Gene Therapy: Human Genes as Drugs

Medical Grand Rounds

WCMC-Q

R. Crystal

Department of Genetic Medicine

11-16-14

Weill Cornell Medical College in Qatar

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RONALD G. CRYSTAL , MD

I DO NOT have a financial interest in commercial products or services.

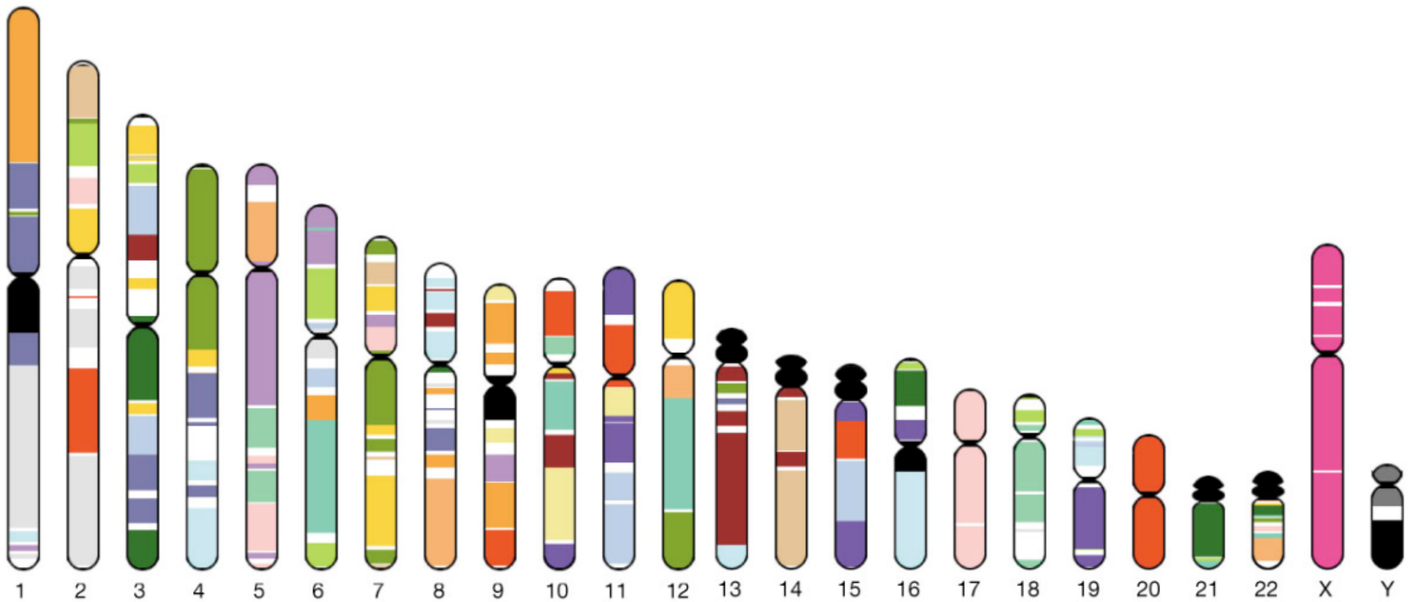
Chemistry

Periodic Table
1869

Biology

Genome
2001

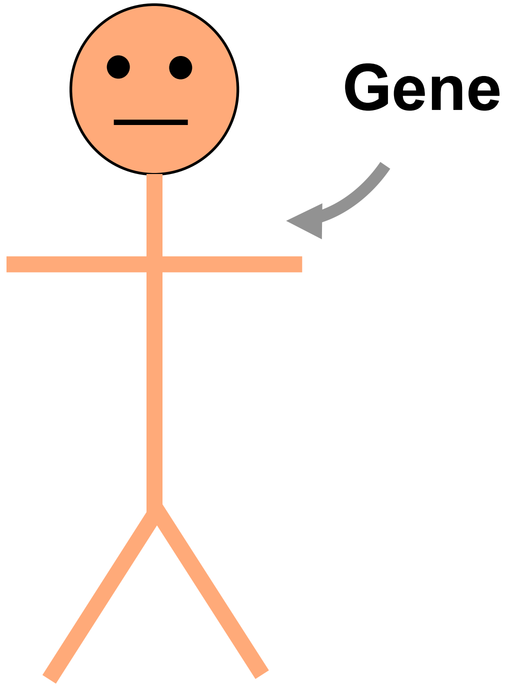
The Human Genome



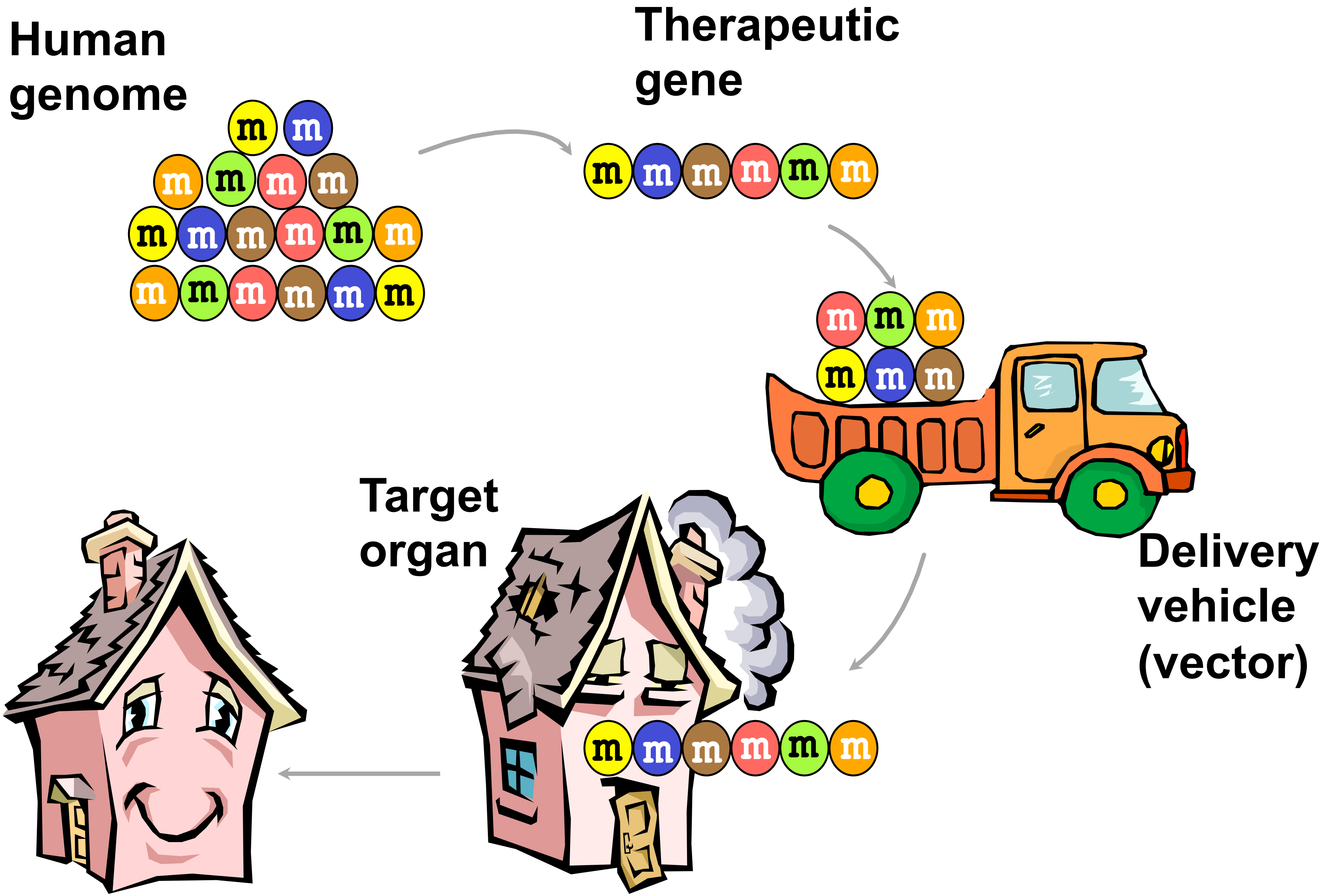
- Composed of DNA – 3.1 billion letters (A, T, G and C) that represent our genetic footprint
- Our genomes are distributed on 2 sets of 23 chromosomes inherited from our parents
- The genome codes for 25,000 genes that tell our cells how to function

Gene Therapy

- Therapies using genetic material to correct, compensate or protect against an abnormal phenotype

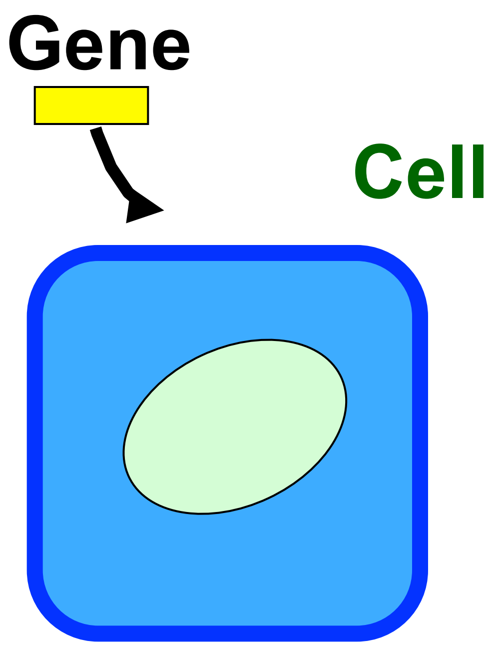


Gene Therapy



Challenges to Gene Therapy

- Delivery vehicle (vector)
- Mode of delivery – *ex vivo* or *in vivo*
- Integration or extra-chromonsomal
- Risks



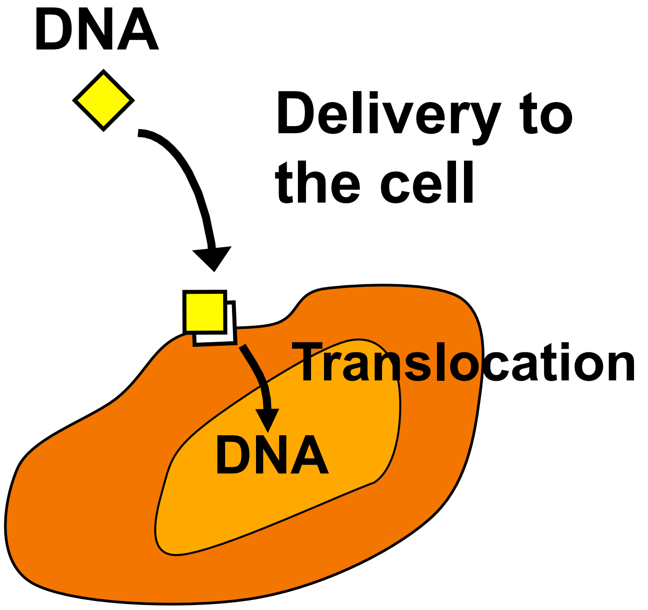
Gene Delivery

***Ex vivo* delivery**

- Modify cells *in vitro,* transfer modified cells *in vivo*

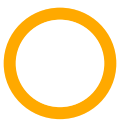
***In vivo* delivery**

- Direct to the organ
- Intravascular

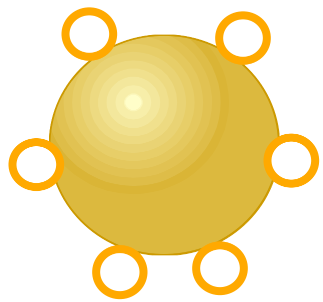


Gene Therapy Vectors

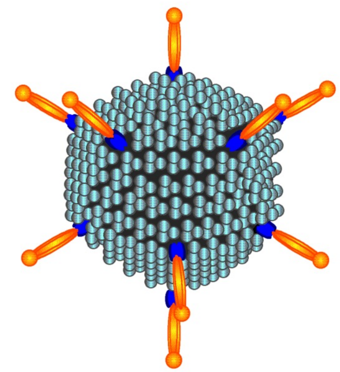
Plasmid



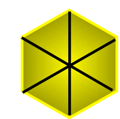
Liposome + plasmid



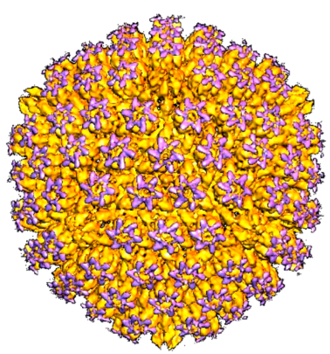
Adenovirus



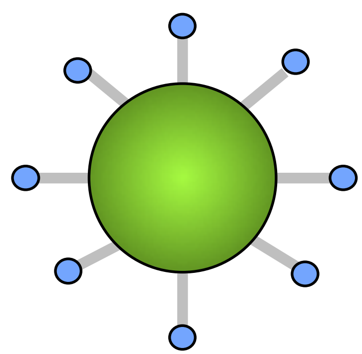
Adeno-associated virus



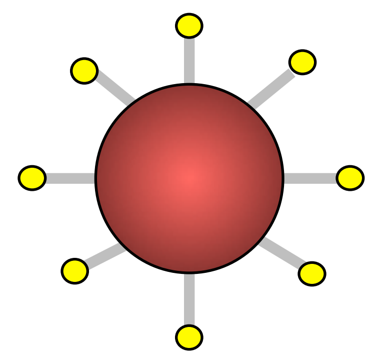
Herpesvirus



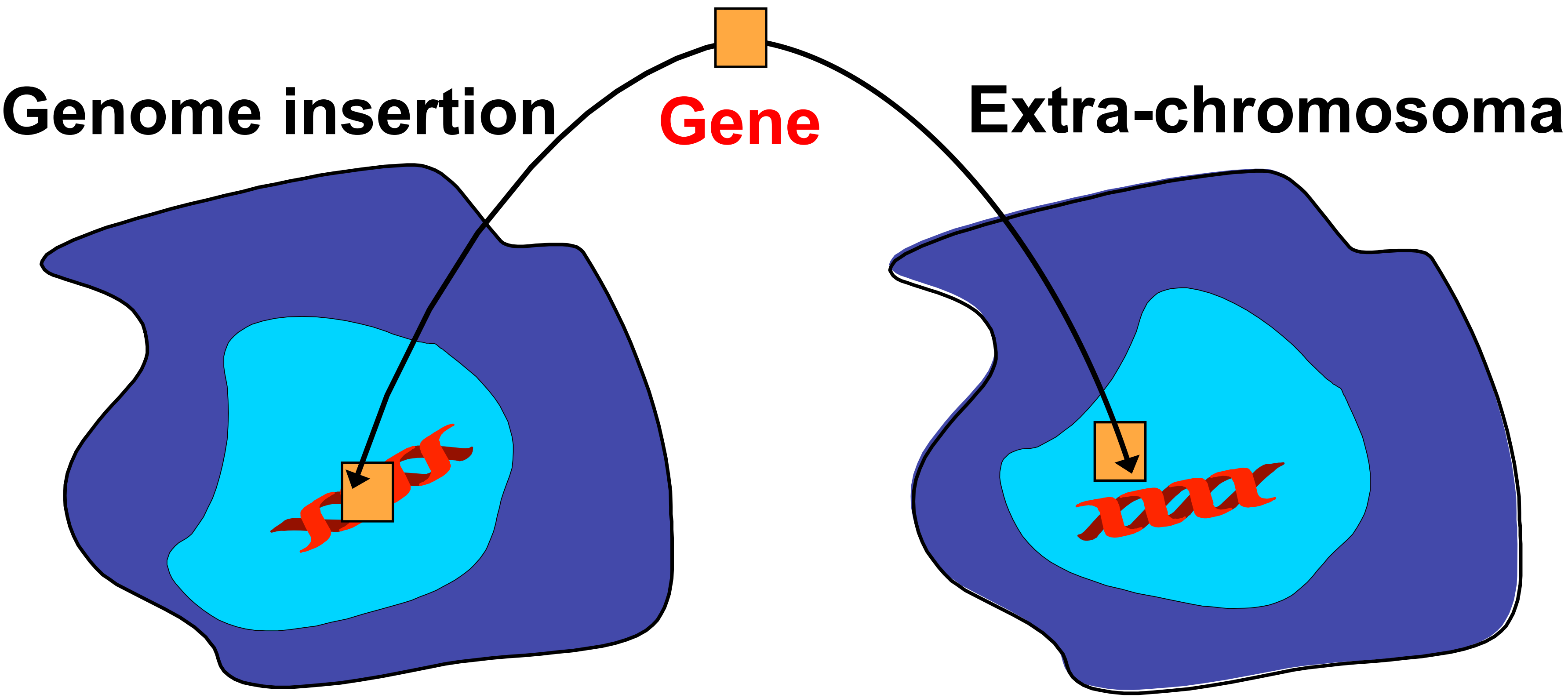
Retrovirus



Lentivirus



Gene Therapy with Viral Vectors: Integration or Extra-chromosomal?

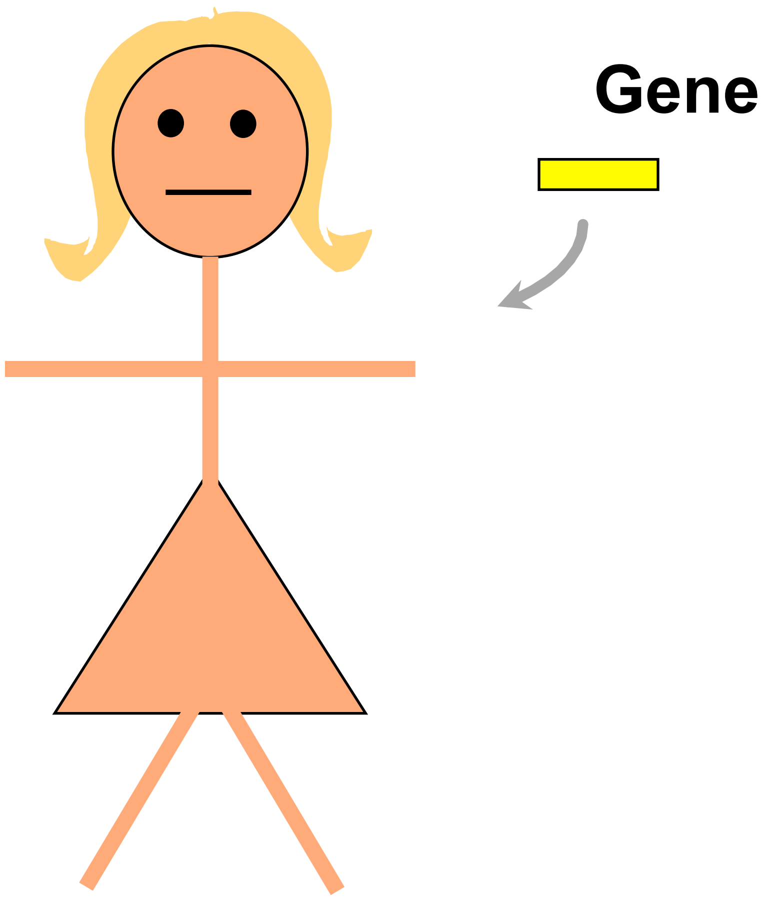


- Required for proliferating cell targets
- Retrovirus/lentivirus

- Ideal for cell targets that are not proliferating
- Adenovirus, adeno-associated virus

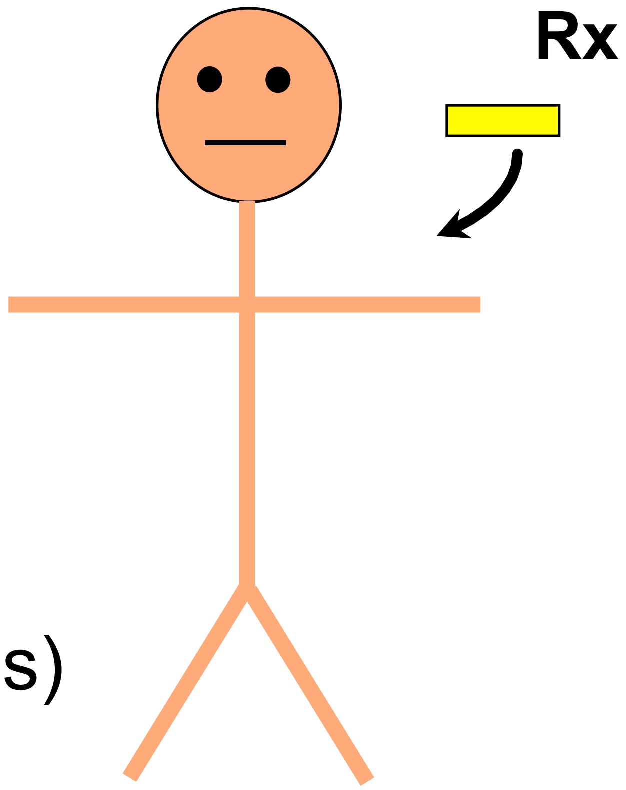
Risks to Gene Therapy

- Persistent overexpression
- Recombination
- Germ line gene transfer
- Contamination of the environment
- Systemic or organ-specific anti-vector immunity
- Insertional mutagenesis



Safety of Nucleic Acid Therapies in Humans

- 100s of studies
- 1000s of humans
- Fatal disorders to normals
- 1 death (adenovirus)
- 4 leukemias, 1 death (retrovirus)



Brief History of Human Gene Therapy (1)

Early "Wow" period

- 1989-90 – first human *ex* vivo gene therapy, retrovirus, *ex vivo,* T-cell marking study followed by T-cell correction of adenosine deaminase immuodeficiency (Rosenberg, Blaise, Anderson)
- 1993 – first human *in vivo* gene therapy, adenovirus, *in vivo* transfer of the CFTR gene to airway epithelium, cystic fibrosis (Crystal)

Brief History of Human Gene Therapy (2)

Dark Ages

- 1999 – Jesse Gelsinger case, adenovirus intravascular ->liver, ornithine transcarbamylase deficiency, innate immune response -> death
- 2002 – retrovirus *ex vivo* T-cells, X-SCID immunodeficiency, insertional mutagenesis -> leukemia

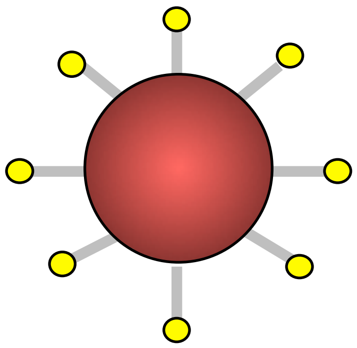
Brief History of Human Gene Therapy (3)

Renaissance - 2014

- Major vector-related advances
  - New serotypes, capsid modifications, genome design
- Success in clinical gene therapy trials
  - CAR therapy for leukemia
  - Retinitis pigmentosa
  - Factor IX deficiency
  - Glybera – lipoprotein lipase deficiency (uniQure, approved in Europe)
- Past yr -> 1 billion commercial investment in gene therapy, total market cap gene therapy companies 2.9 billion

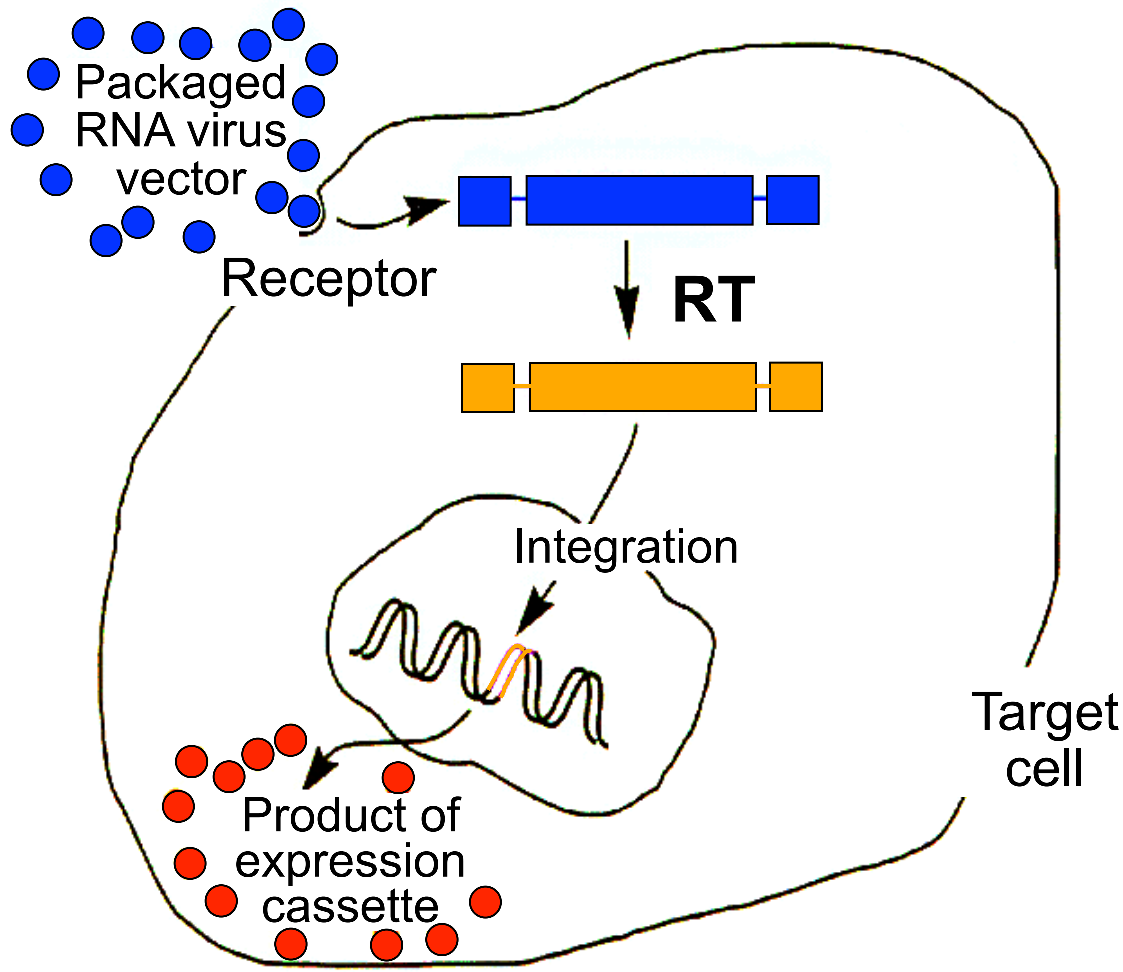
Retrovirus/Lentivirus Vectors

- Gamma retrovirus, lentivirus – RNA viruses, reverse transciptase -> DNA ->integrase -> genome integration
- Expression cassette 8.5-9.0 kb
- Because the vector DNA integrates, excellent for *ex* vivo applications with proliferating cells (e.g., T-cells, bone marrow stem cells)
- Can be used *in vivo*
- Gamma retroviruses have a propensity to integrate into promoters, including that of proto-ongogenes -> insertional mutagenesis
- Extensive engineering of lentivirus vectors prevents replication and insertional mutagenesis



Gene Transfer Using RNA Vectors

- Retrovirus
- Lentivirus

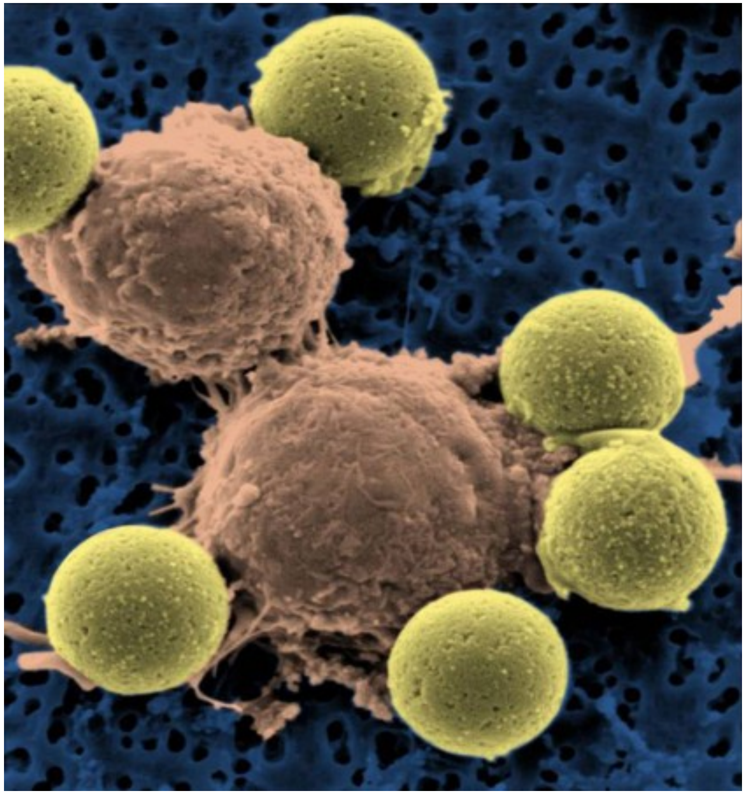


Ongoing Retrovirus/Lentivirus Clinical Trials

- **Hereditary disorders** – adrenoleukodystrophy, thalassemia, sickle cell disease, various immunodeficiencies, X-linked chronic granulomatous disease, Stargardt disease, Usher syndrome,
- **Acquired disorders** – cancer, HIV, macular degeneration, glioblastoma

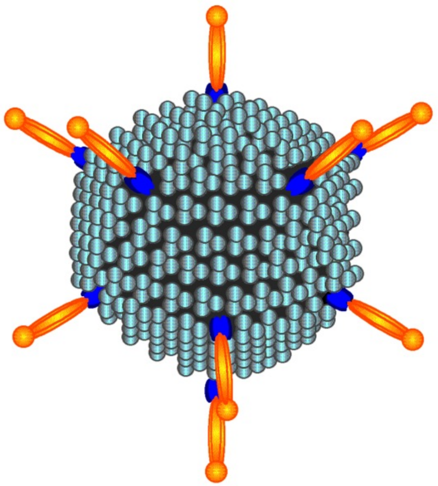
Chimeric Antigen Receptor (CAR) T-cell Therapy

- *Ex vivo*, lentivirus-mediated transfer of a synthetic T-cell receptor to target T-cells to tumor cells
- Example – modify autologous T-cells with a single chain monoclonal sequence to target CD19 on the surface of B-cell leukemia cells
- Binding of the CAR-modified T-cell to CD19 triggers the T-cell to kill the cancer cell
- Applications – hematologic malignancies, ?solid tumors and chronic viral infections

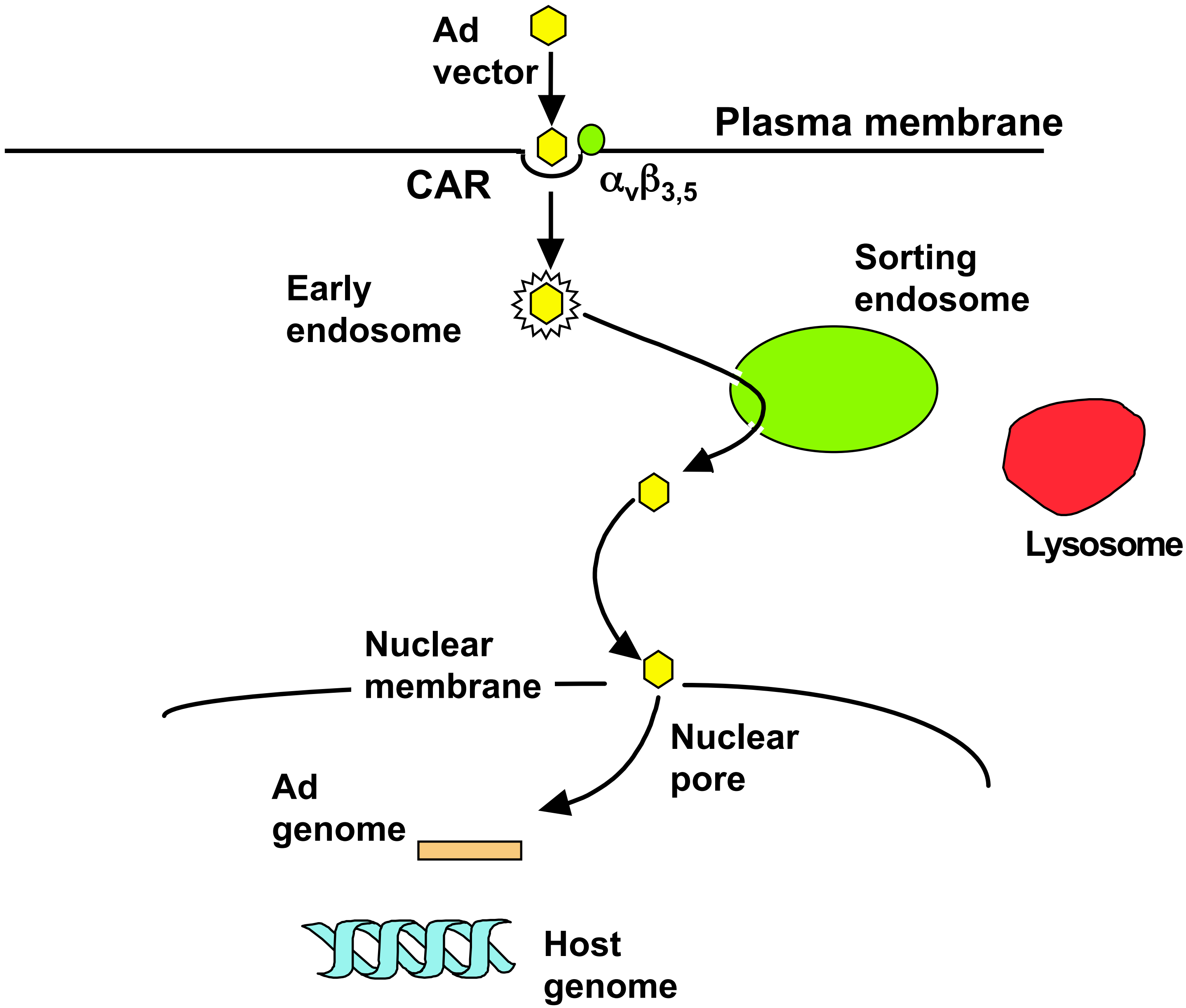


Adenovirus Vectors

- Double stranded, 36 kb DNA virus
- >50 serotypes, human and non-human
- Delete E1 genes to render replication incompetent and E3 genes to increase space for the expression cassette (7.5 kb)
- Immunity against adenovirus proteins limits expression to 2 wk
- Ideal for *in vivo* applications to build new structures or transiently express toxic genes

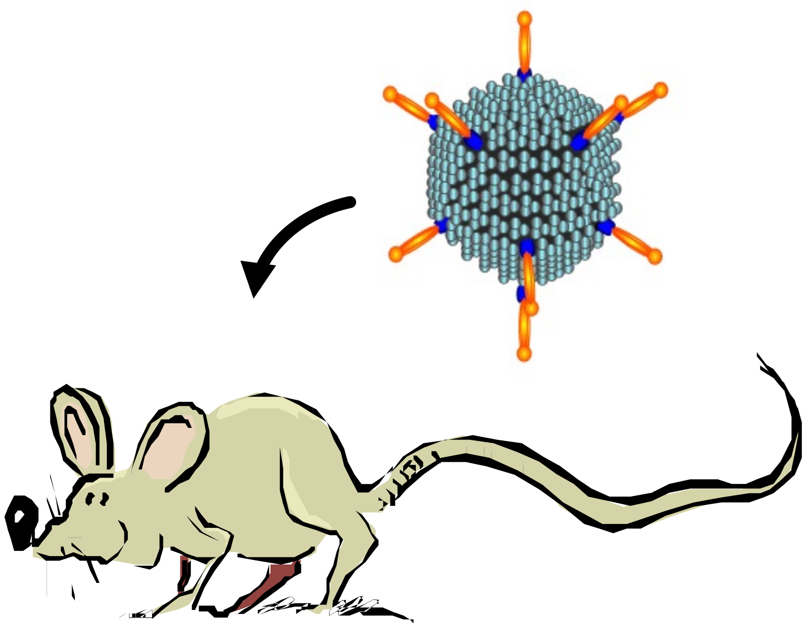


Gene Transfer Using Adenovirus Vectors

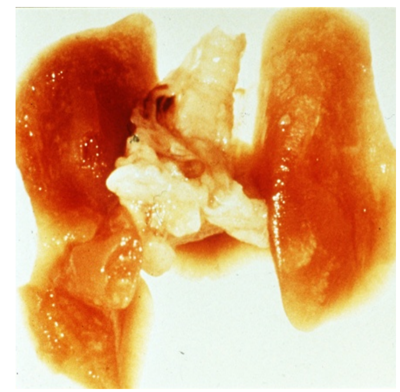


Gene Transfer with a Marker Gene

Adenovirus
coding for "blue" gene
(β-galactosidase)



Uninfected



Ad.RSVβgal

Gene Therapy for Cystic Fibrosis

Recessive disorder

↓

CFTR gene defined

↓

Airway epithelial CFTR deficiency

↓

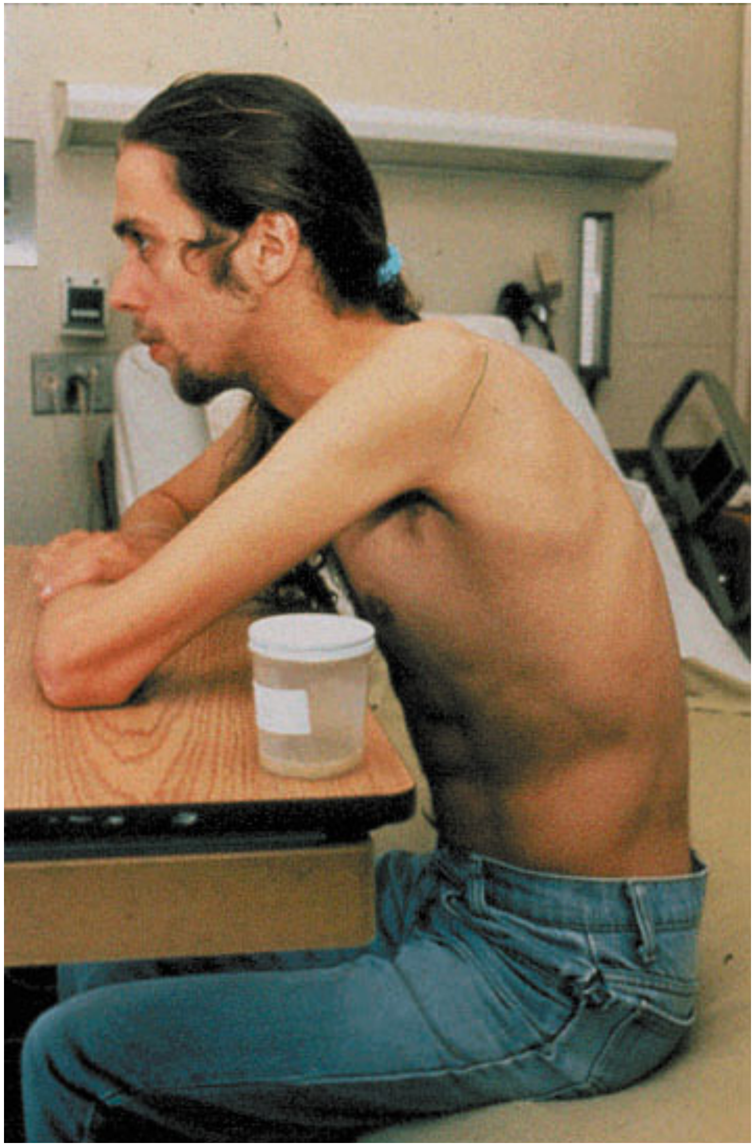
CFTR cDNA corrects *in vitro*

↓

Vectors transfer CFTR cDNA *in vivo*

↓

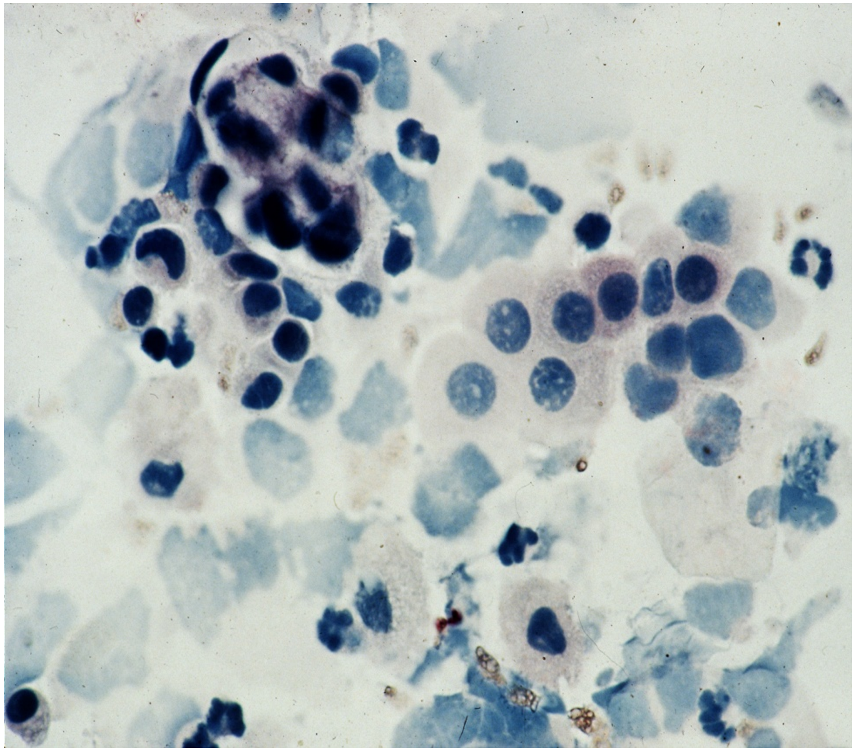
Cure CF?



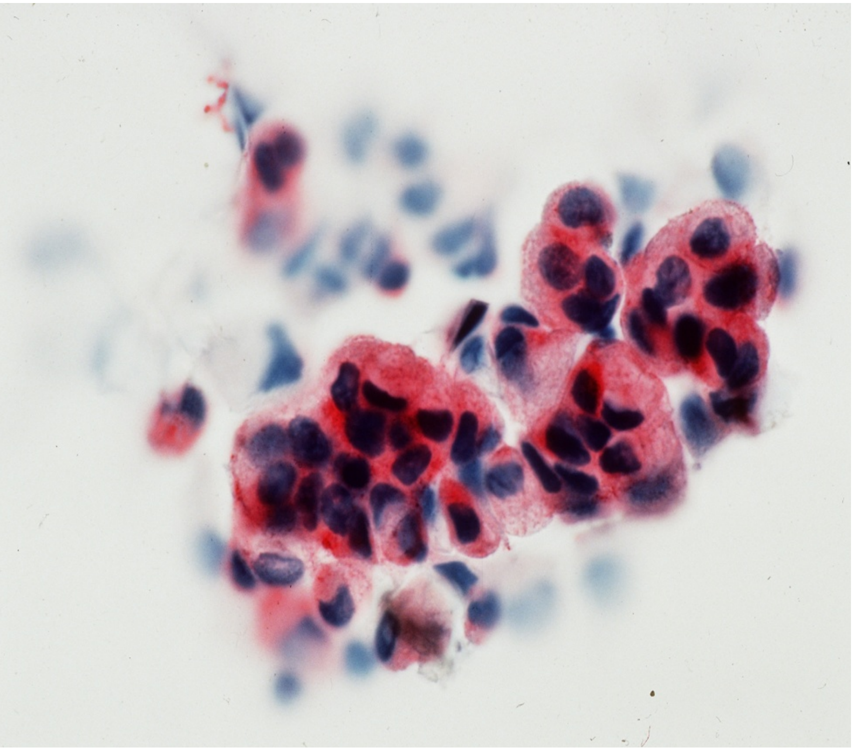

OLYMPUS KEYMED
VIDEO PROCEDURES CART TV-2

In Vivo Adenovirus-mediated Transfer of the Normal CFTR cDNA to the CF Airway Epithelium

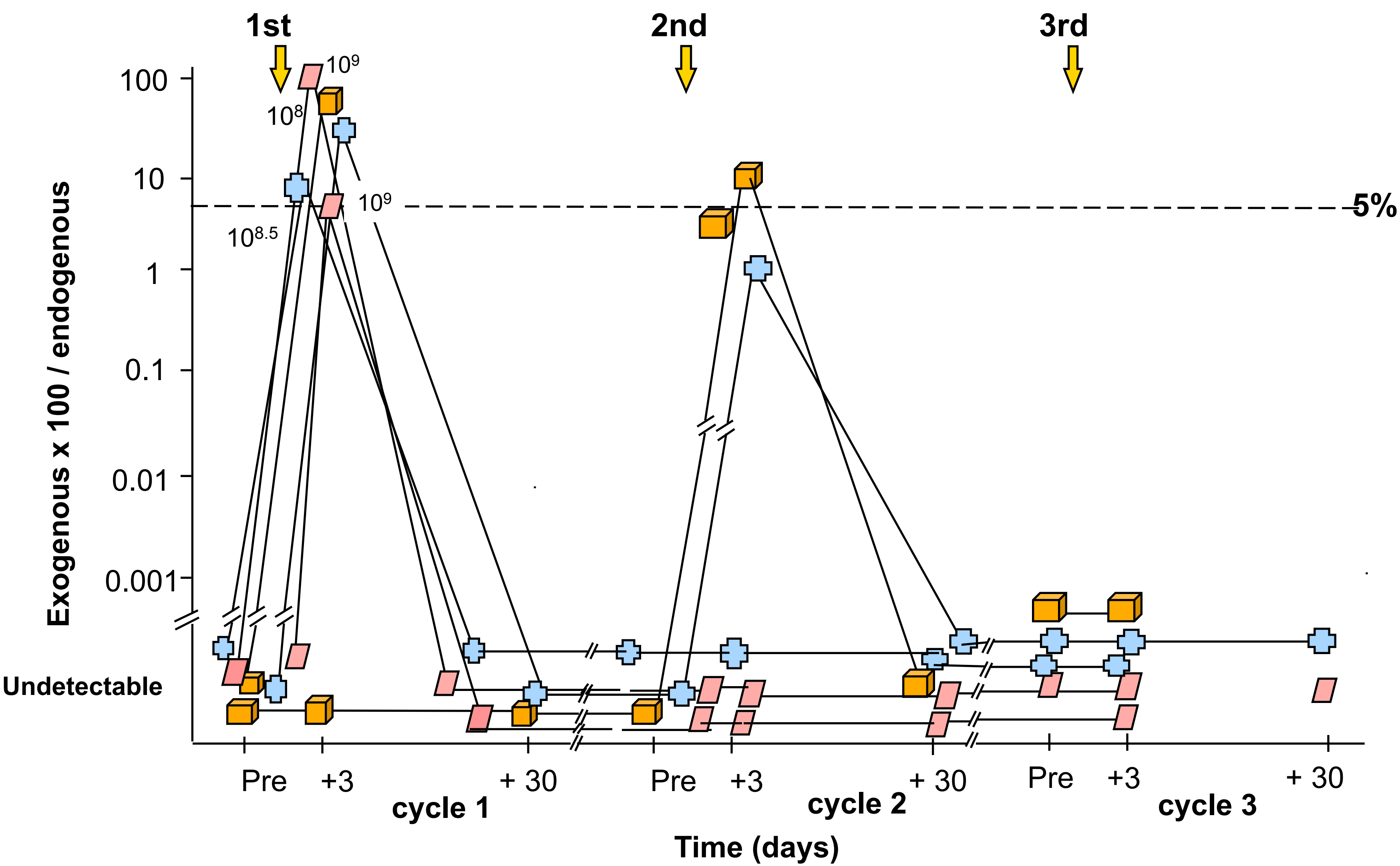
Pre-therapy



4 days



Expression of Vector-derived CFTR mRNA in the Airway Epithelium (10^8 - 10^9 pfu)

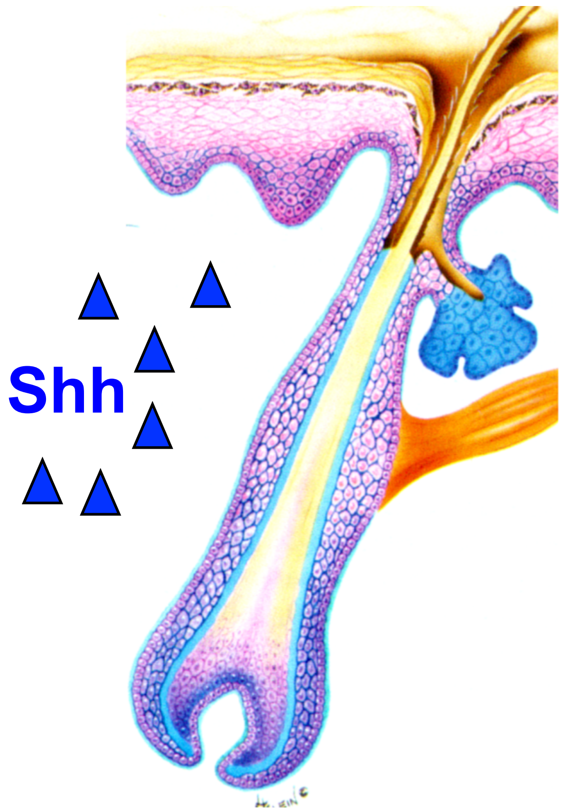


Effective Use of Adenovirus Gene Transfer Vectors

- Adenovirus vectors are safe and highly effective in transferring and expressing genes *in vivo*
- But anti-adenovirus immunity limits expression to ~2 wk
- The effective use of adenovirus vectors takes advantage of these properties to build new biologic structures or to destroy abnormal biologic structures (cancer)

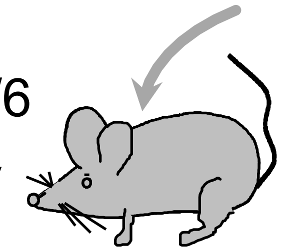
Sonic Hedgehog Gene and Hair Follicles

- Expressed in cells associated with developing embryonic hair follicles
- Transgenic mice overexpressing Shh in skin have hyperproliferation of basal cells
- Shh knockout mice do not develop mature hair follicles



Effect of Intradermal AdShh Administration on Hair Growth

C57Bl/6
19 day

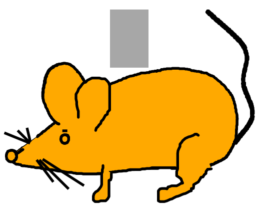


Intradermal

- AdNull
- AdShh

(10^8 pfu)

5 days



Blond hair dye

7 - 14 days

Clip hair

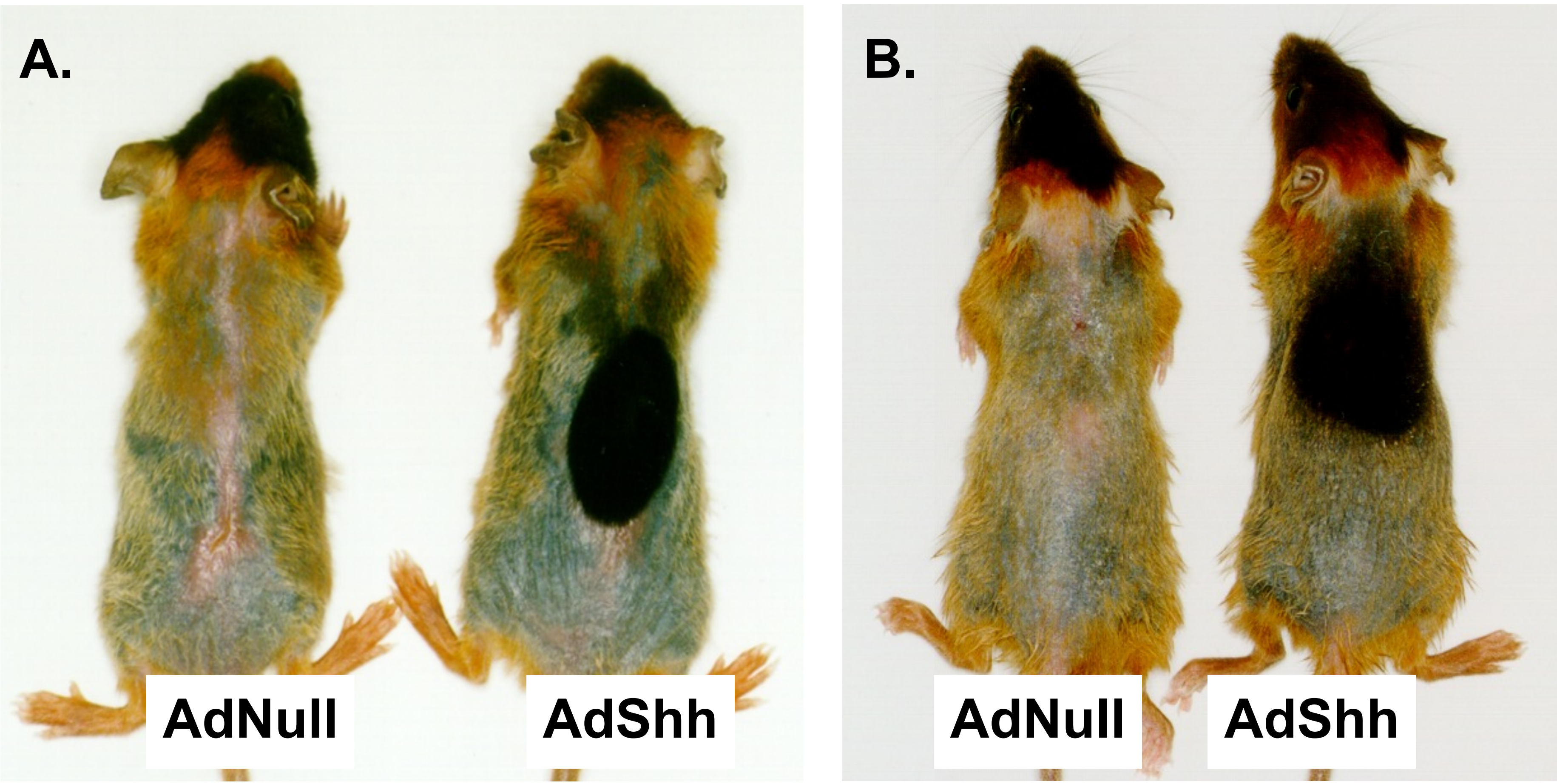
Evaluate

- Black 1hair (new growth) in background of dyed blond hair

Pre-dye **Blond dye**

Day 5

AdShh-mediated Hair Growth
14 days

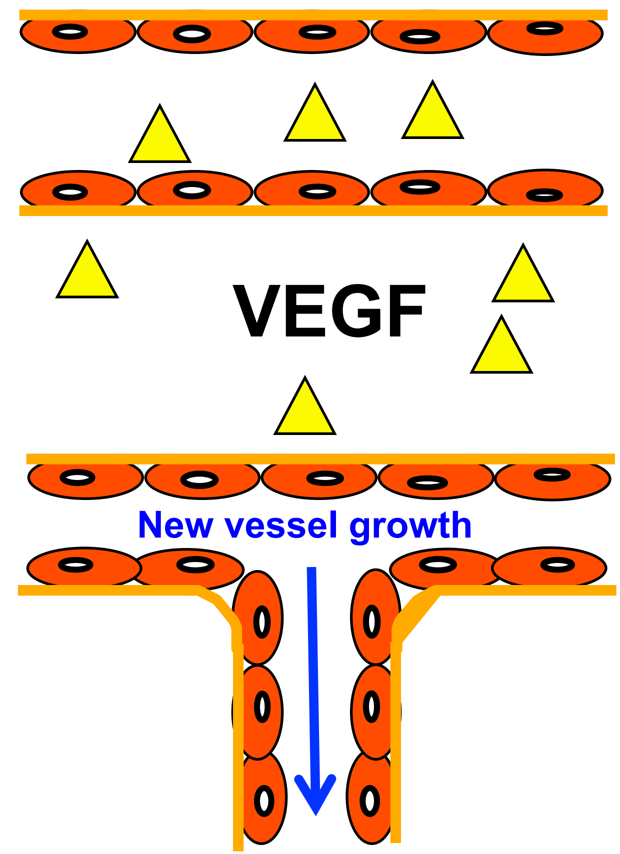


Coronary Artery Disease

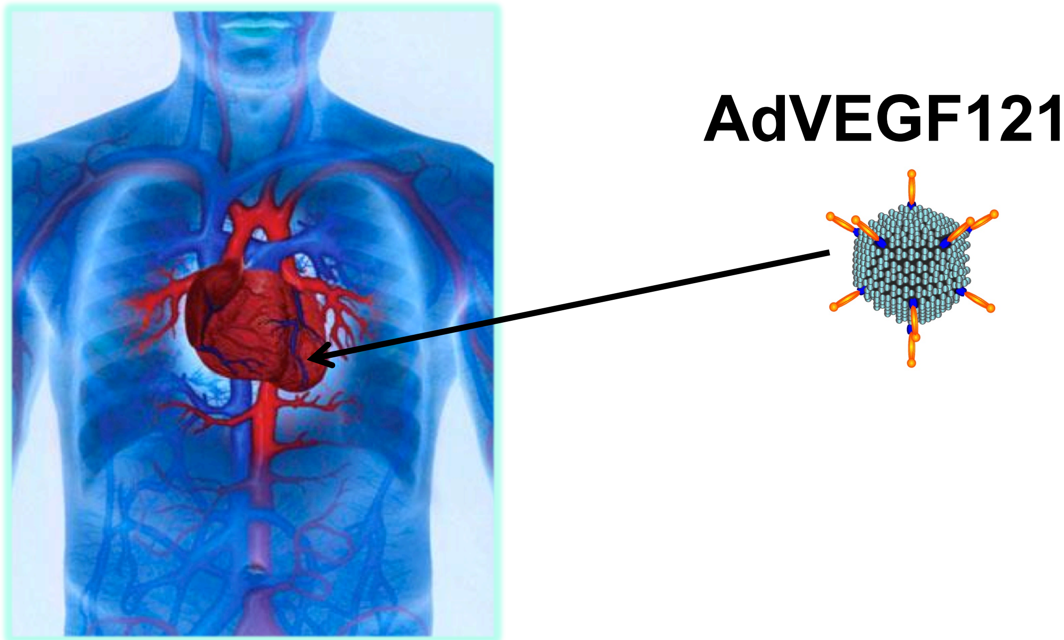
- Responsible for 1 of 6 deaths in US (386,000 deaths/yr)
- 950,000 stents and 400,000 bypass procedures in the US annually
  - Complete revascularization achieved in only about 50% of cases
- Diffuse coronary artery disease
  - Common - 5 million Americans with resulting heart failure
  - Not effectively treated by stents or surgical bypass
  - High mortality, 5 yr risk for end-stage >50%, equivalent to many cancers
  - No effective therapy; only 5,000 receive ventricular assist device and/or cardiac transplant
- Candidates for cardiac angiogenic gene therapy - 1,000,000+ patients annually in the US

Cardiac Gene Therapy with Vascular Endothelial Growth Factor (VEGF)

- Potent mediator that initiates new blood vessel growth (angiogenesis) through receptors localized on endothelial cells lining coronary blood vessels
- VEGF gene codes for 3 isoforms, VEGF 121, 165, 189



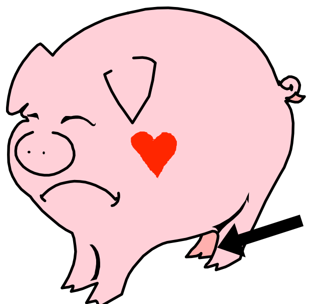
Treatment of Diffuse Coronary Artery Disease with Vascular Endothelial Growth



Strategy

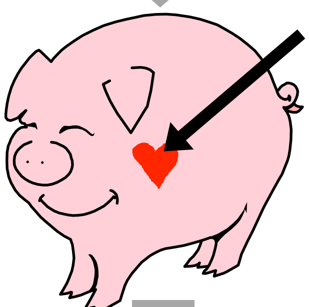
- Using direct cardiac administration of AdVEGF121 using an adenovirus gene transfer vector, induce new blood vessel growth in patients with moderate to severe diffuse coronary artery disease on optimal medical therapy with no other therapeutic options

AdVEGF121-Induced Angiogenesis in the Ischemic Pig Myocardium



Induce ischemia left circumflex

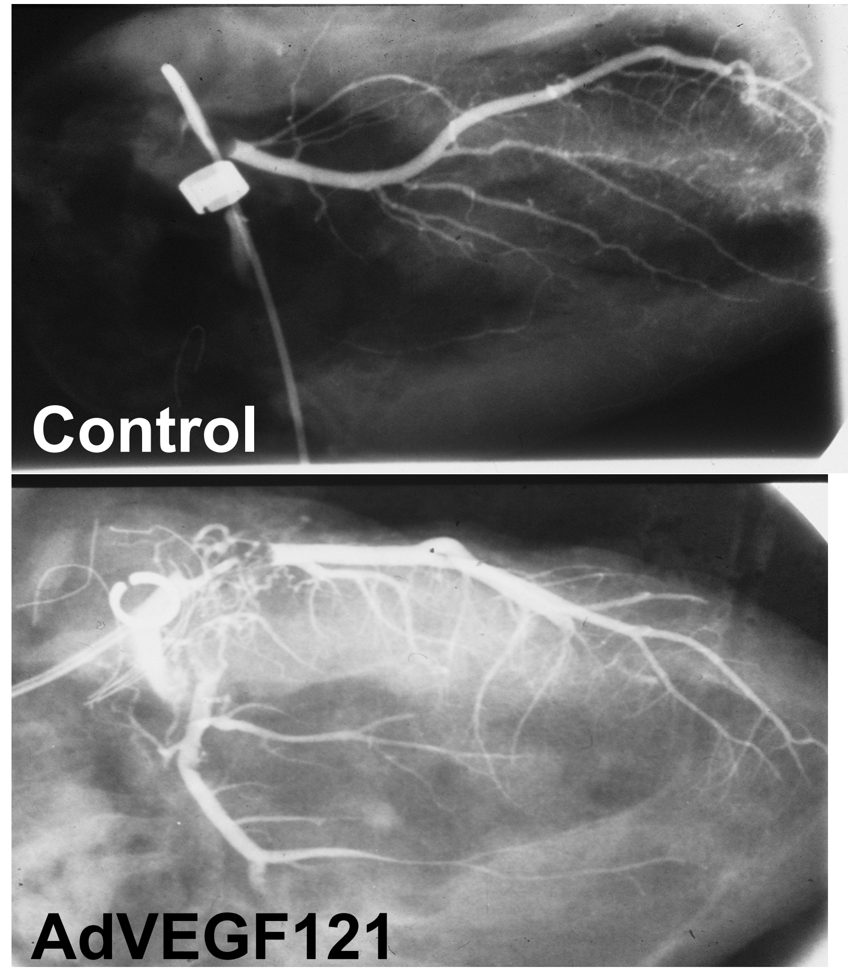
3 wk



Administer AdVEGF121

4 wk

Evaluate
- **Safety**
- **Efficacy**



Example of One Subject in the Phase I Trial

FIRST PERSON

I'm Superstitious About Calling It a Miracle

I run little tests. This afternoon I hauled and stacked wood for an hour—big fireplace logs. Then I did a three-mile quick march with my dog along the road. I felt terrific.

Trying this a year ago, I would have been tempting that ominous stirring that I think of as the Shadow—the dark, incipient something in my chest, bad news that used to arrive with sweats, shortness of breath and pressures and pains wisping about the chest bones like evil electricity. A year ago, hauling the firewood might have killed me.

I am superstitious about calling it a miracle: I don't want to invite further attention from the evil eye. But let me whisper that as far as I am concerned, the news about gene therapy is very good.

Because of severe coronary-artery blockage, I have had two heart attacks, two multiple-coronary-bypass operations (1976 and 1993) and a couple of angioplasties

JAMES KEYSER FOR TIME

PLAYING SQUASH A year ago, this might have killed me

(1998). Last year, when I began having symptoms again, my choices—with further bypass impossible—were 1) to treat the trouble with continued medication (beta-blockers, ACE inhibitors, aspirin, furosemide and so on), hoping, further down the line, for a heart transplant; or 2) to try to sign up for one of the new, experimental operations (gene therapy or laser therapy) designed to encourage the growth of new blood vessels in the heart.

My cardiologist, Dr. Robert Ascheim, put me in touch with Dr. Todd Rosengart, then leader of a team at Weill Medical College of Cornell University in New York City tha
both las
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Rosengart p
operation in mid
made a 5-in. inc
back—the scar t
could mail letter
and pried open
ache when I sne
the heart, which
20 times with a
DNA that instruc
Grow vessels he
A month lat
to the hospital f
angiogram, thali
other tests. The
was that the new

would grow in the first month—or not at all. The tests detected no new vessels. Failure. I reverted to Plan A and resigned myself to the prospect of, at best, a much restricted life.

But my face—once the color of a sidewalk, with a nasty eggplant underglow—began to turn almost rosy. It

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TIME, MARCH 19, 2001

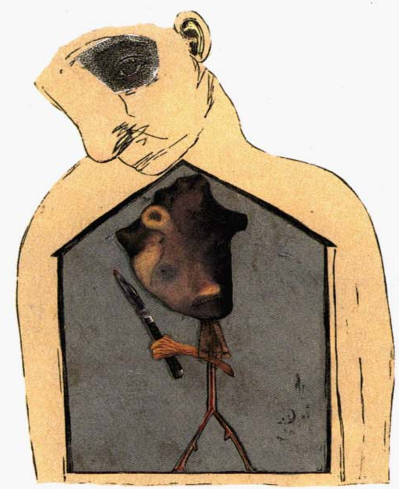
ESSAY

Lance Morrow

Lessons of a Bad Heart

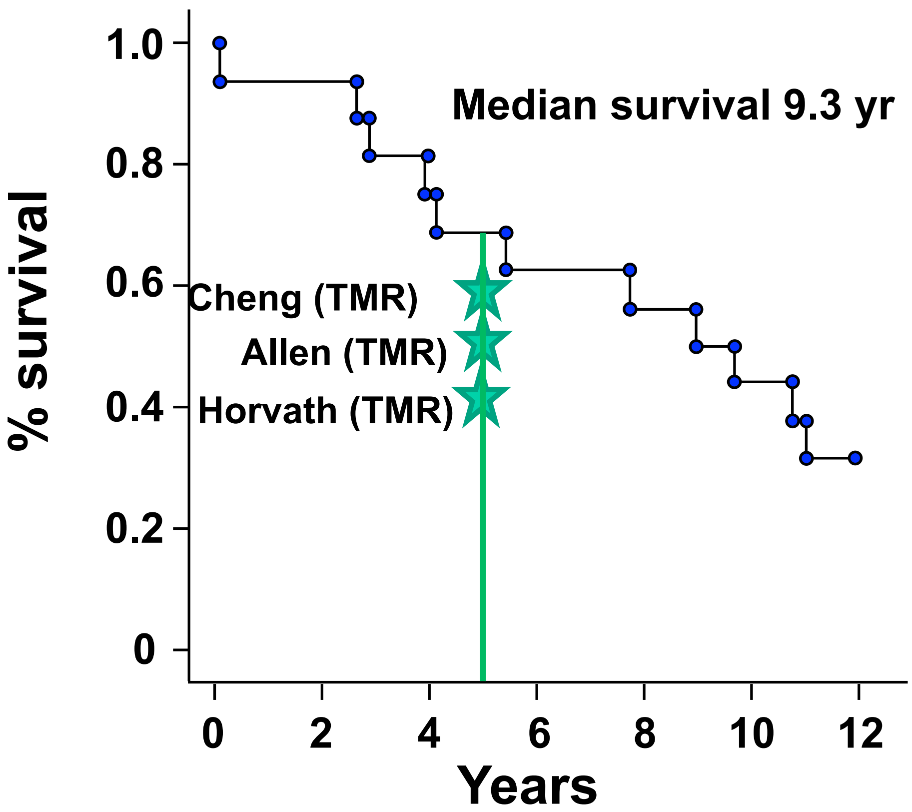
How Dick Cheney and I live on the edge and quiet the killer in the basement

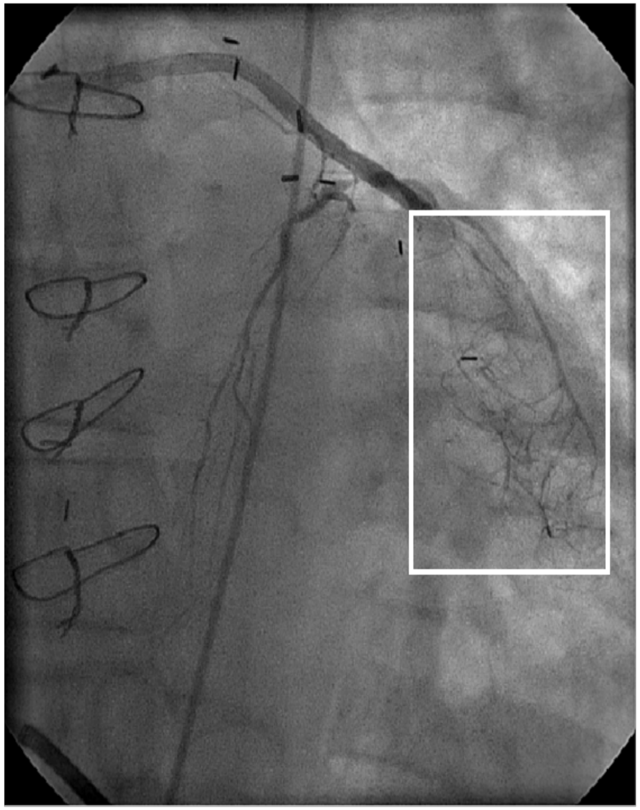
FROM TIME TO TIME, I HAVE FELT DICK CHENEY'S PAIN. We are both about the same age—I am some months older—and we both had our first heart attacks in our mid-30s. Over the years, we have been similarly inconvenienced by heart attacks. The elephant has stepped on his chest four times, and on mine twice. Cheney has had one multiple-bypass operation; I have had two of them. We have both had angioplasties, with stents. A couple of years ago, I drew ahead of Cheney in the fancy-therapy category by having DNA injected into my myocardium in order to induce the growth of new vessels—angiogenesis, a still experimental but highly promising technique that has, in my case, worked miraculously well.



Five Year and Median Survival after Adenovirus VEGF121 Cardiac Gene Therapy Exceeded Expectations

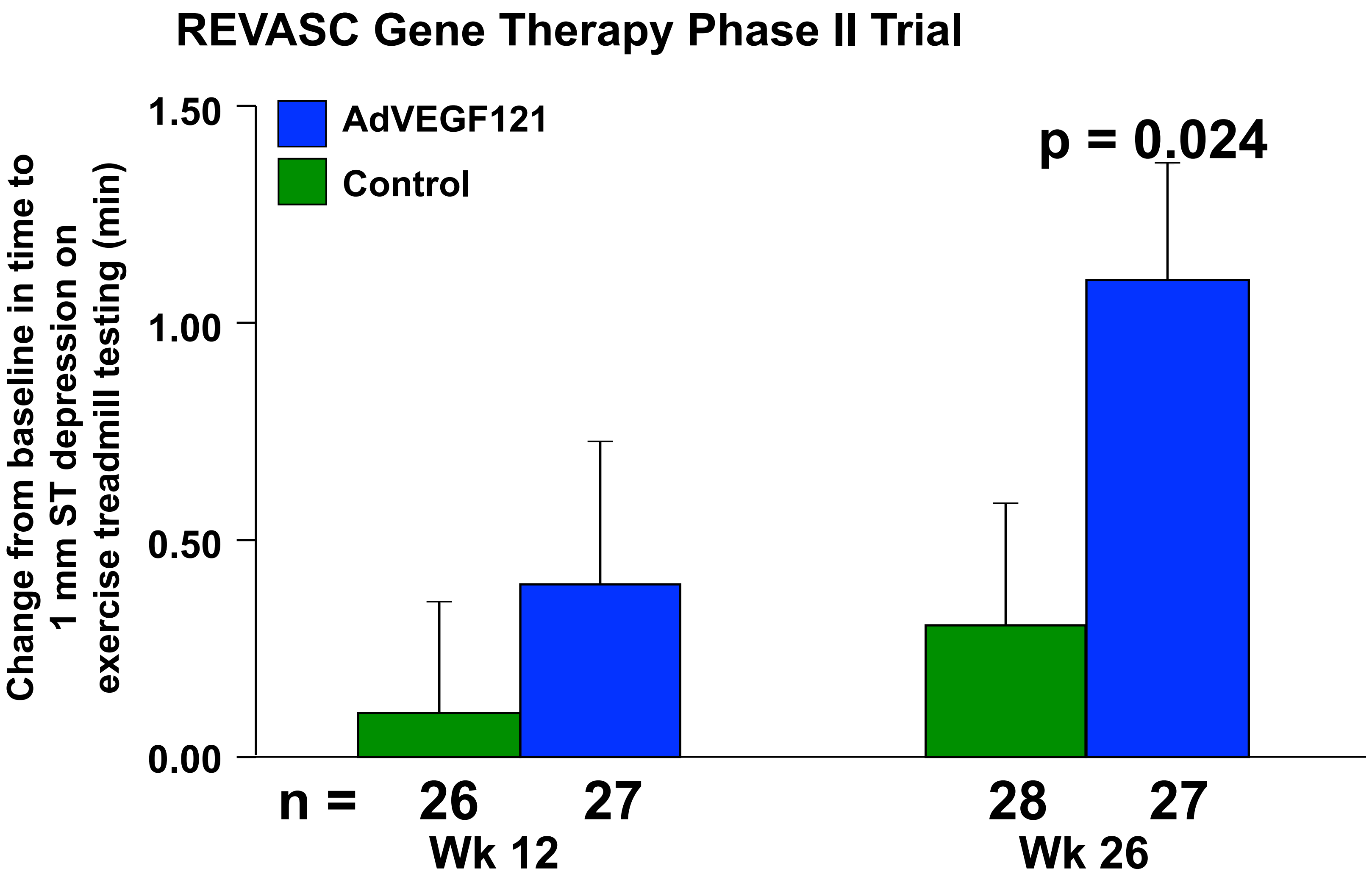
AdVEGF121 WCMC trial 10 yr followup*





Rosengart et al, *Hum Gene Ther.* 2013;24:203; Allen et al. *Ann Thorac Surg*. 2004; 77, 1228-34; Cheng et al. *Innovations*. 2006; 1: 295; Horvath et al. *Circulation*. 2001; 104: I81.

Statistically Significant Clinical Improvement in Phase II

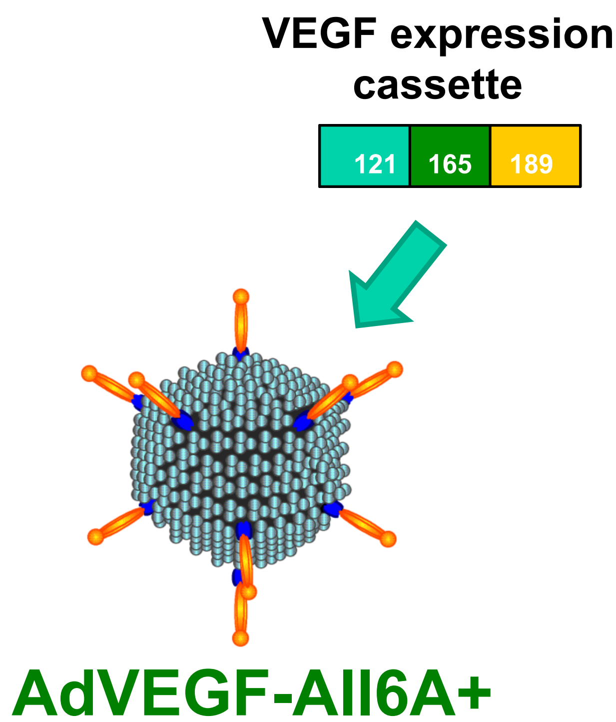


Stewart et al. *Gene Ther*. 2006;13:1503-11

New, More Effective Strategy – Instead of One VEGF Isoform, Use All Three Isoforms

AdVEGF-All6A+ vector

- Expression cassette coding for all 3 VEGF isoforms (121, 165 and 189)
- 10 to 100-fold more effective than an adenovirus vector coding for a single isoform
- Safer - designed to stay within the myocardium, reducing systemic administration
- FDA approved investigational new drug application to carry out studies in Qatar

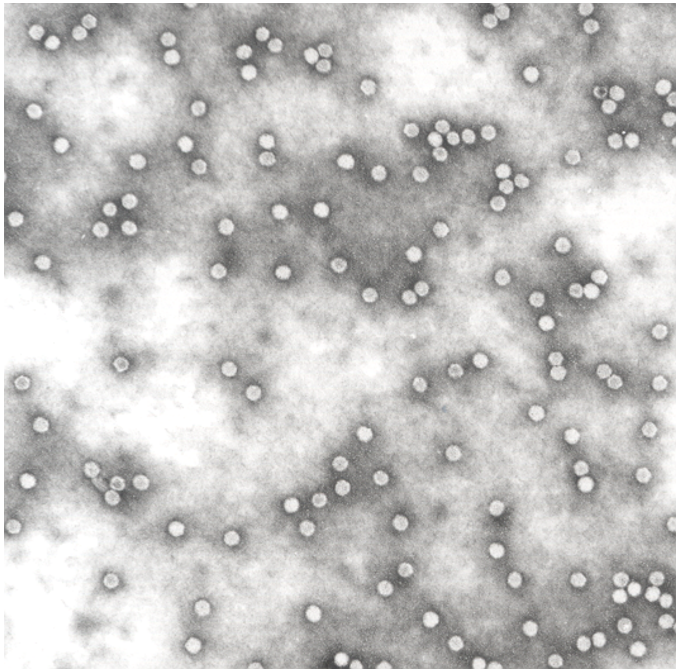


Ongoing Adenovirus Clinical Trials

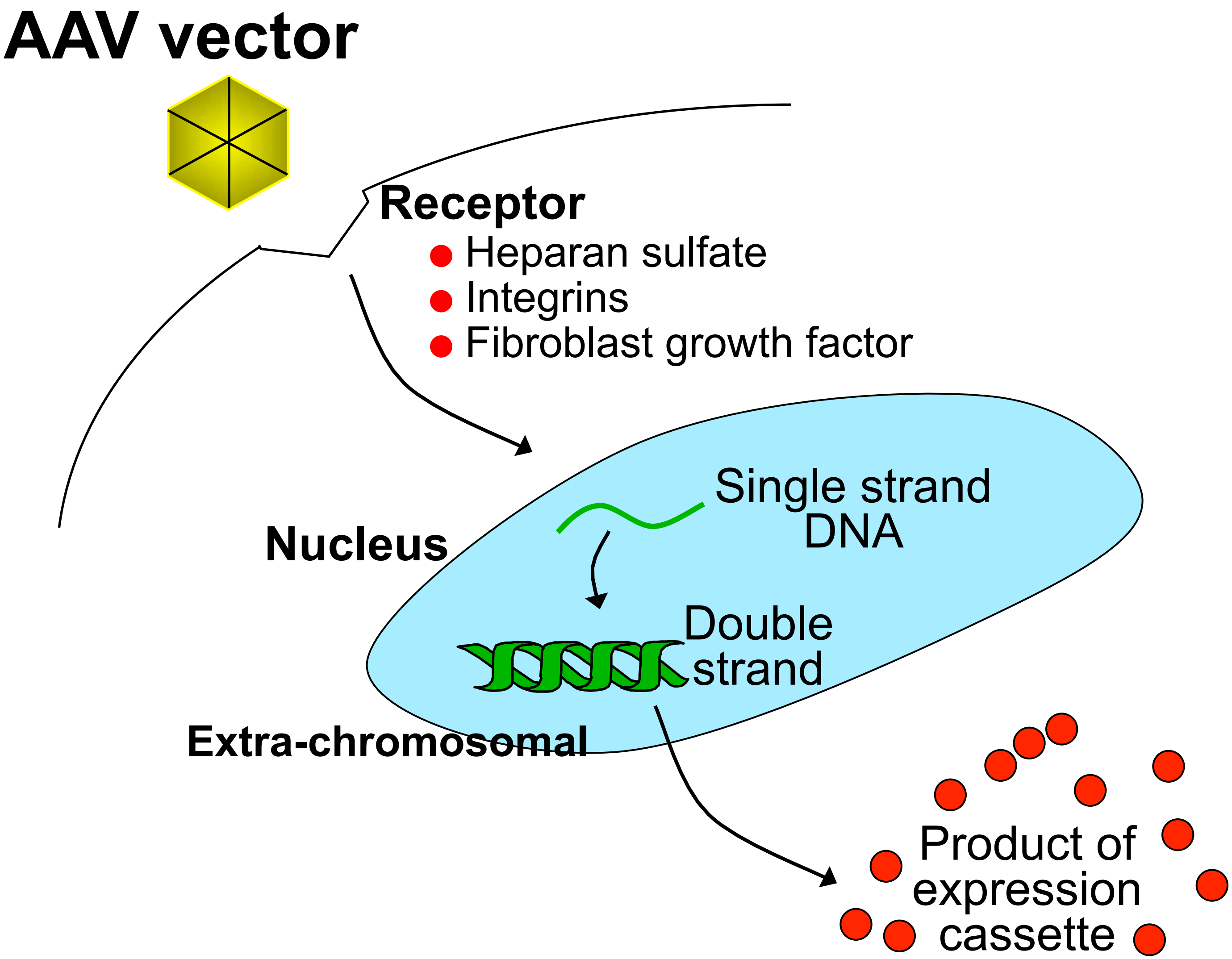
- **Acquired disorders** – cardiac angiogenesis, heart failure, various cancers

Adeno-associated Virus Vectors

- Small (20 nm) parvovirus, single strand DNA
- 6 human serotypes, >50 non-human serotypes
- Capsid can be modified to alter cell specificity
- Delete all viral sequences, insert promoter + therapeutic transgene
- 4.5-5.0 kb expression cassette
- Vector of choice for *in vivo* applications, but only in cells that are not proliferating

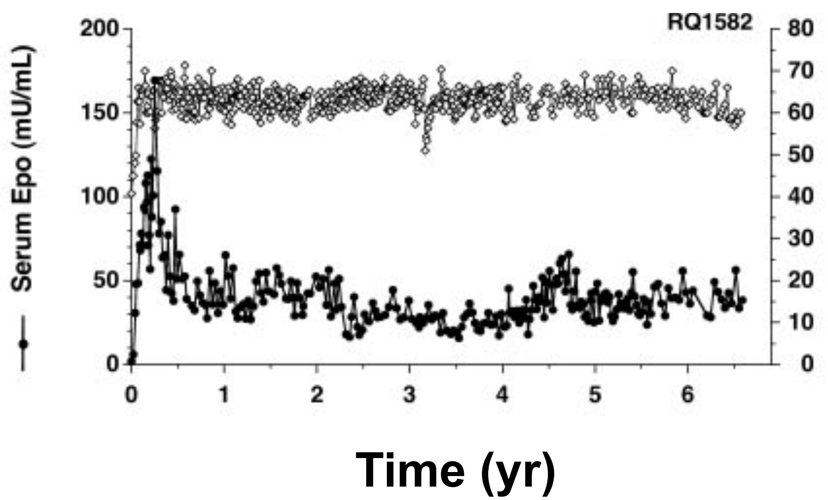


Gene Transfer Using Adeno-associated Virus Vectors

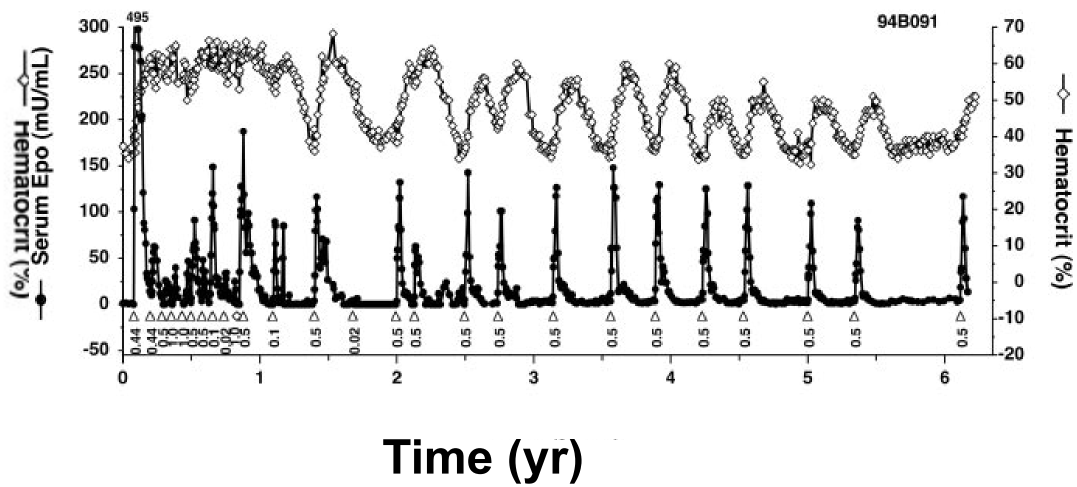


Long-term Expression of Erythropoietin Mediated by AAV1 Skeletal Muscle-transduced Primates[1]

A. Constitutive



B. Regulated



[1] Rivera V et al Blood 2005; 105, 1424

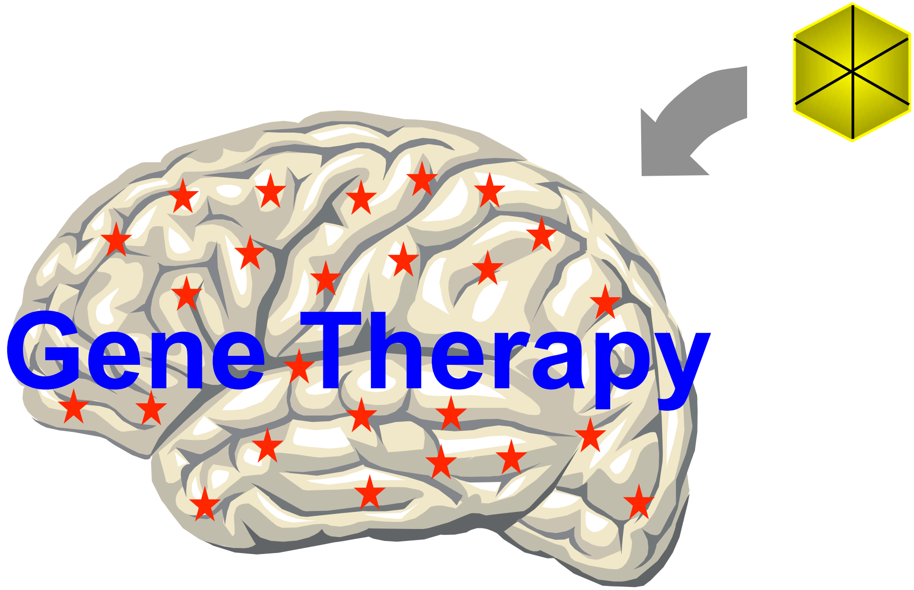
Success with Adeno-associated Vectors

- Lipoprotein lipase deficiency (Glybera, uniQure) – liver gene transfer, approved in Europe
- Factor IX hemophilia – liver gene transfer
- Leber congenital amaurosis (hereditary retinitis pigmentosa) – retina gene transfer

Ongoing AAV Clinical Trials

- **Mendelian disorders** – hemophilia B, Batten disease, metachromatic leukodystrophy, alpha 1-antitrypsin deficiency, spinal muscular atrophy, Pompe disease, retinitis pigmentosa, choroideremia
- **Acquired disorders** - macular degeneration, Alzheimer’s, Parkinson’s, cardiac failure

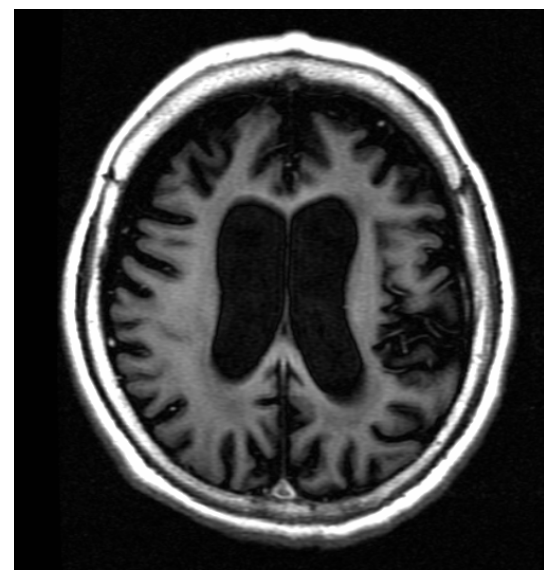
Gene Therapy for Metabolic CNS Disorders



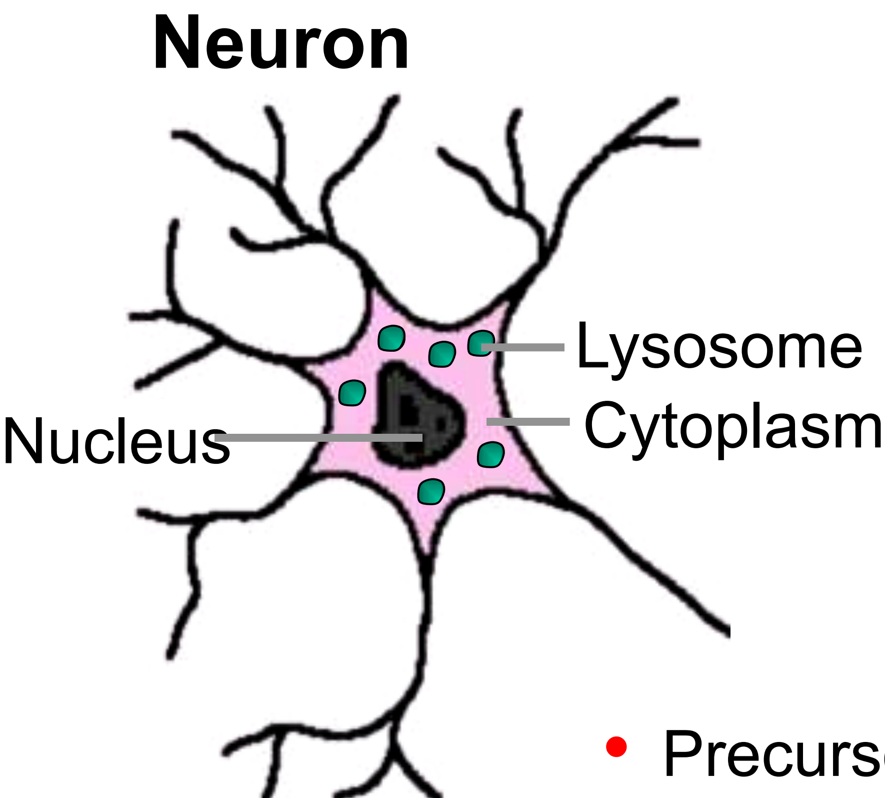
- **Requirement – most of the metabolic CNS disorders affect most of the CNS, the goal is delivery the gene diffusely throughout the brain**

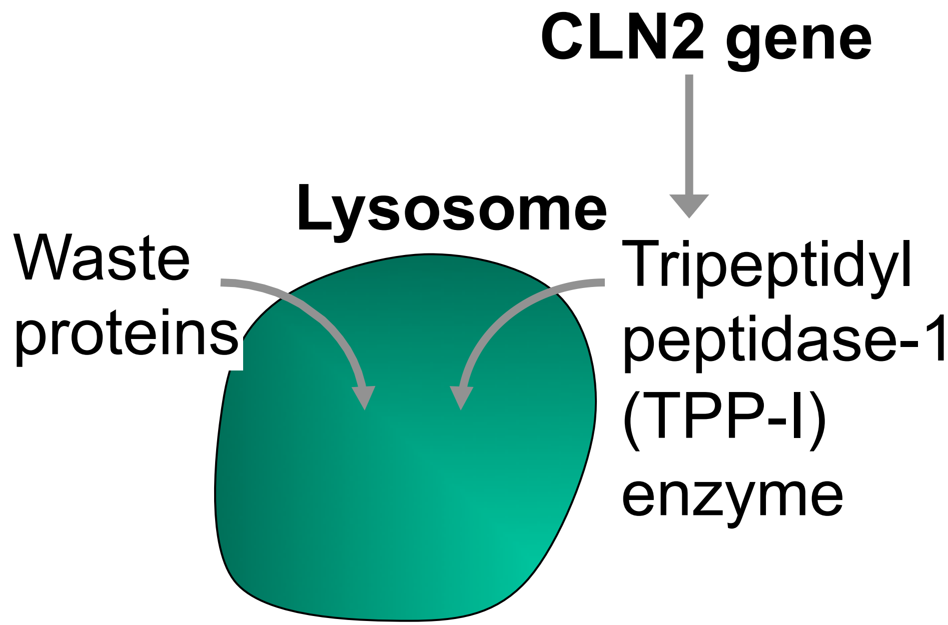
Late Infantile Neuronal Ceroid Lipofuscinoses (LINCL, Batten Disease)

- Autosomal recessive,
  ~ 400-600 cases worldwide
- Disease onset ages 2-4
- Cognitive impairment, visual failure, seizures, and deteriorating motor development, leading to a vegetative state and death by ages 8-12



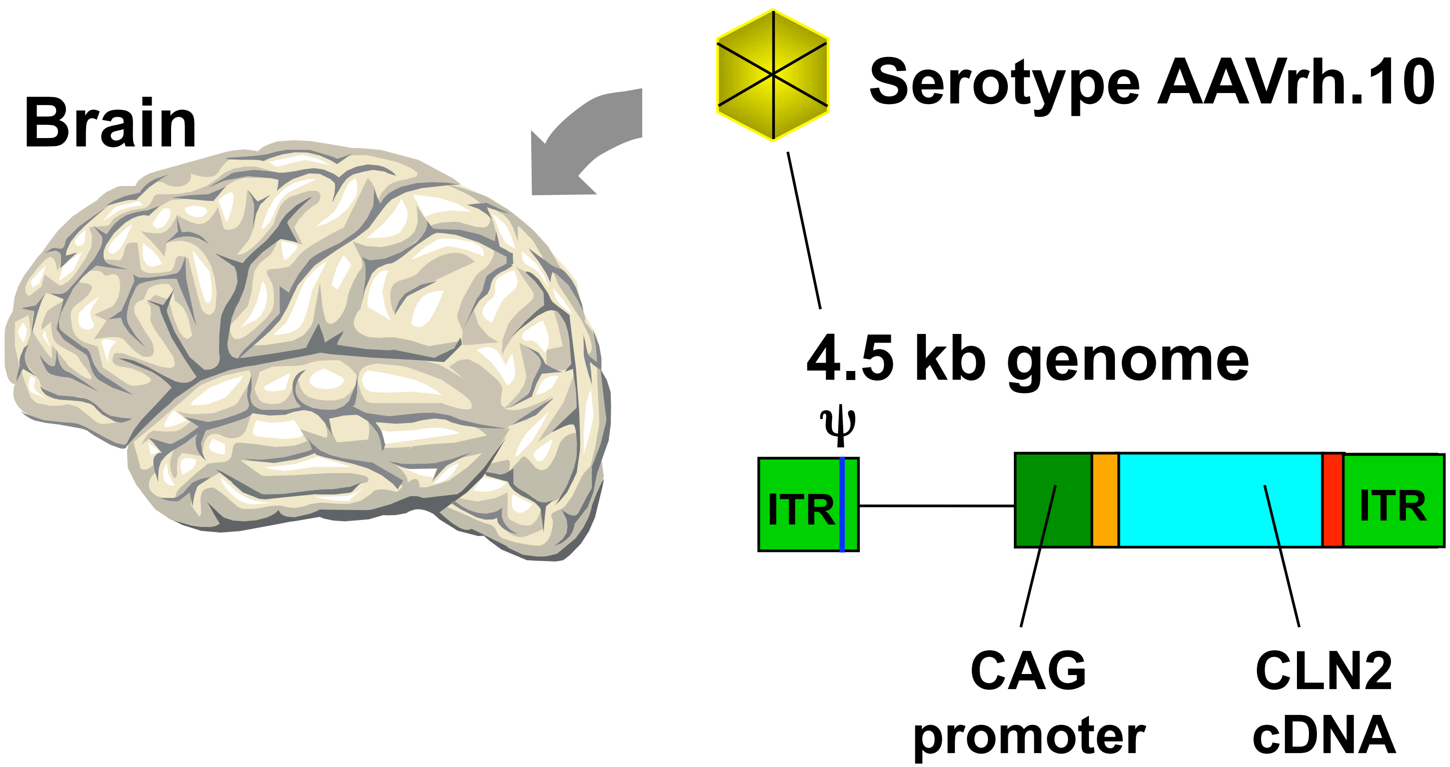
LINCL Is Caused by Mutations in the CLN2 Gene



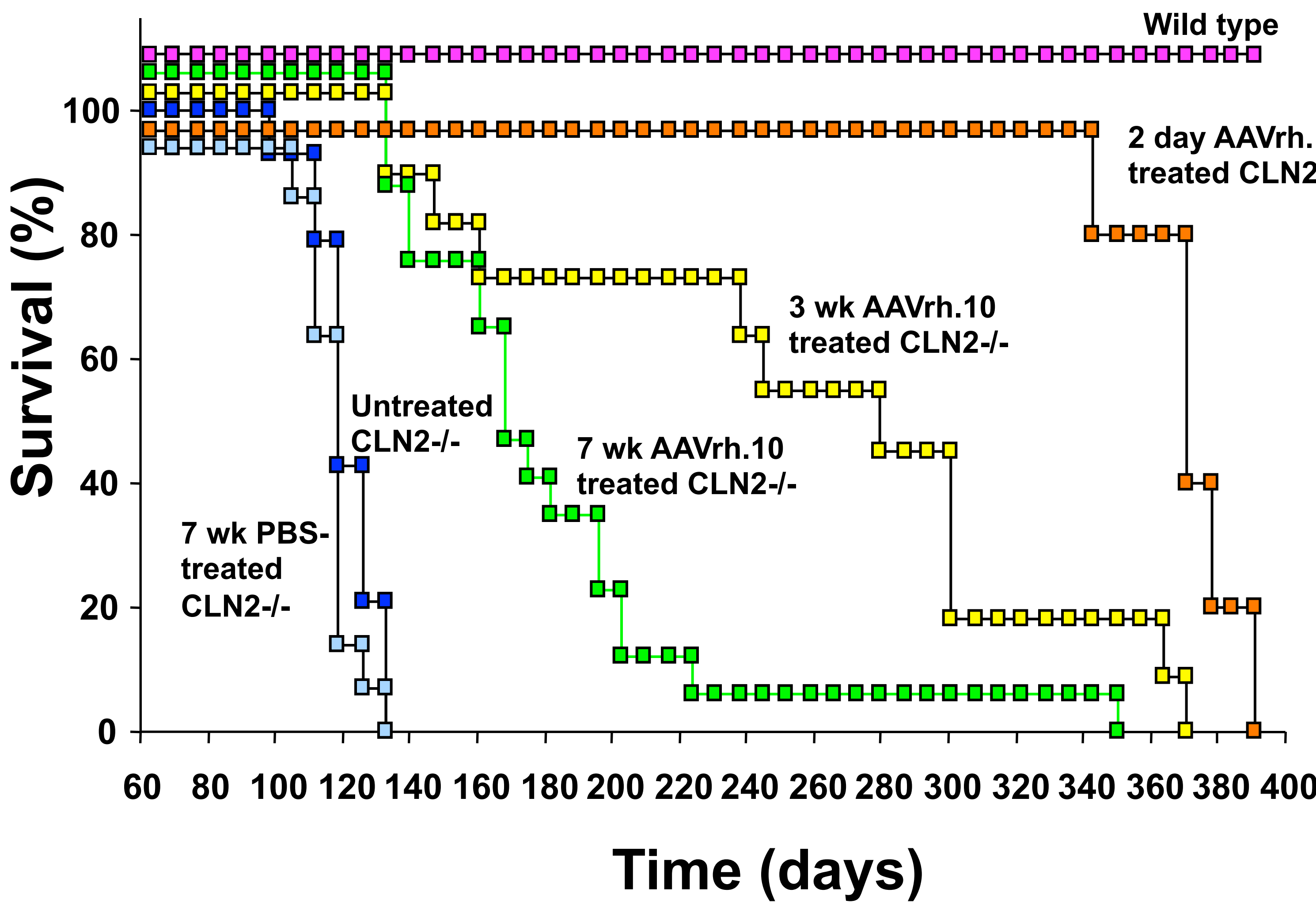


- Precursor TPP-1 is secreted and taken up by the mannose-6-phosphate pathway of neighboring cells

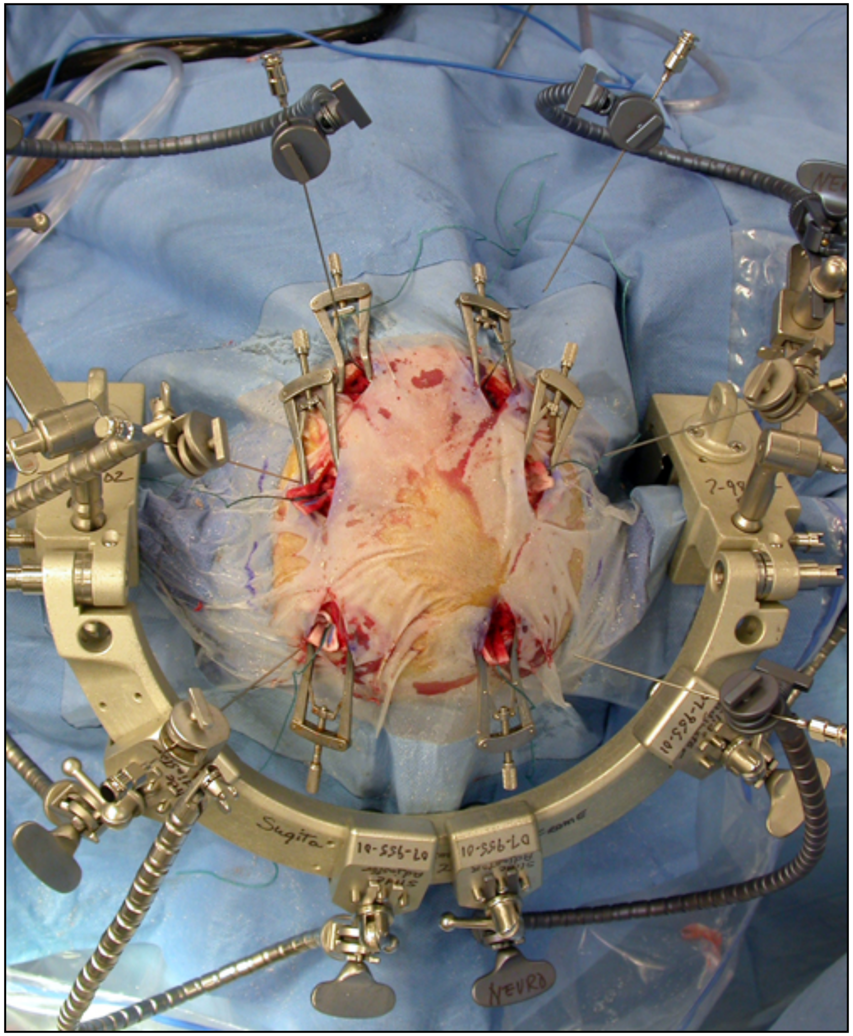
2^{nd} Generation Gene Therapy for LINCL

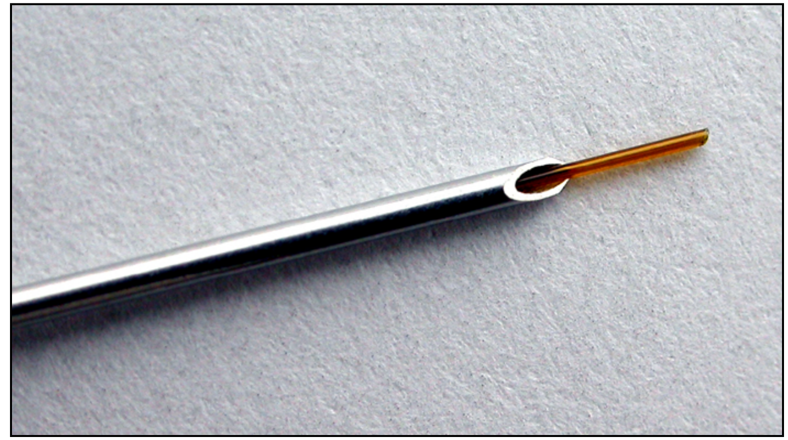


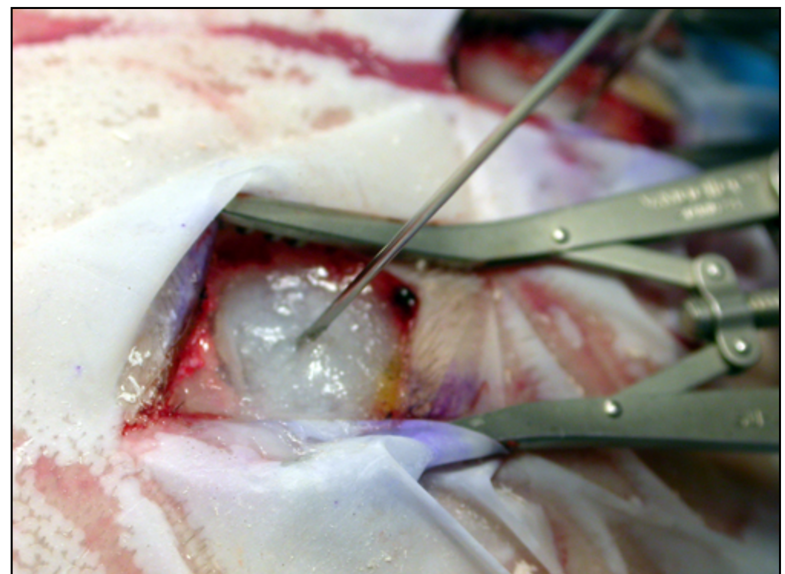
Survival of CLN2-/- Mice Treated at Different Times with AAVrh.10hCLN2



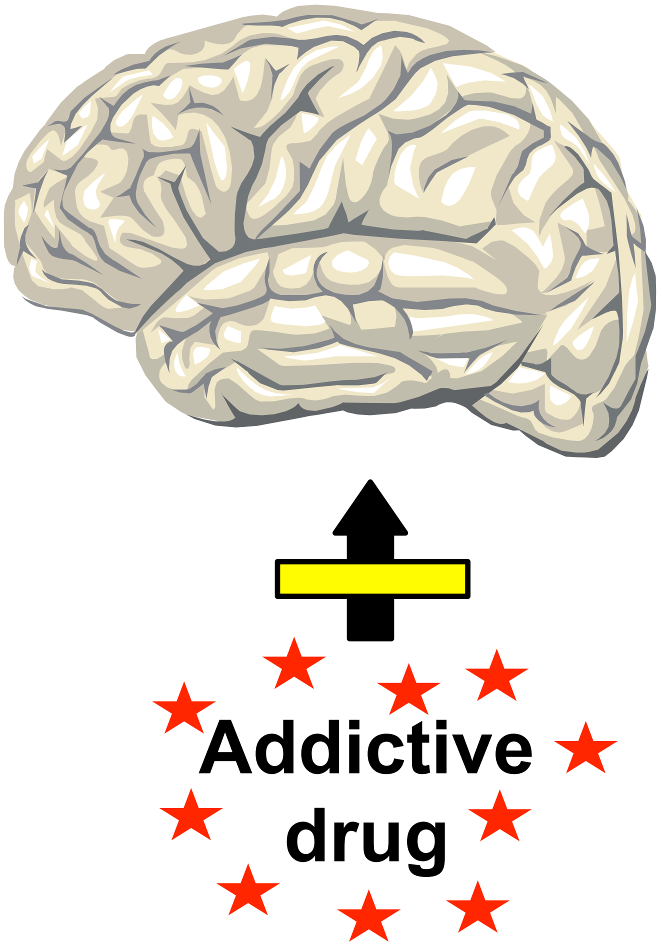
AAV Vector CNS Administration

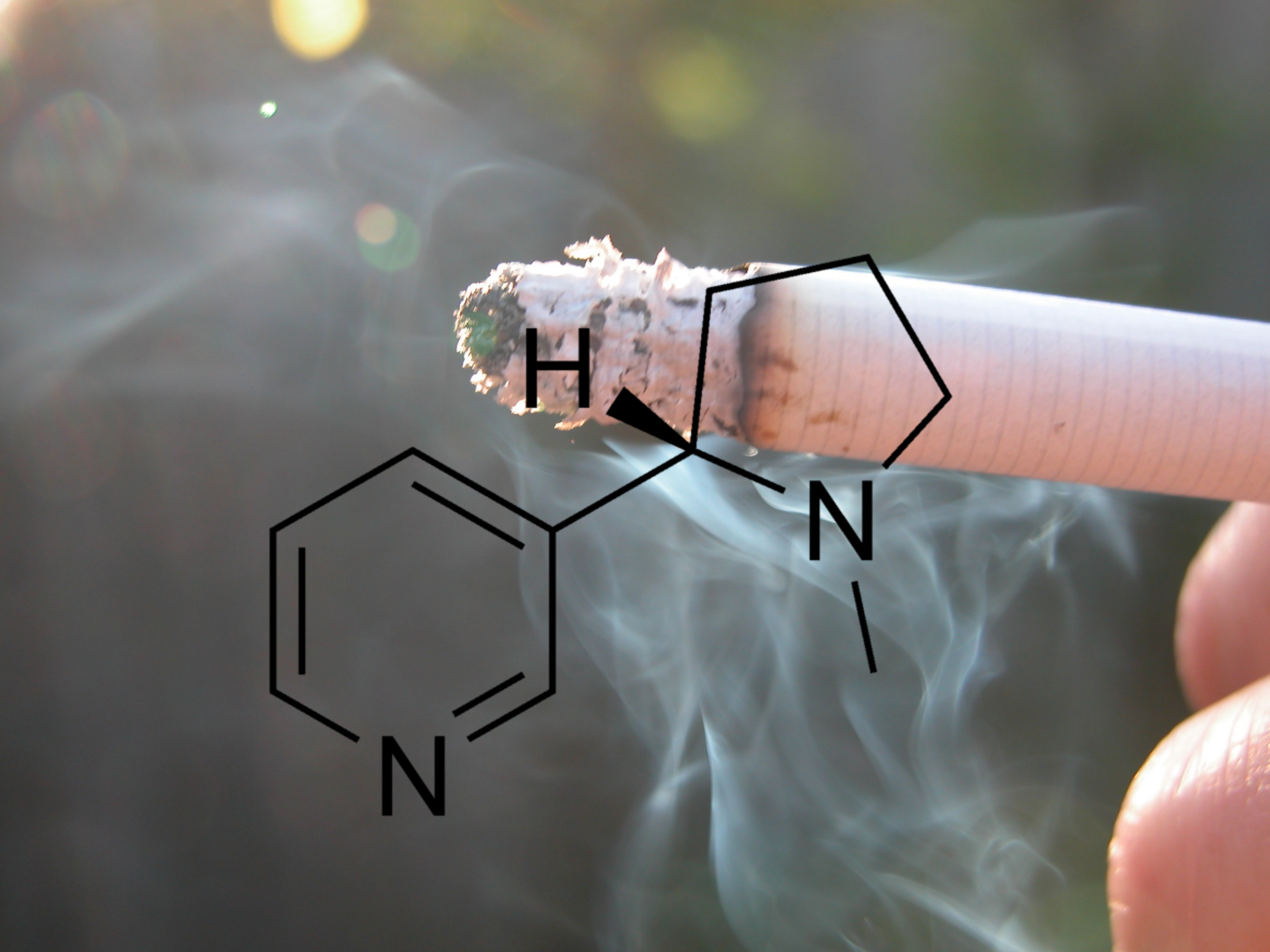






Vaccines to Shield the CNS from Addictive Drugs

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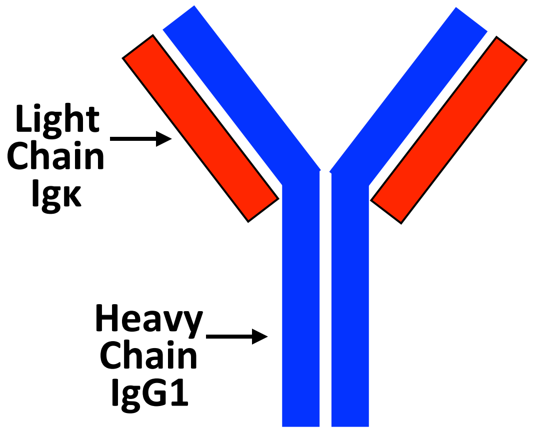
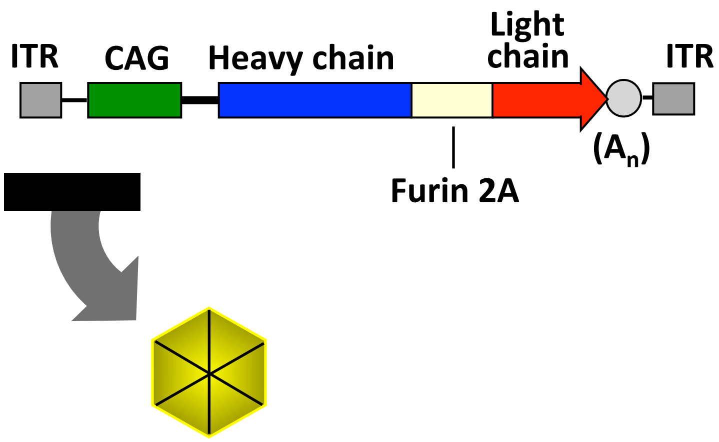
Generation of an Adeno-associated Virus Gene Transfer Vector Coding for an Anti-nicotine Monoclonal Antibody



- Anti-nicotine antibody (NIC9D9)

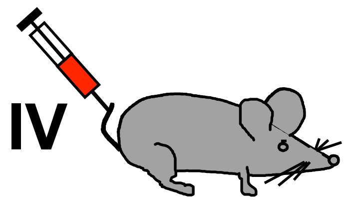
Expression cassette



AAVantiNic

Persistence of AAVantiNic-directed Expression of an anti-Nicotine Monoclonal Antibody

- AAVantiNic
  (10^{11} genome copies)

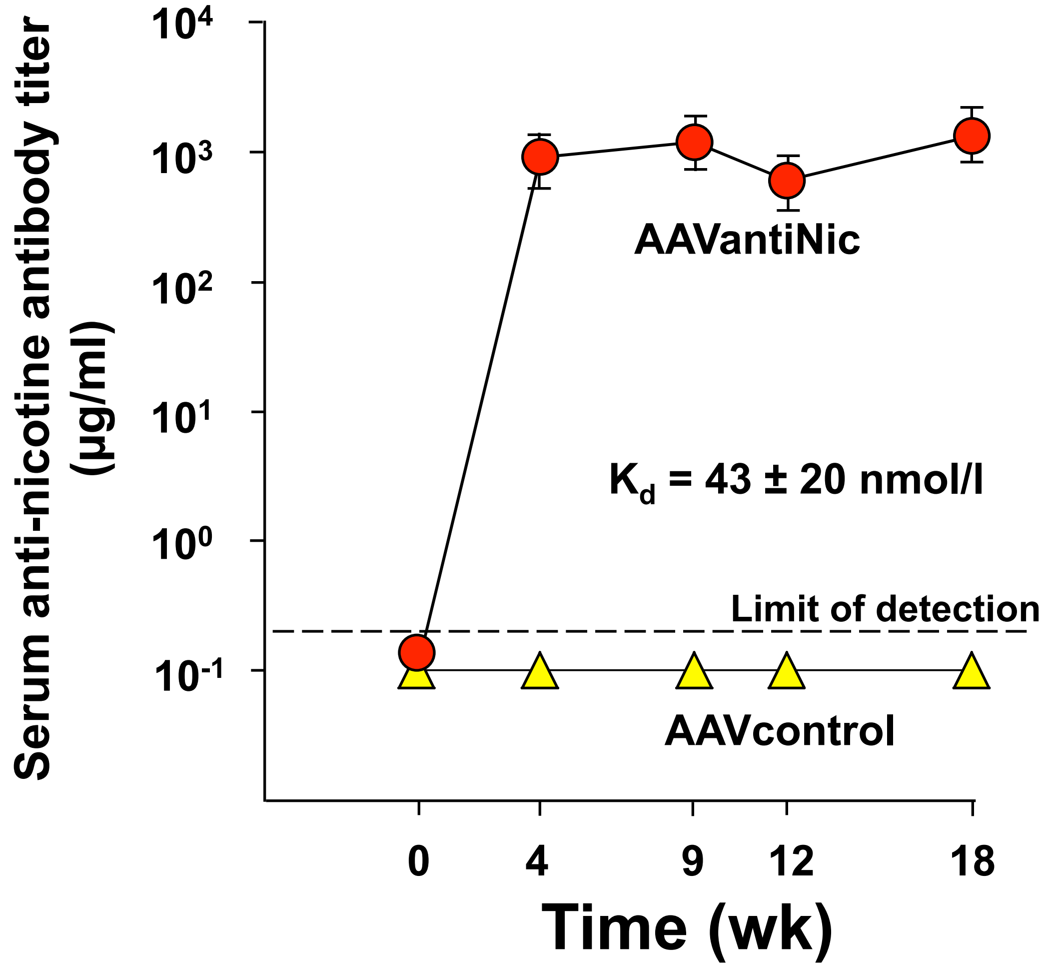


C57Bl/6
n=5

0-18 wk

Evaluate

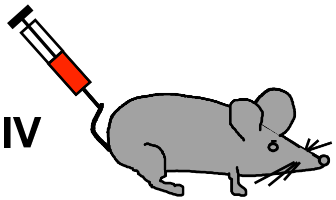
- Serum anti-nicotine antibody titers (ELISA)



AAVantiNic Shields the Brain from Systemic Nicotine

- **AAVantiNic**
  (10^{11} genome copies)

IV



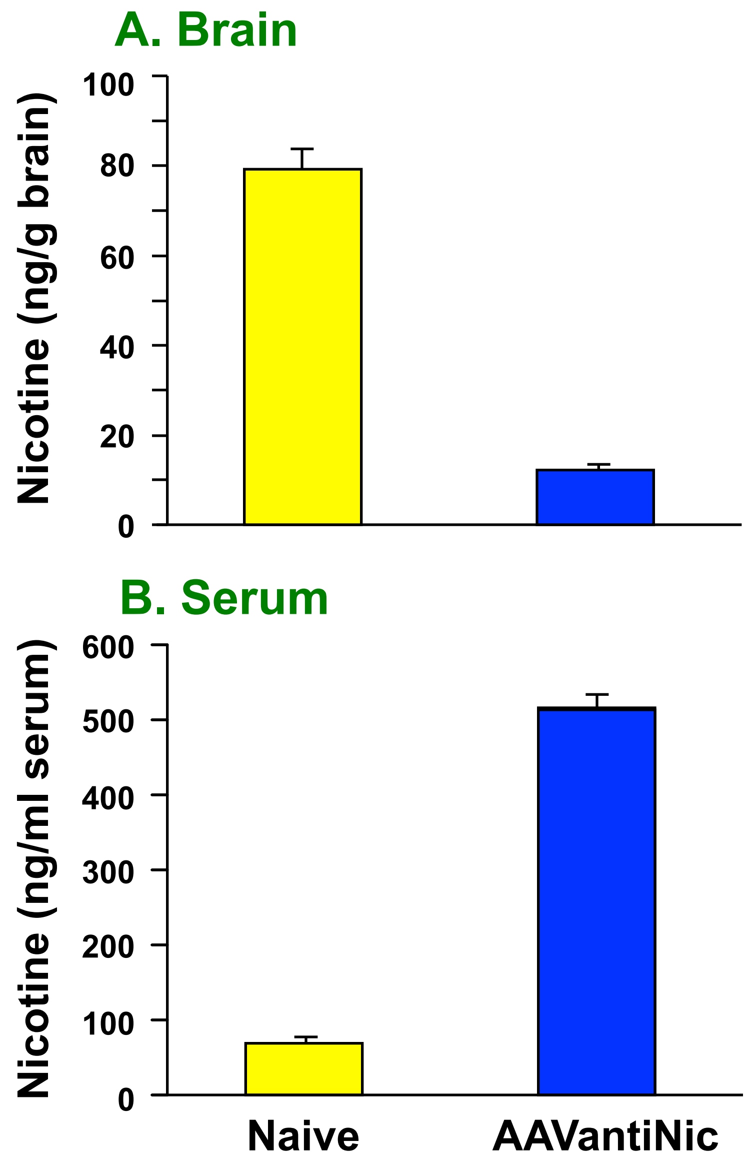
C57Bl/6
n=5

4 months

Nicotine
(0.03 mg/kg, IV)

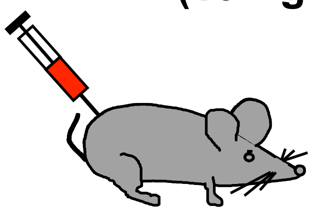
1 min

Assess
- **Brain and blood nicotine levels**



AAVantiNic Immunization Blocks Nicotine-induced Hypo-locomotion

- **AAVantiNic (10^{11} genome copies)**



C57Bl/6
n=10

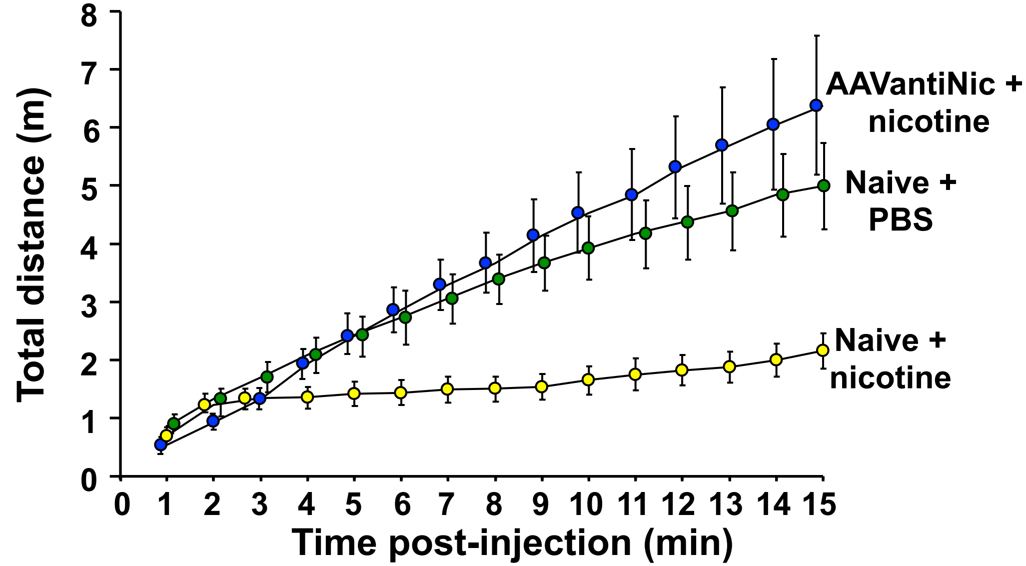
7 wk

Nicotine (0.5 mg/kg, subcutaneous)

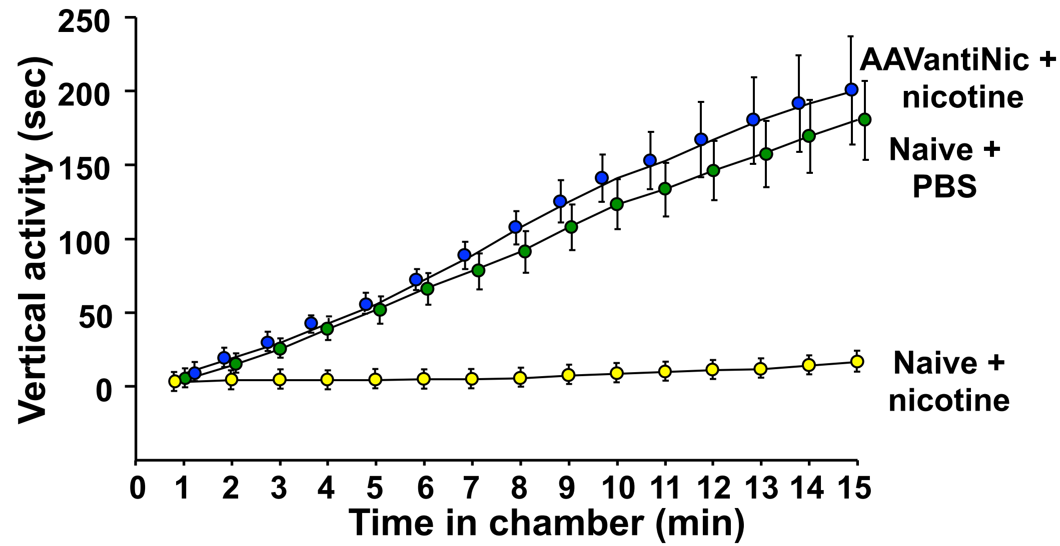
Assess

- **Cumulative distance traveled (15 min)**
- **Vertical activity time (15 min)**

A. Total distance

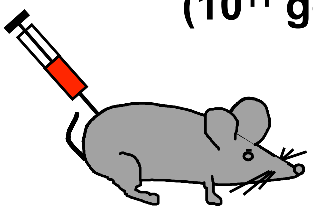


B. Vertical activity



AAVantiNic Prevents Cardiovascular Effects of Nicotine

- AAVantiNic (10^{11} genome copies)



C57Bl/6
n=4

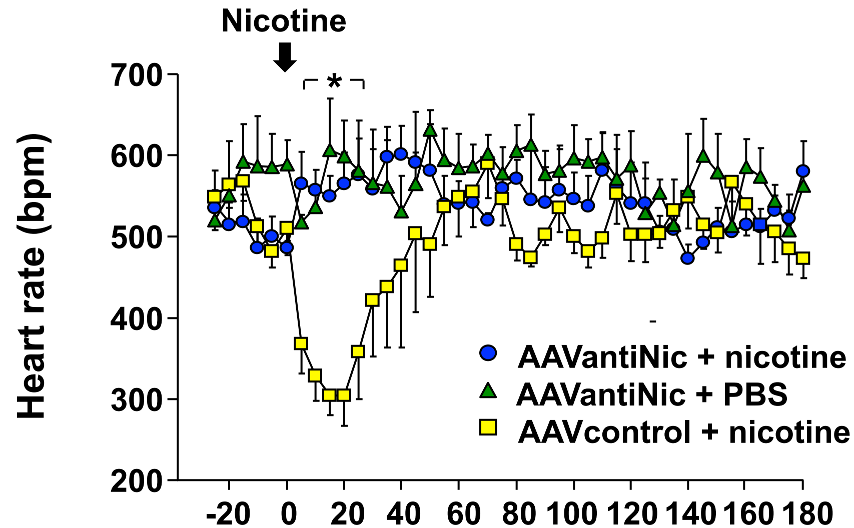
5 wk

Nicotine
(1.0 mg/kg, subcutaneous)

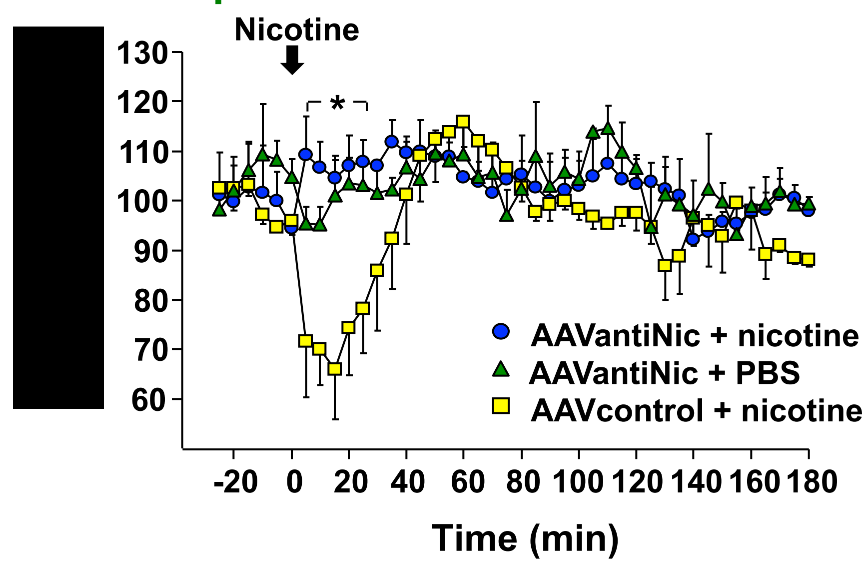
Evaluate

- Heart rate and mean arterial pressure

A. Heart rate



B. Blood pressure



Gene Therapy 2014