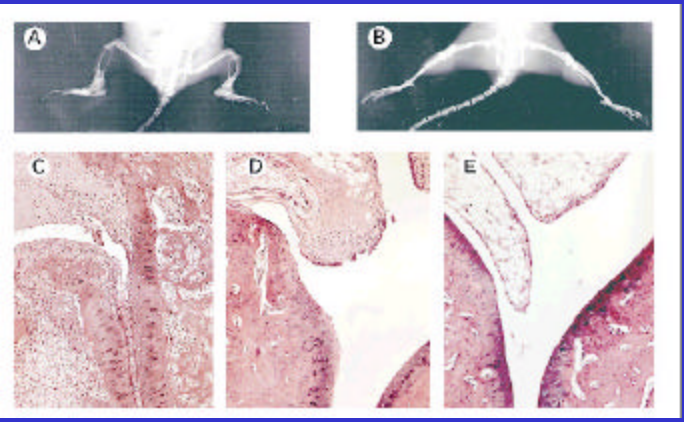




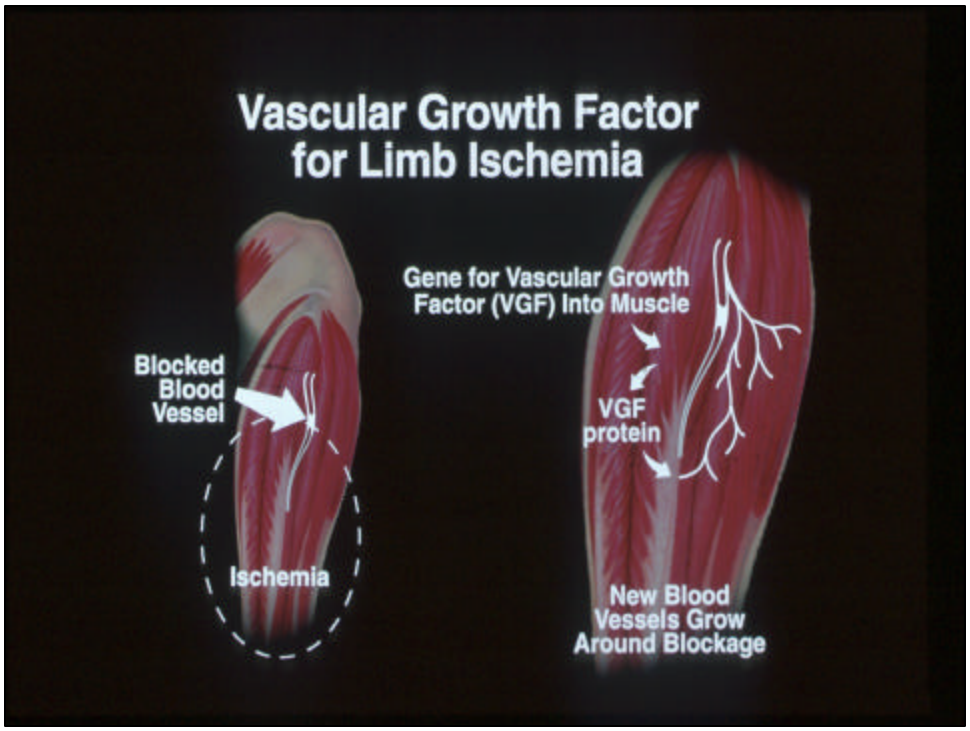
Tripathy, S.K., et al., PNAS, Vol. 93, pp. 10876–10880, 1996



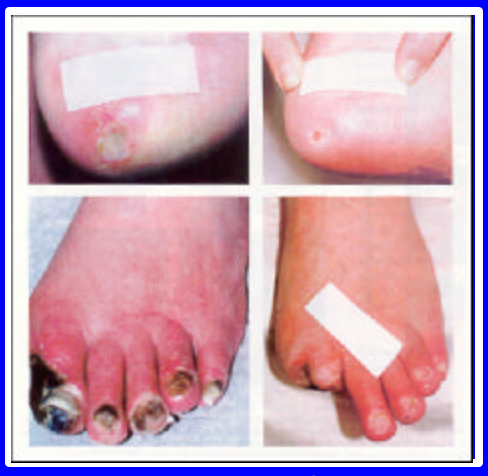
Plasmid DNA Encoding Transforming Growth Factor-ß1 Suppresses Chronic Disease in a Streptococcal Cell Wall-induced Arthritis Model



Song, Xiao-yu, et al., J. Clin. Invest. 12:2615-2621, 1998

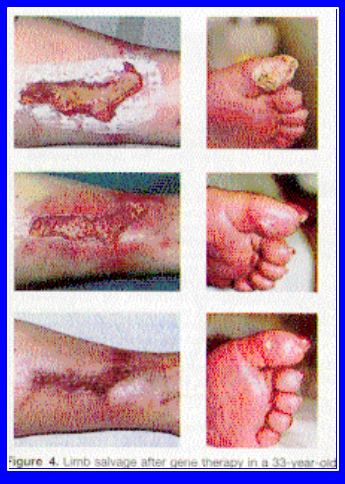


Limb Salvage After Gene Therapy



Baumgartner et al., 1998

Limb Salvage After Gene Therapy



igure 4. Limb salvage after gene therapy in a 33-year-old

Baumgartner et al., 1998

Intramuscular Gene Transfer

BASELINE, HEMODYNAMIC, ANGIOGRAPHIC, LABORATORY, AND OUTCOME FINDINGS BEFORE AND AFTER INTRAMUSCULAR phVEGF$_{165}$ GENE TRANSFER

No.	Sex	Age, y	Clinical History and Findings Before Gene Therapy: Claud, pkfy	DM	Previous Treatment	Signs/Symptoms	Outcome After Gene Transfer: Limb Status	DSA Findings	VEGF Level, pg/mL: Bl	1st	2nd	Molecular Findings
1	F	53†	30	0	4 bypass grafts, 3 rev., prostaglandins	Calf ulcer, toe gangrene (digit I)	ABI +0.34; complete healing→limb salvage	New collaterals, 200–400 μm	47	223	507	
2	F	63	0	+	3 bypass grafts, 1 PTA, prostaglandins	Toe gangrene (digit V)	ABI +0.12; complete healing	New collaterals, 200–400 μm	36	ND	ND	
3	M	77	0	+	None	Toe gangrene (digits I, IV)	TBI +0.11; gangrene/ osteomyelitis→BKA	New collaterals, 200–400 μm	45	131	790	
4	F	39†	22	0	Sympathectomy	Forefoot gangrene	ABI +0.27; forefoot necrosis→BKA	New collaterals, 200–400 μm	30	59	688	PCR pos. in skin + muscle specimens; Southern pos. in muscle specimens
5	M	74	50	0	1 PTA	Rest pain	ABI +0.16; rest pain resolved	New collaterals, 200–800 μm	62	300	96	
6	F	84	40	0	6 bypass grafts, 1 PTA	Toe gangrene (digits I–V)	ABI +0.22; toe amputation—limb salvage	None	25	164	80	
7	F	80	20	0	1 bypass graft	Rest pain	ABI unchanged, rest pain resolved	New collaterals, 200–800 μm	40	44	223	
8*	F	39	20	0	Sympathectomy	Heal ulcer, toe gangrene (digits I–V)	ABI +0.22; toe amputation—limb salvage	New collaterals, 200–>800 μm	ND	ND	ND	
9	M	54	30	0	4 bypass grafts, 2 rev., 1 PTA	Rest pain	TBI +0.16; rest pain resolved	None	0	113	63	
10	M	54	70	0	5 bypass grafts, 1 PTA	Toe gangrene (digits I, III, IV)	No change in ABI/TBI, BKA	None	ND	ND	ND	PCR pos. in skin + muscle specimens; Southern pos. in muscle specimens

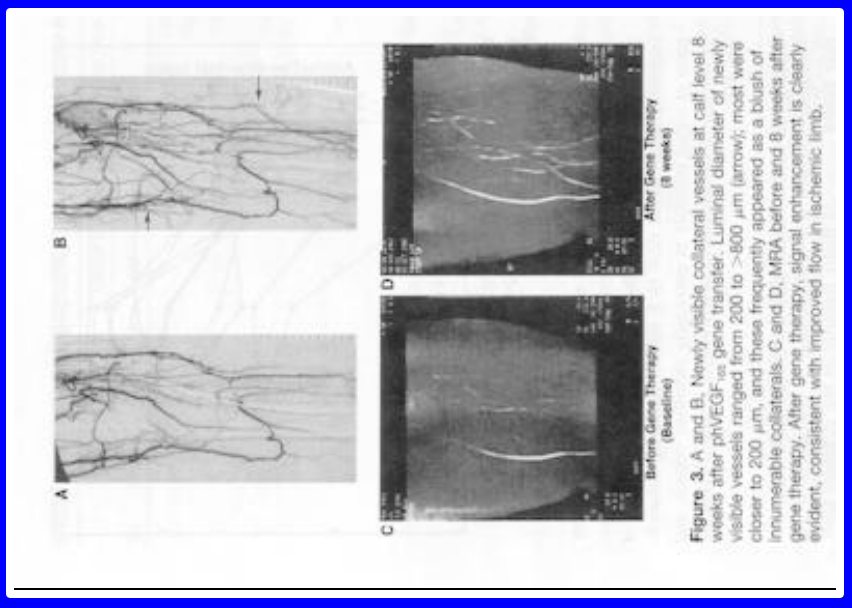
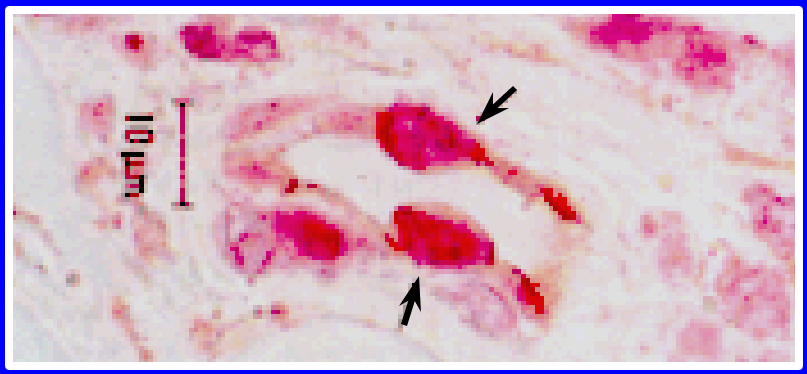


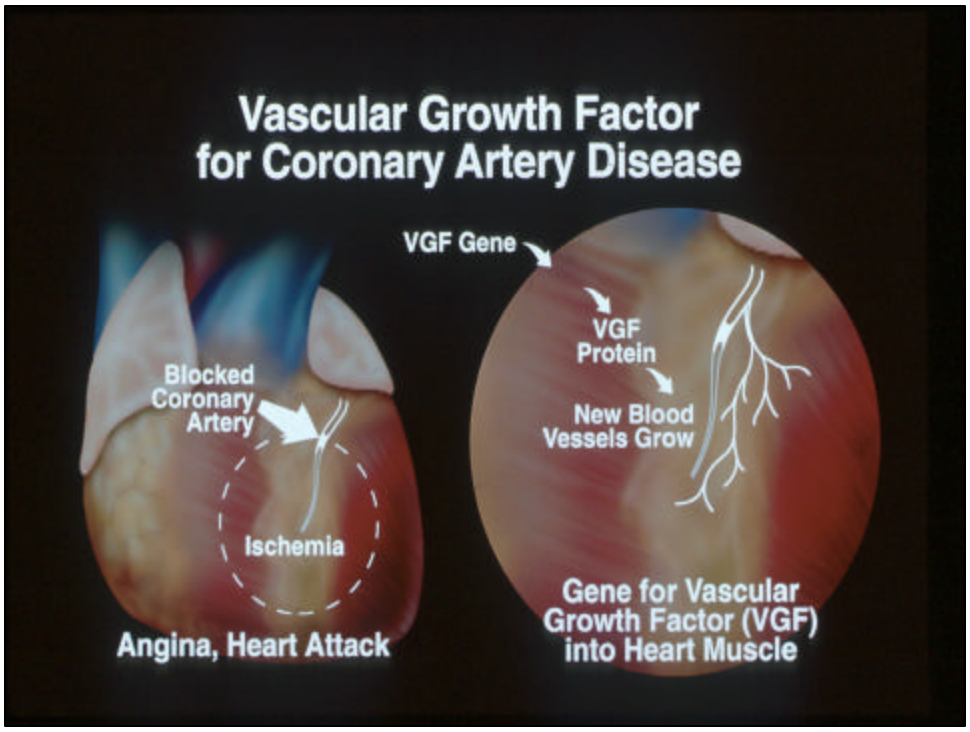
Figure 3. A and B, Newly visible collateral vessels at calf level 8 weeks after phVEGF$_{165}$ gene transfer. Luminal diameter of newly visible vessels ranged from 200 to >800 μm (arrow); most were closer to 200 μm, and these frequently appeared as a blush of innumerable collaterals. C and D, MRA before and 8 weeks after gene therapy. After gene therapy, signal enhancement is clearly evident, consistent with improved flow in ischemic limb.

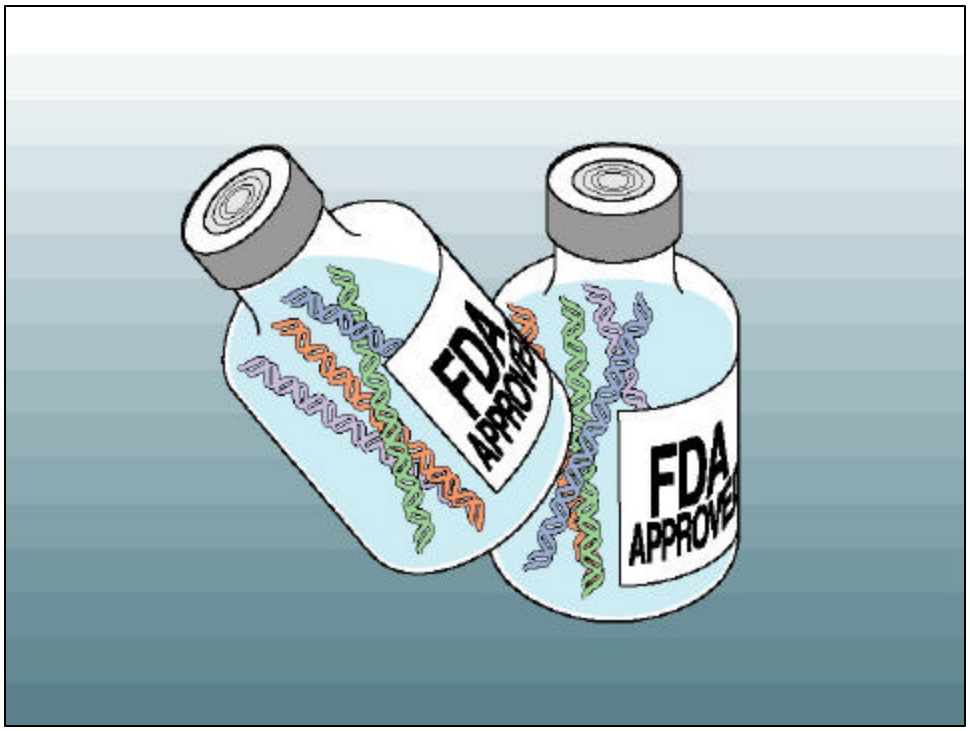
Baumgartner et al., 1998

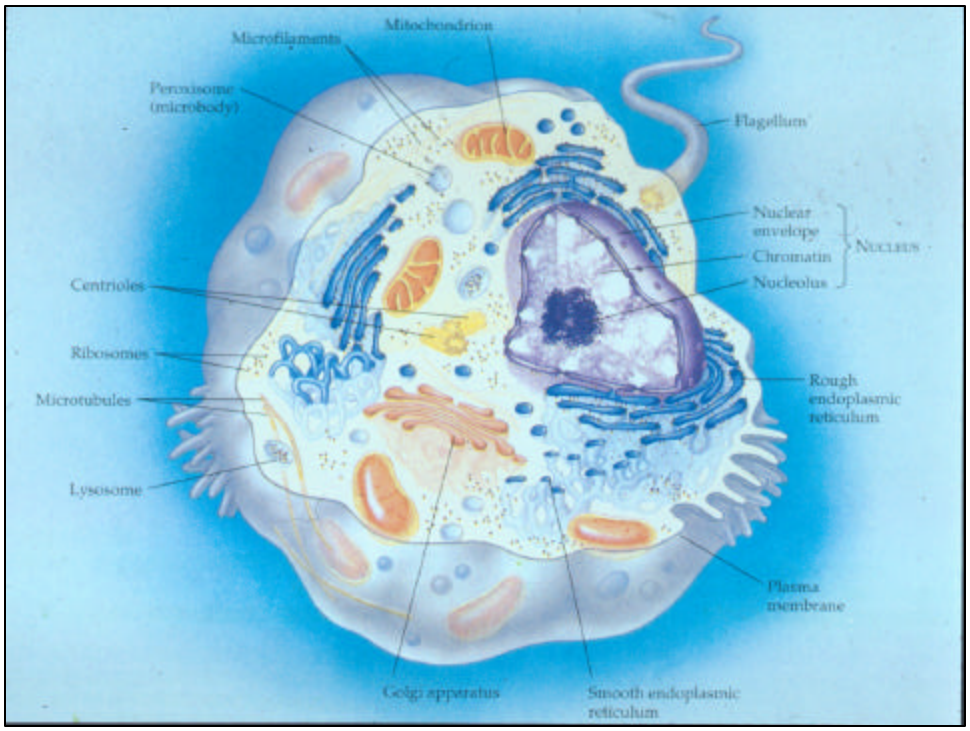
Proliferating Microvascular Endothelial Cells



Baumgartner et al., 1998




FDA
APPROVED
FDA
APPROVED


Microfilaments
Mitochondrion
Peroxisome (microbody)
Flagellum
Nuclear envelope
Chromatin
Nucleolus
Nucleus
Centrioles
Ribosomes
Microtubules
Rough endoplasmic reticulum
Lysosome
Plasma membrane
Golgi apparatus
Smooth endoplasmic reticulum