Cardiovascular Gene Therapy

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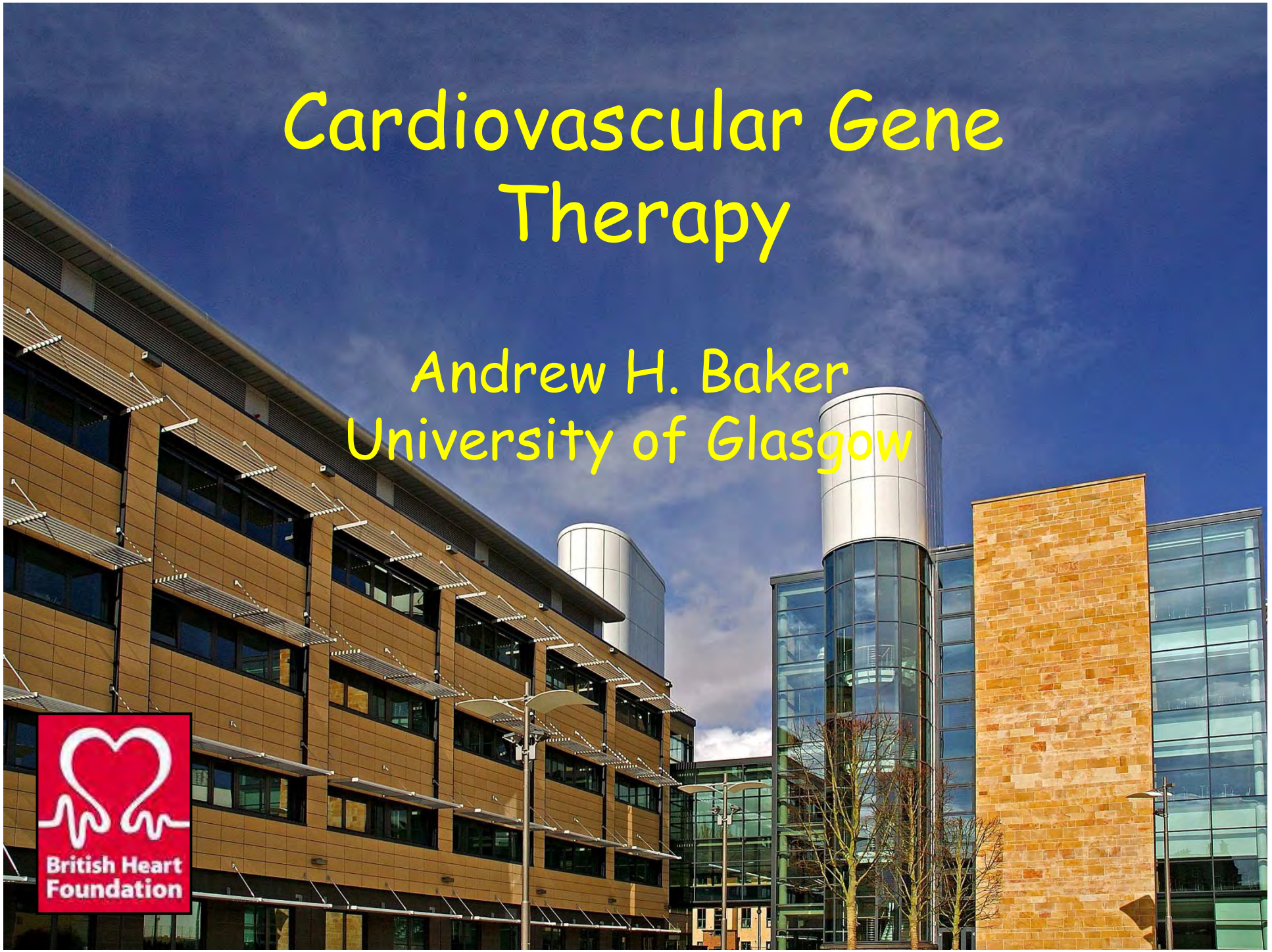
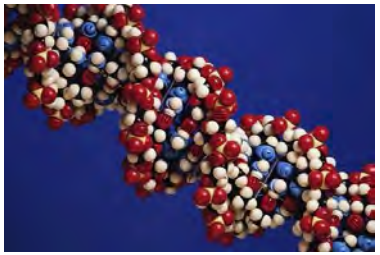




Why gene therapy?

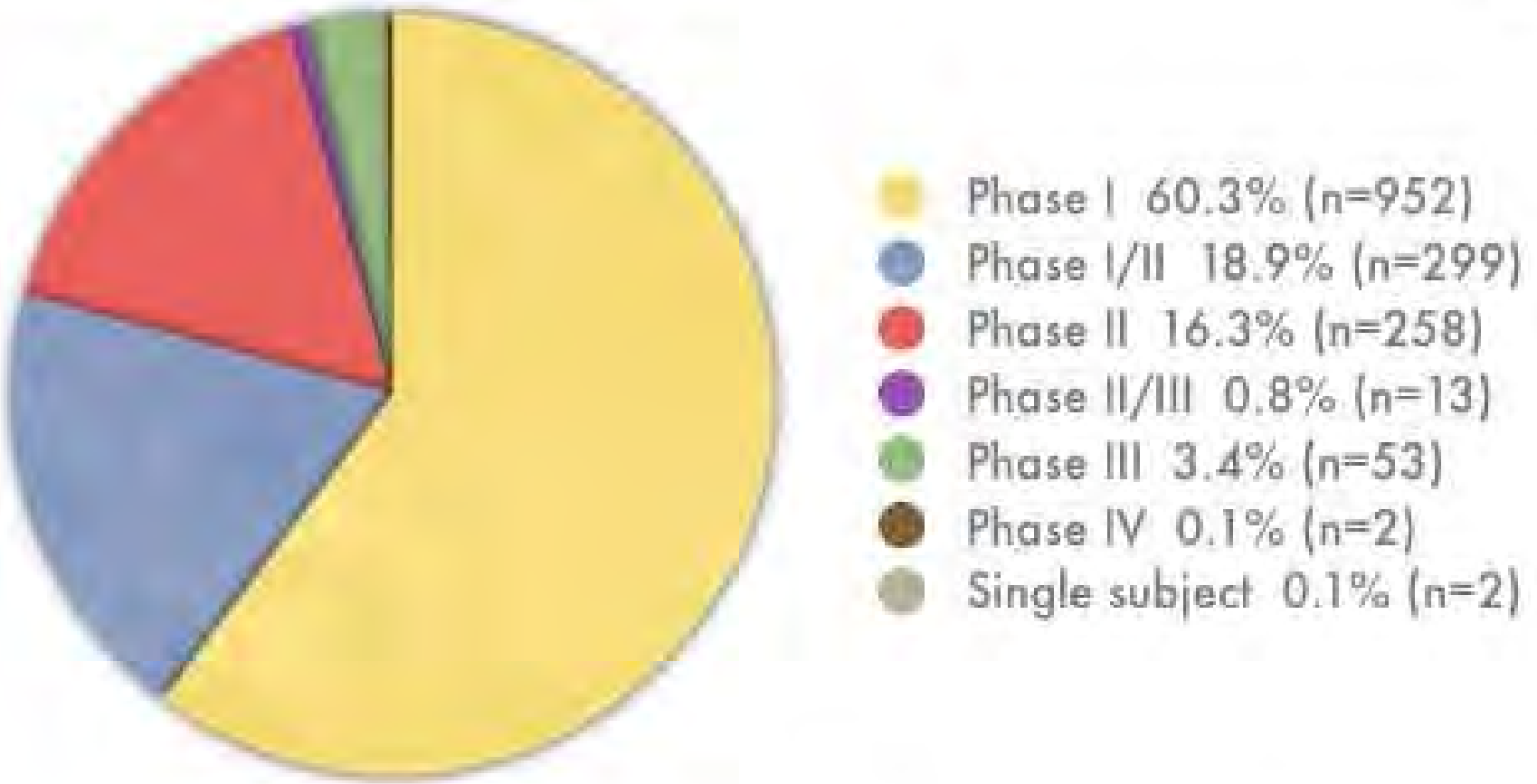
- Diseases with no pharmacological therapy.
- Diseases with sub-optimal drug therapies.
- Identification of genetic deficiencies.
- Increased understanding of molecular mechanisms of diseases.
- Increased identification of candidate therapeutic genes.
- Procedures that allow gene delivery at the time of surgery.
- Gene transcription cassettes can easily be engineered.
- Many diverse vehicles for gene delivery to the vasculature.
- Clear opportunity to beneficially treat diverse CVDs.

Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 65.2% (n=960)
- Cardiovascular diseases 9.3% (n=137)
- Monogenic diseases 8.2% (n=120)
- Infectious diseases 7.6% (n=112)
- Neurological diseases 1.2% (n=17)
- Ocular diseases 0.8% (n=12)
- Other diseases 2.2% (n=32)
- Gene marking 3.4% (n=50)
- Healthy volunteers 2.2% (n=32)

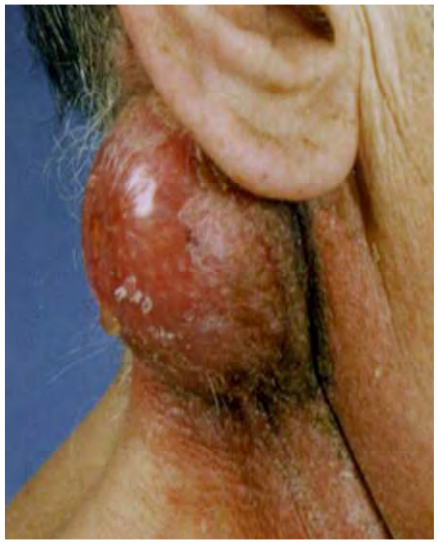
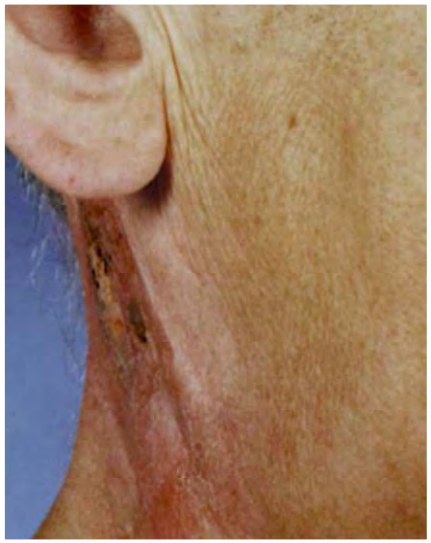
www.wiley.co.uk/genmed/clinical

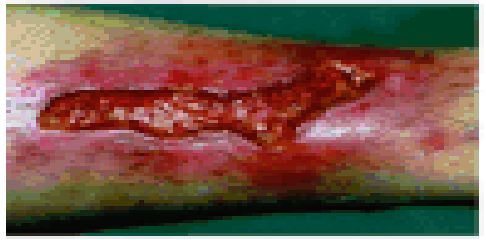
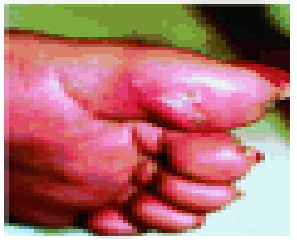
Phases of Gene Therapy Clinical Trials



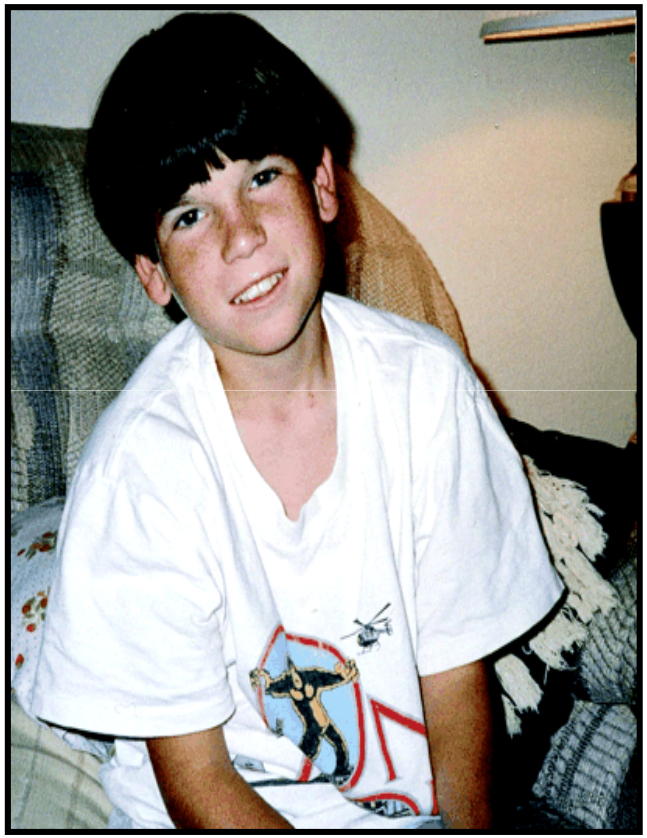
Gene therapy has suffered from:

A – over hype in the early days
B – premature advancement to poorly controlled clinical trials
C – Safety concerns with viruses

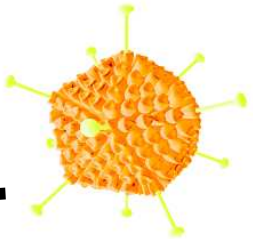




Death of Jesse Gelsinger in 1999



Jesse Gelsinger: his death sent shockwaves through the scientific community

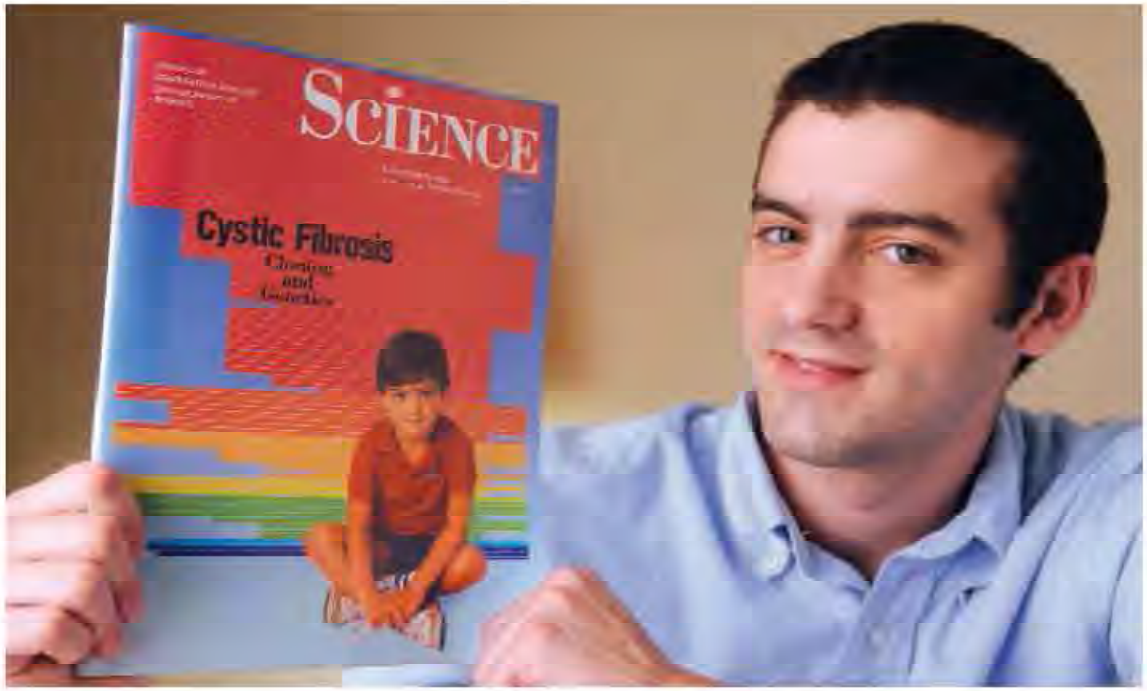
1999 - Philadelphia



Injected into the bloodstream
to target the liver and express the
corrective gene

- Substantial induction of cytokines
- DIC
- Multi-organ failure
- Death

- Poor knowledge of virus:host interactions and dosing regimens/patient variability.
- Not predictable from mouse data



All grown up. Danny Bessette, a 24-year-old with CF, was 4 years old when he appeared on the cover of *Science* announcing the discovery of the CF gene.

The Promise of a Cure: 20 Years and Counting

The discovery of the cystic fibrosis gene brought big hopes for gene-based medicine; although a lot has been achieved over 2 decades, the payoff remains just around the corner

after signing up with Collins, in the spring of 1989. In collaboration with a large research group in Toronto, Canada, that had started an aggressive chase for the CF gene years earlier, the team cloned the CF gene—called the cystic fibrosis transmembrane conductance regulator (*CFTR*)—and nailed a crucial, disease-causing mutation (*Science*, 8 September 1989, pp. 1059, 1066, 1073).

Everyone in the CF community recalls the electric moment when they heard the news. "I remember seeing it roll off the fax machine, gathering people in the lab, and thinking, 'What did we need to know' " now? says Michael Welsh, a pulmonary physician at the University of Iowa, Iowa City. Most believed that the disease had grown vastly less complex overnight and would soon be eliminated, probably by gene therapy.

On the 20th anniversary of the identification of the CF gene, as new gene discoveries pile up weekly and hype over the power of genes to transform medicine flows fast, CF offers an object lesson in how difficult it is, and how long it takes, to convert genetic knowledge into treatments. Every CF expert agrees that the gene discovery transformed their understanding of the disease's pathol-

nature

www.nature.com/nature

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Gene therapy deserves a fresh chance

Initial interest in gene therapy waned after the technology failed to live up to expectation. Progress made since has received little attention, but suggests that the pervading sense of disillusionment is misplaced.

In the early 1990s, when the first human trials got under way, it seemed to many that the era of gene therapy was at hand: the techniques of modern molecular biotechnology would make it possible to repair genetic defects by inserting healthy DNA directly into a patient's cells. The excitement was short-lived. Lasting effects proved difficult to obtain in early trials, and the community quickly grew sceptical. Then, in 2003, when it was announced that several gene-therapy patients in a Paris-based clinical trial had developed leukaemia, and that one of them had died, the mood became bleak. Subsequent reports of successful and effective gene-therapy trials have done little to lift the prevailing sense of doom. For most researchers, gene therapy now seems like a dead end.

But it doesn't have to be a dead end — not if scientists shift their perspective on the risks of gene therapy to be more in line with that of clinicians.

Scientists are trained to focus on understanding the systems that they study in great detail. And when they devise therapeutic interventions — for example, harnessing a viral shell to insert a therapeutic gene into a patient's DNA — they naturally want those systems to be engineered with equally great care, and for them to be as near to risk-free perfection as possible.

From that perspective, the fact that, collectively, the Paris trial and others carried out since have produced positive results in some 20 patients out of a total of two dozen looms at least as large as the handful of leukaemia cases. To clinicians, such results suggest a treatment that is risky, but potentially life-saving — a new option for people for whom there are no alternatives.

However, this was not the view that prevailed. When the viral delivery vehicle itself turned out to be responsible for the leukaemia cases in the Paris trial, scientists deemed the trial a failure. Bad press ensued, proposals for gene-therapy clinical trials came under increased regulatory scrutiny and standards for demonstrating safety were set higher than for other approaches. Unsurprisingly in such a climate, the biotechnology and pharmaceutical industries gradually dropped out of the gene-therapy pursuit. This corporate disinterest slowed clinical progress: academic centres are ill-equipped to make gene-therapy vectors of clinical grade and scale, and research funding is typically insufficient to support clinical trials. More insidiously, it has become harder to recruit young talent to a field that is perceived

"The results suggest a treatment that is risky, but potentially life-saving."

Therapeutic Strategies - to 2011

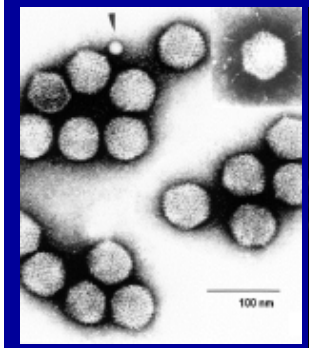
- Lipid lowering therapy
- Hypertension
- Post-angioplasty restenosis
- In-stent restenosis
- Vein graft failure
- Peripheral ischaemia
- Myocardial ischaemia
- Heart Failure

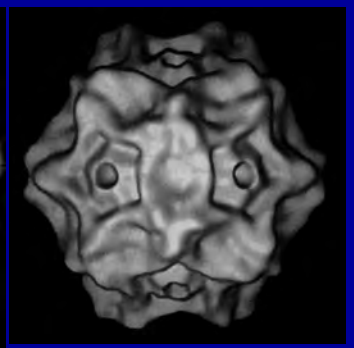
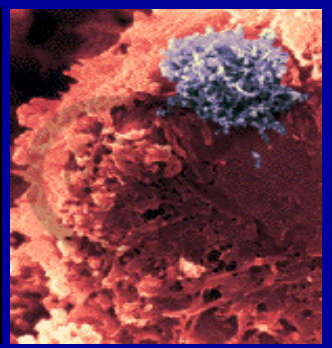
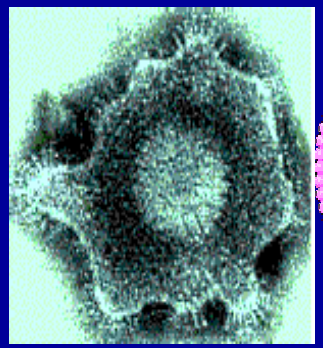
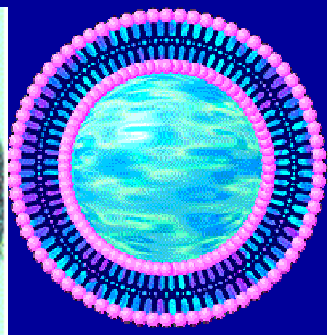
Building a gene therapy vector

- The therapeutic gene
- Vector delivery (virus/DNA)
- Transcriptional control
- Device/system for delivery?

Delivery Vectors

- Efficient
- Selective for the target cell/organ
- Non-toxic
- Stable *in vivo*
- Easy to produce
- Non-immunogenic
- Minimal dissemination for local applications
- Homing ability for systemic applications
- Longevity for the appropriate disease
- Cell-selective gene expression
- Regulatable gene expression



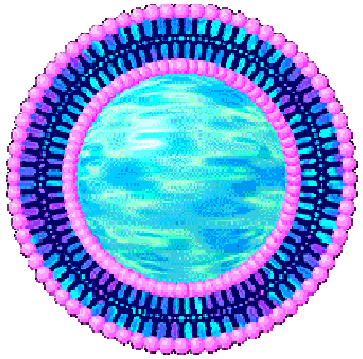






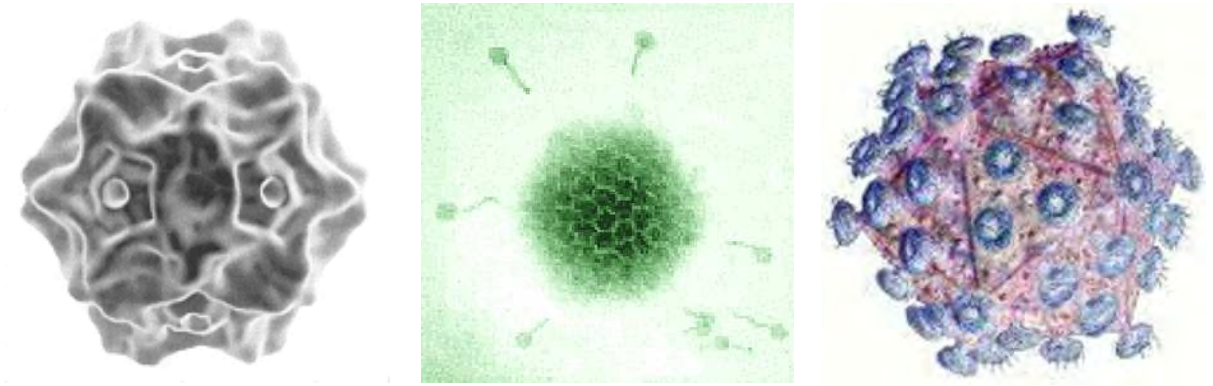
Vectors Used in Gene Therapy Clinical Trials

- Adenovirus 26% (n=305)
- Retrovirus 24% (n=288)
- Naked/Plasmid DNA 17% (n=206)
- Lipofection 8.3% (n=99)
- Poxvirus 6.9% (n=82)
- Vaccinia virus 6.5% (n=78)
- Adeno-associated virus 3.4% (n=40)
- Herpes simplex virus 3.4% (n=40)
- RNA transfer 1.3% (n=15)
- Others 2.4% (n=29)
- Unknown 3.1% (n=37)

www.wiley.co.uk/genmed/clinical

Gene therapy: Choice of disease target and vector are both critical





Non- viral

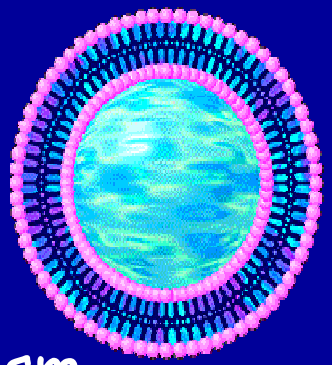
- lower efficiency
- poor selectivity
- poor nuclear targeting
- toxicity?

Viral

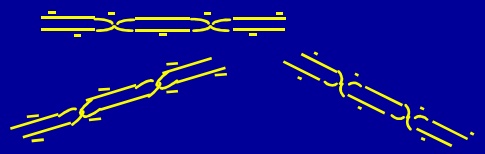
- generally higher efficiency
- variable nuclear targeting
- toxicity?
- Immunogenicity

Regulatory Issues

Liposome-Mediated Gene Transfer

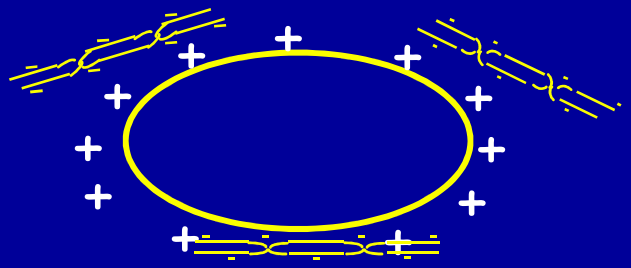


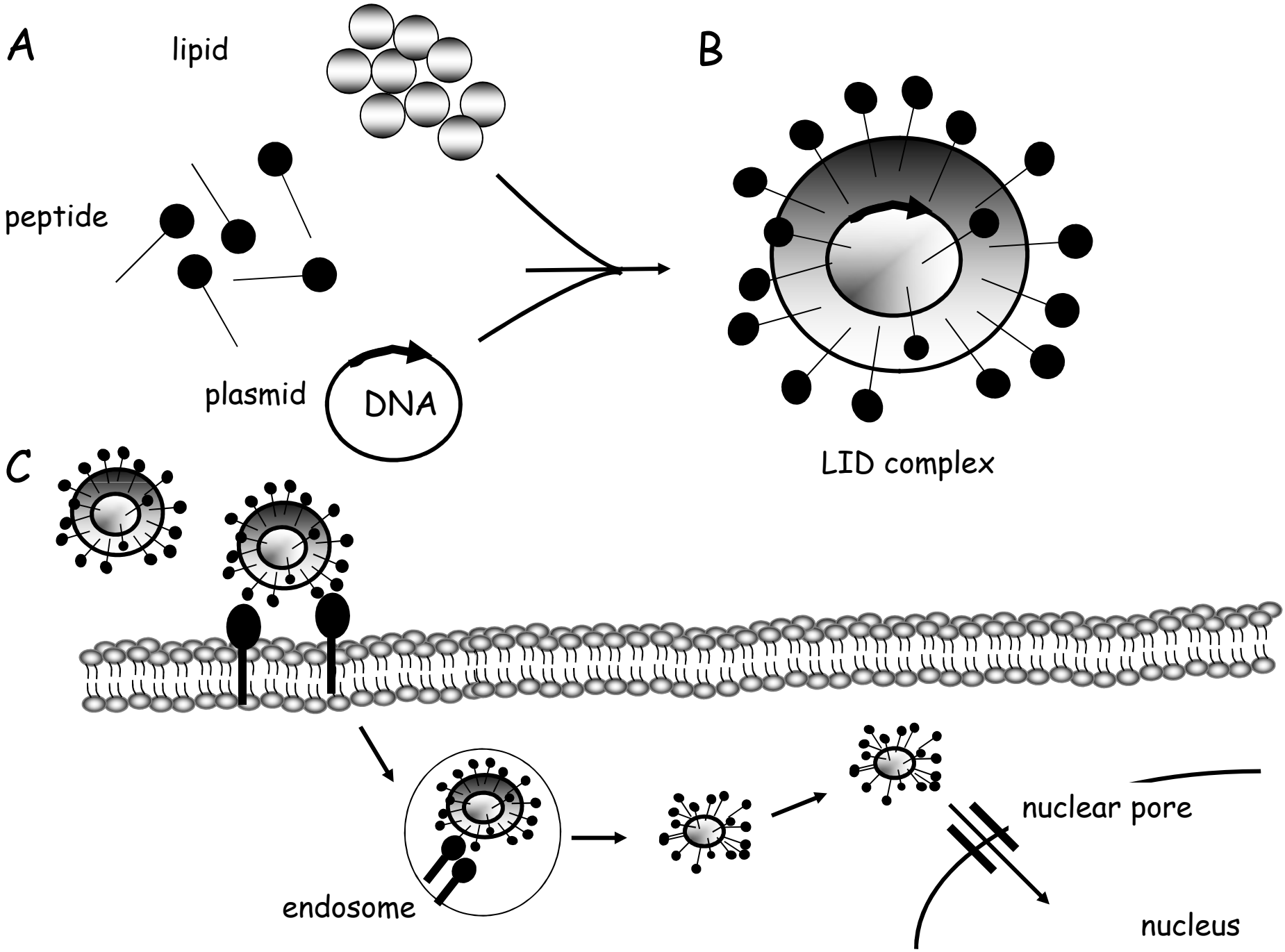
- Cationic lipids mixed with DNA.
- Wide variety.
- Efficient *in vitro (not* primary cells).
- Inefficient release of DNA into cytoplasm.
- Poor nuclear targeting.
- Low efficiency *in vivo*.

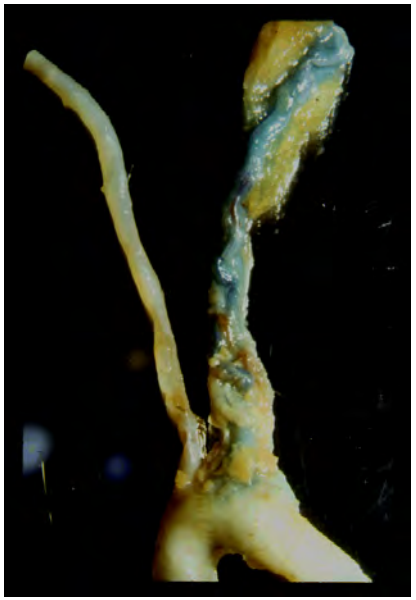


<u>Applications:</u>

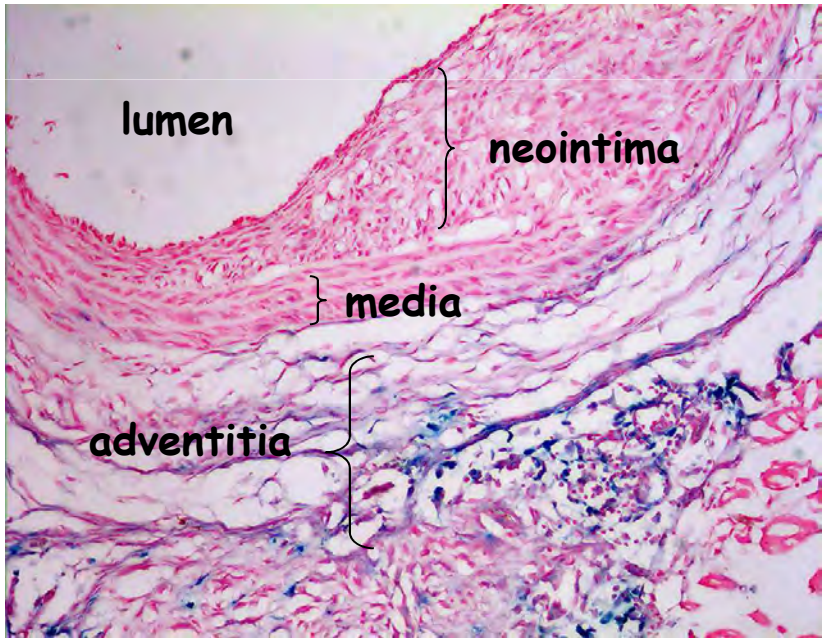
Limited due to inefficiency



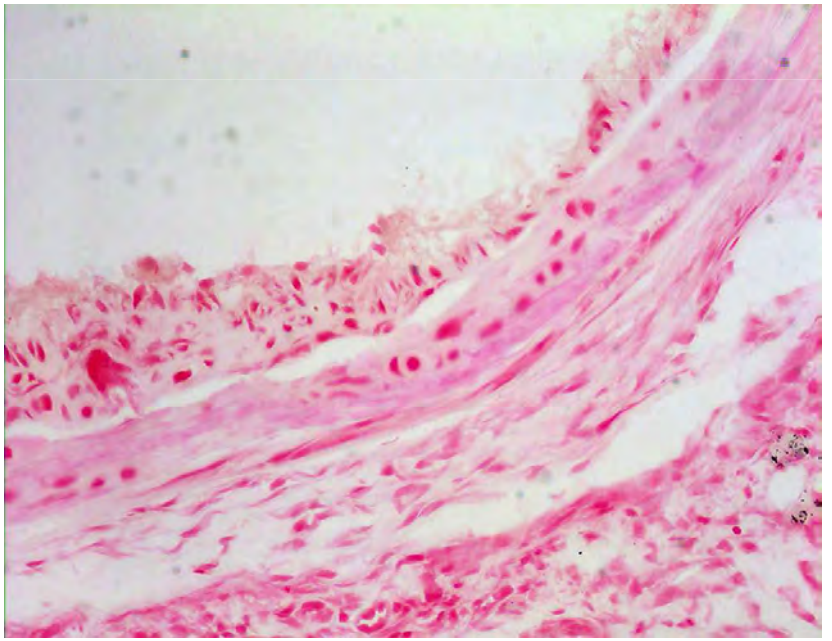

A
lipid
peptide
plasmid
DNA
B
LID complex
C
endosome
nuclear pore
nucleus



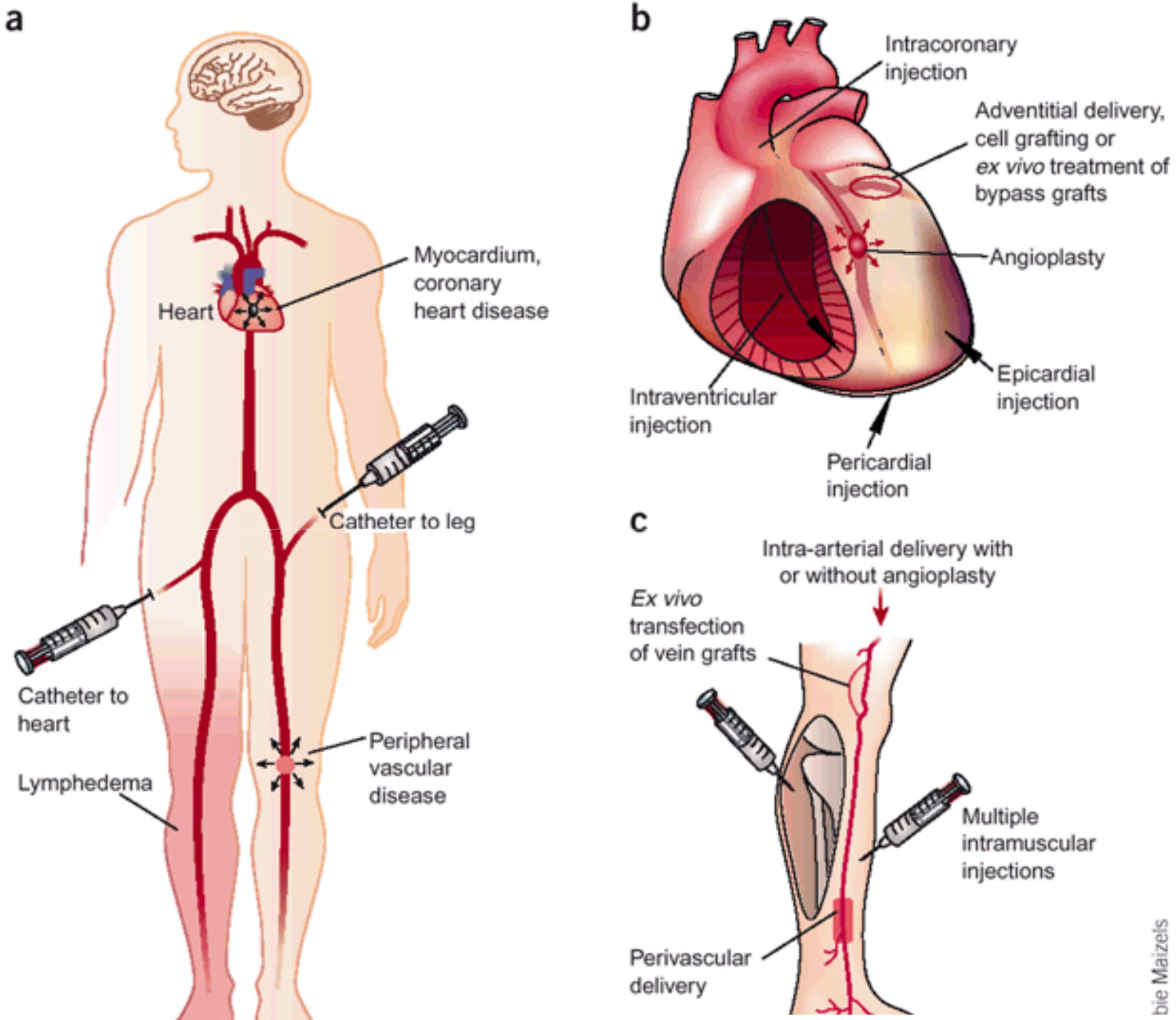
In vivo transfection of balloon-injured rat carotid artery



pCI-with peptide

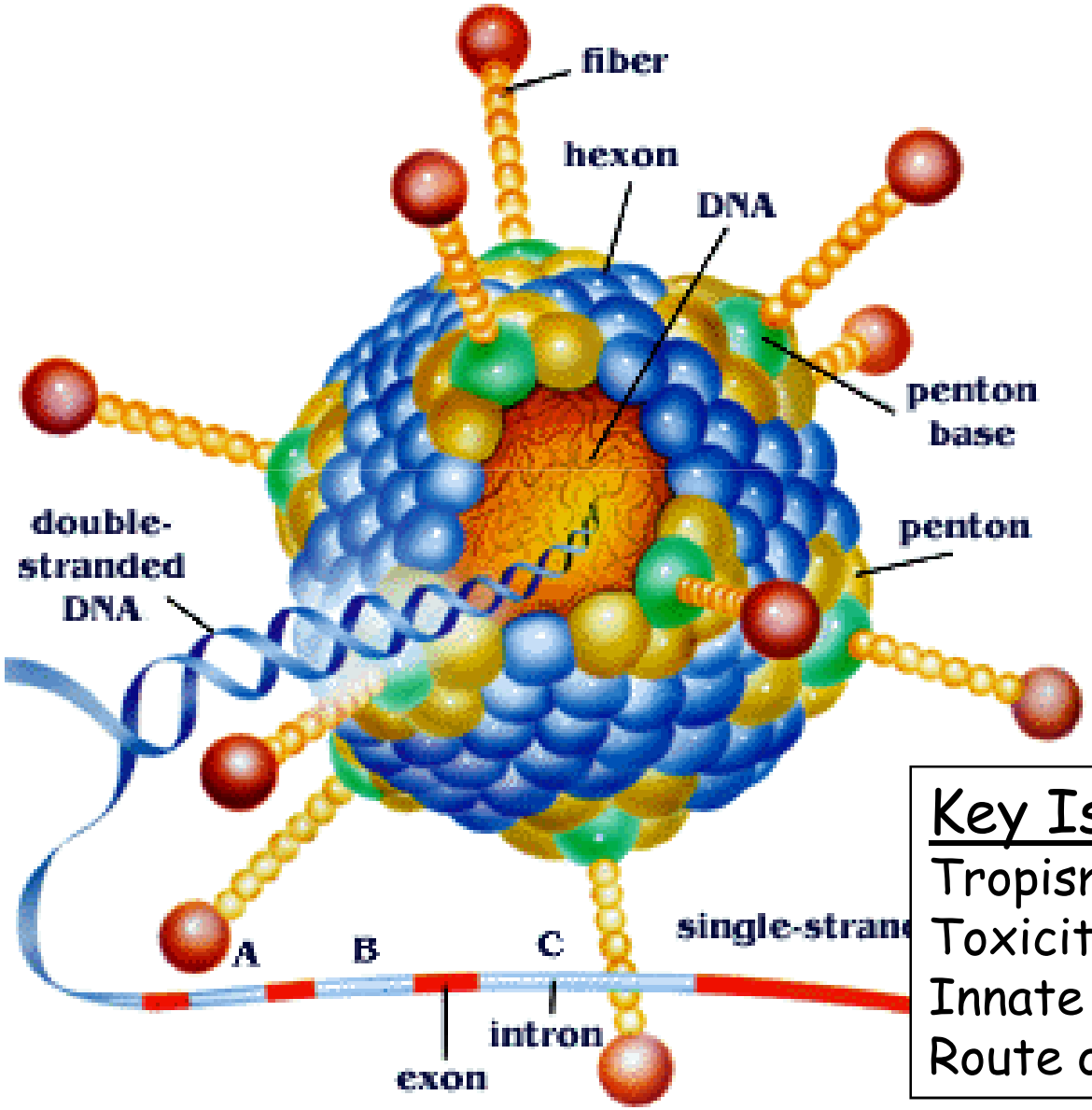


pCI plasmid



Yla-Herttuala and Alitalo, *Nat Med*, 2003

Adenovirus – a flexible vector system for vascular gene transfer



Key Issues:

Tropism

Toxicity

Innate Immune Response

Route of Delivery defines above

Adenoviruses

Common pathogens

-30-50% of people Adeno +

-50 different serotypes

-occular and respiratory problems

-non-oncogenic (ct. retroviruses)

Disadvantages

- immunogenic
- transient gene expression
- some cells non-infective
- clinical trial death

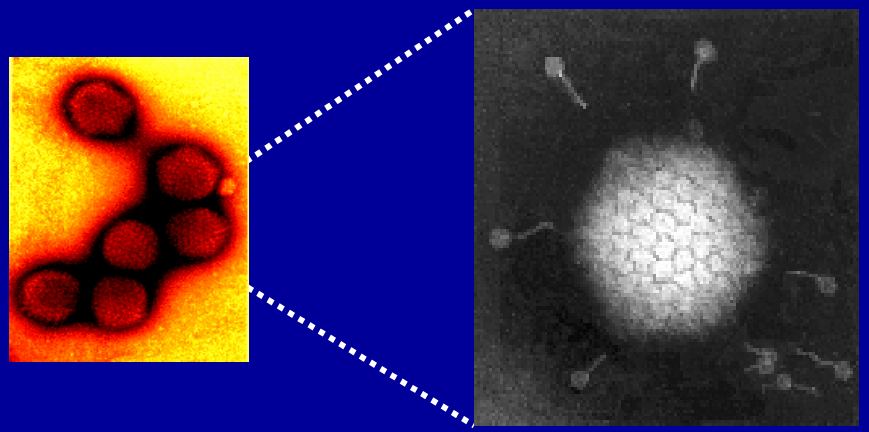
Highly efficient

-infect all types of cells

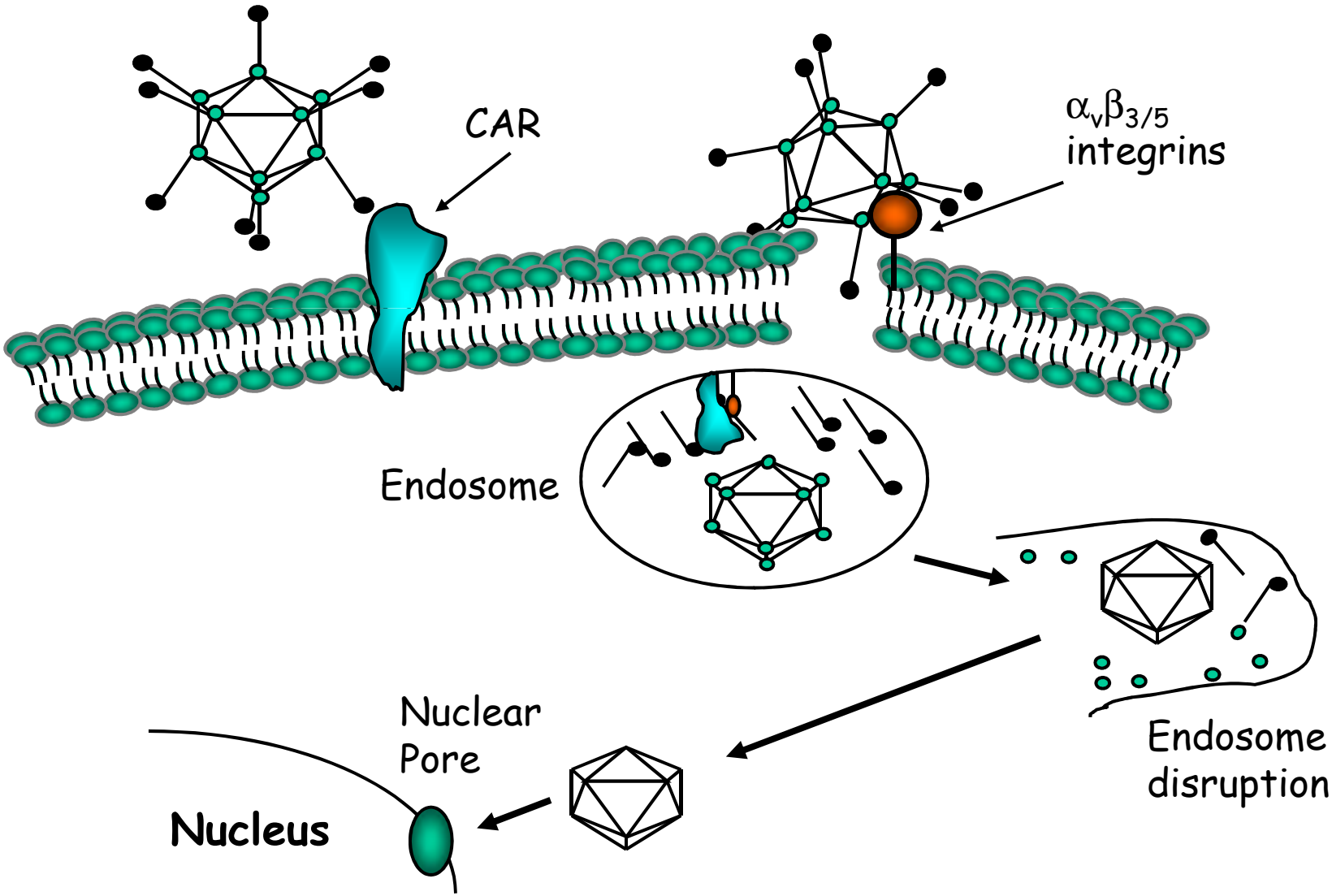
-efficient cell entry mechanism

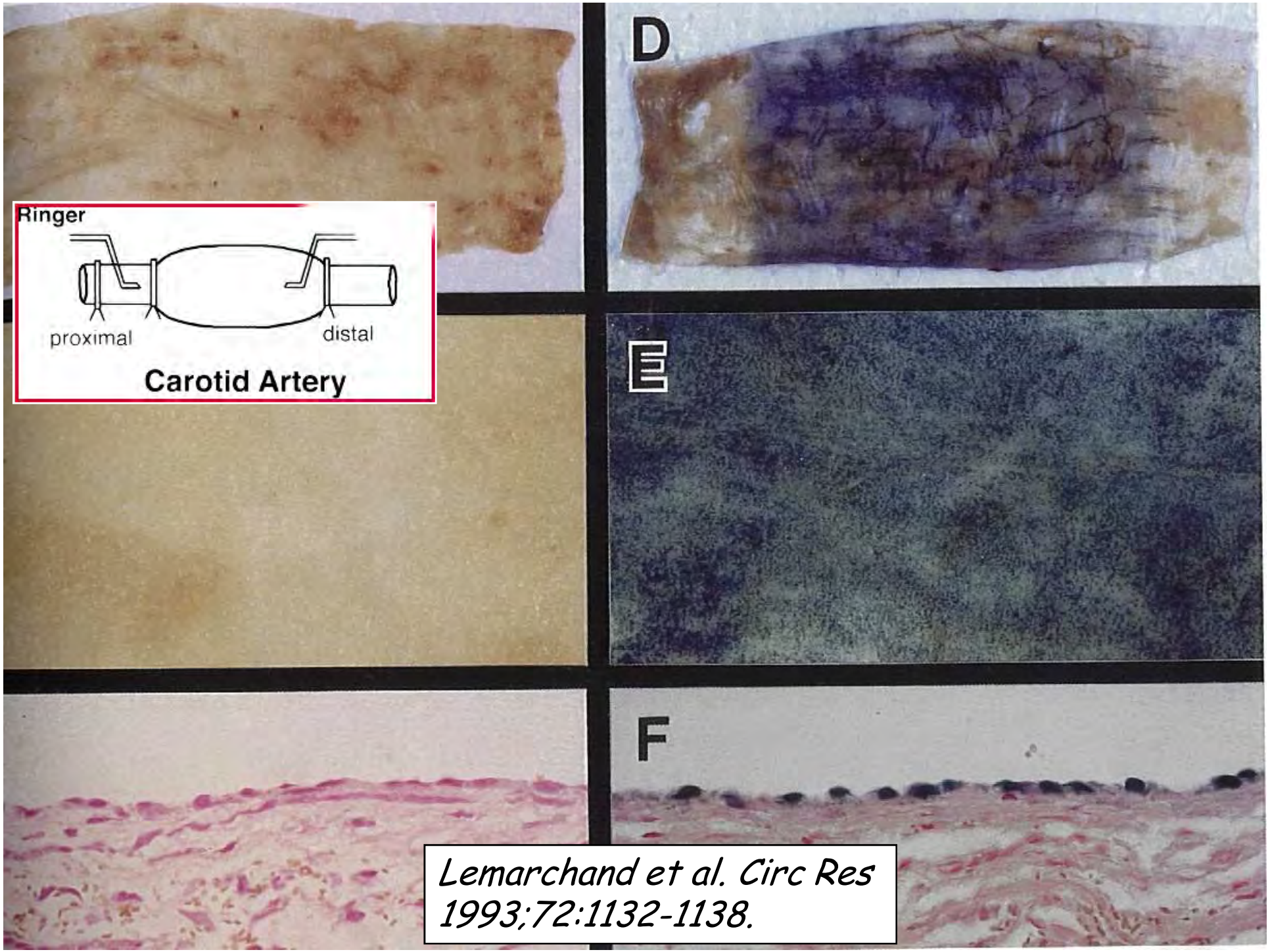
-organised delivery to the nucleus

-based on replication-deficiency

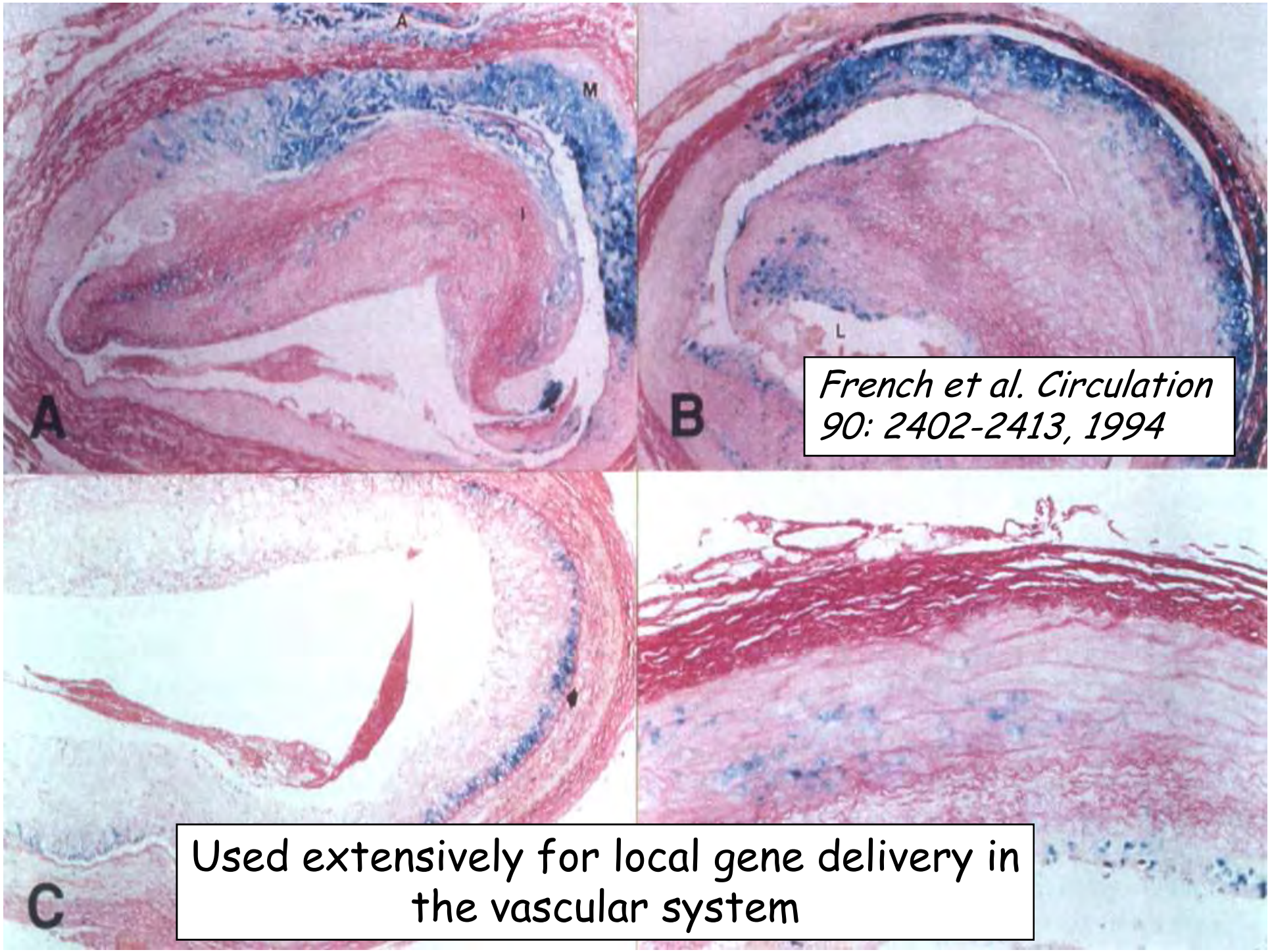


Adenovirus Transduction in vitro and in vivo – local access to target

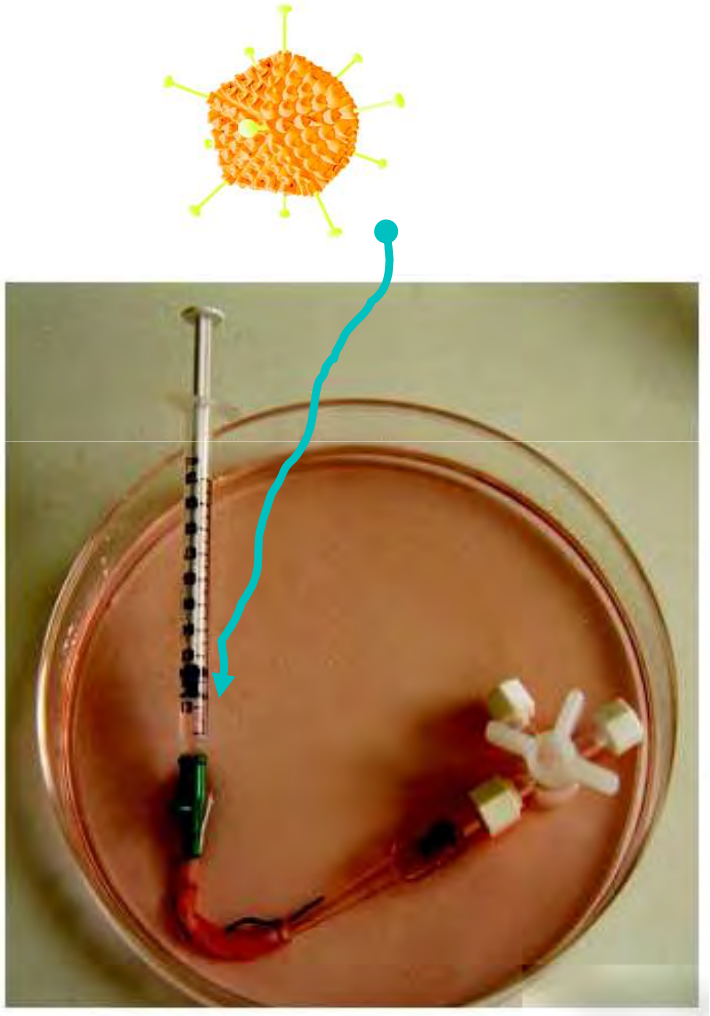


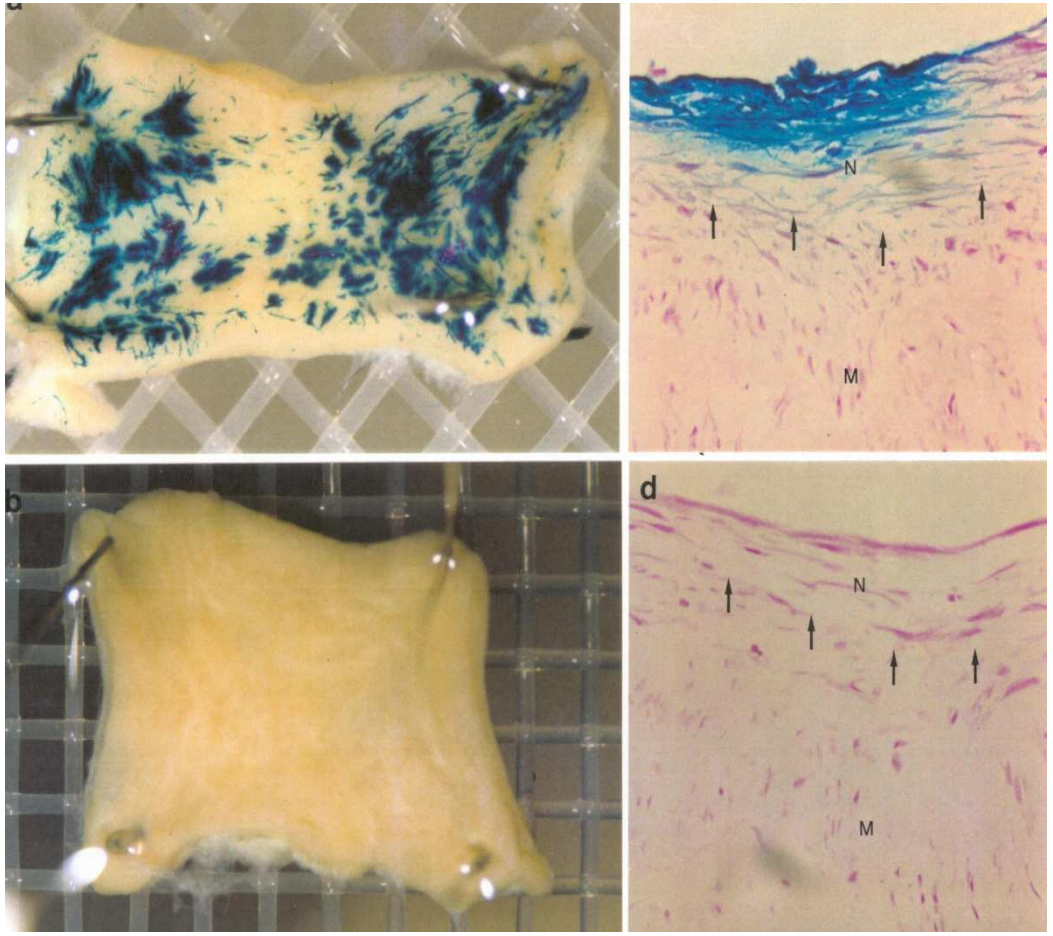


Lemarchand et al. Circ Res 1993;72:1132-1138.


A
M
I
A
L
B
French et al. Circulation
90: 2402-2413, 1994
C
Used extensively for local gene delivery in
the vascular system

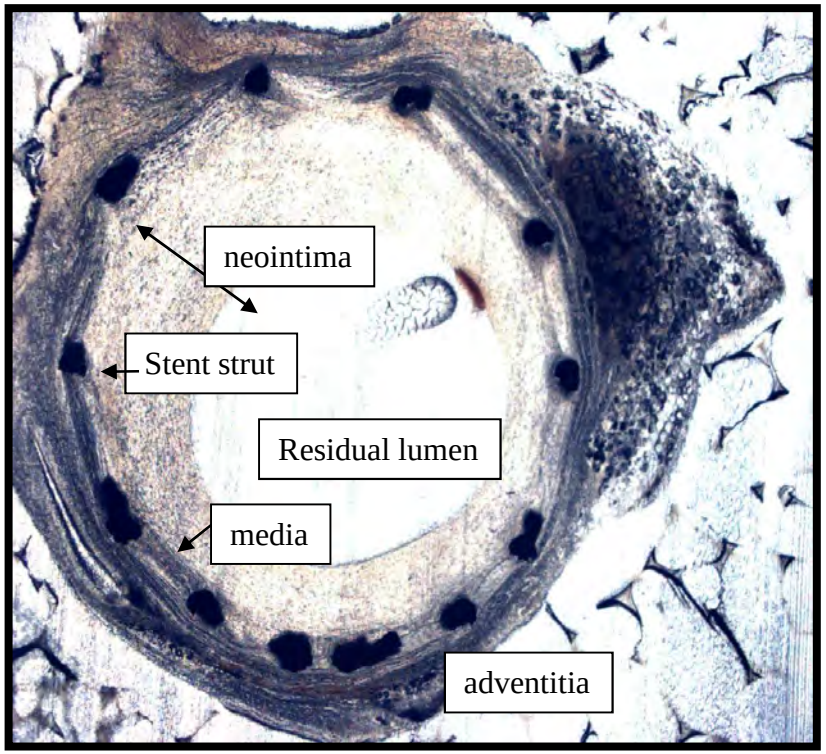
Human Vein *Ex Vivo*





George et al., Hum Gene Ther **9**;867-877, 1998

Adenovirus delivery using stents



High restenosis rate
In-stent restenosis
DES (late thrombosis)
Proven therapies?

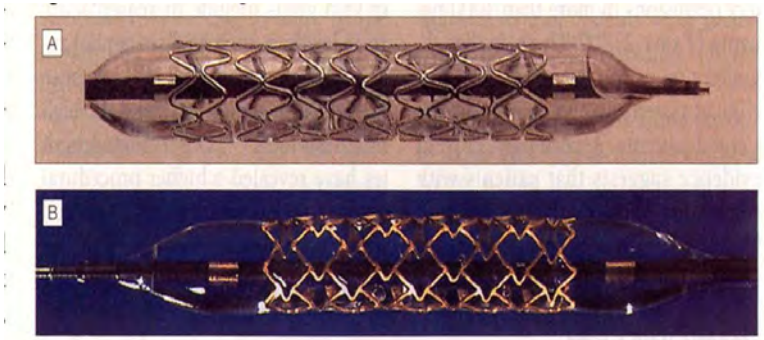
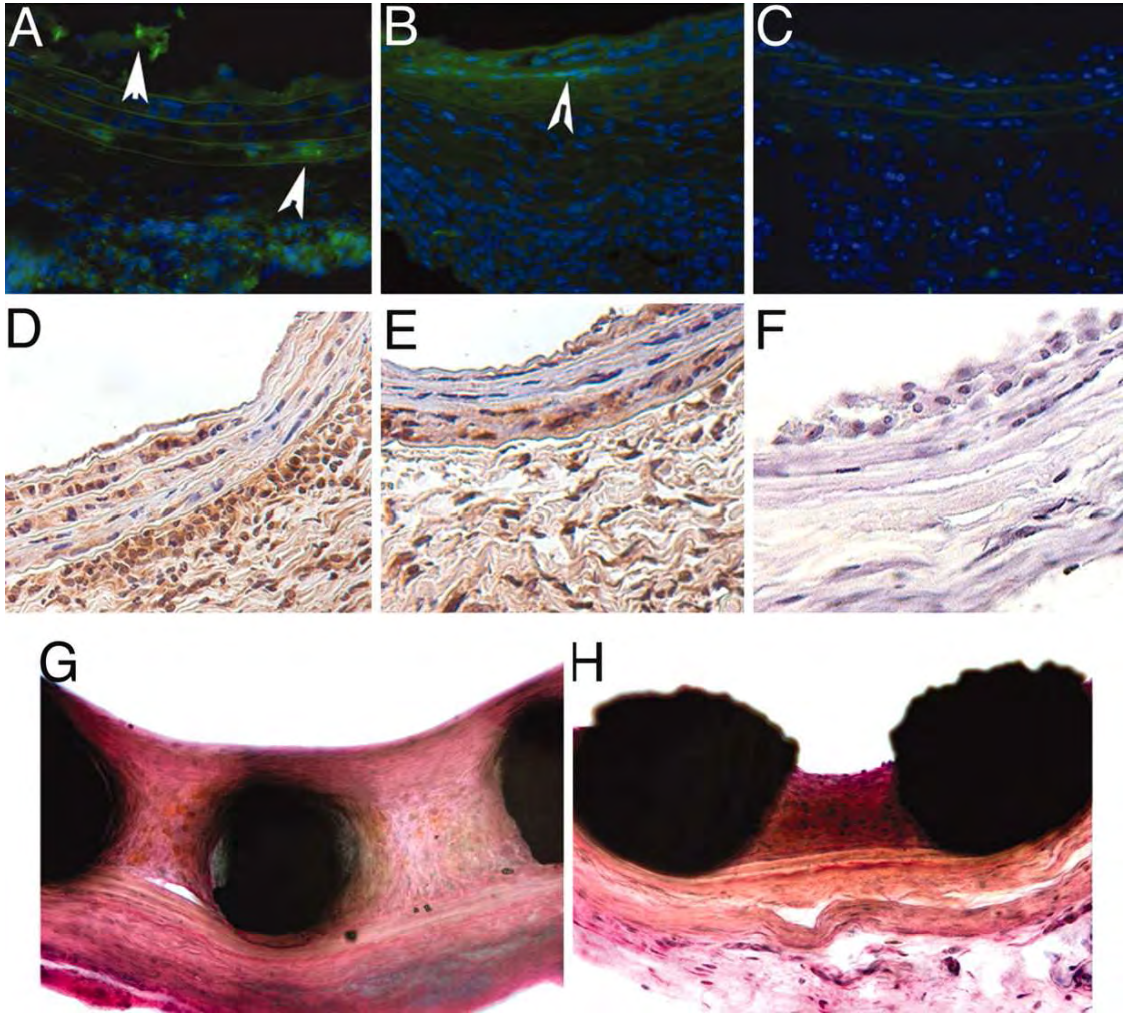
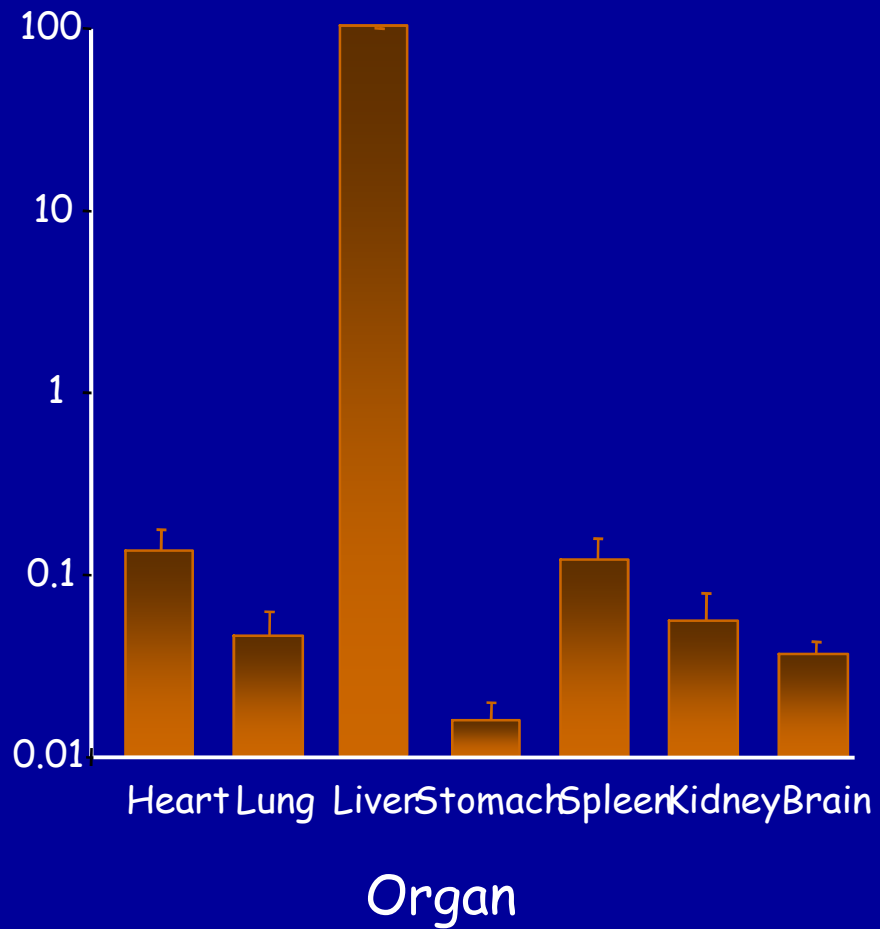


Fig. 5. PAA-BP-vector binding agent mediated tethering of Ad to steel surfaces in vivo: reporter (GFP) and Ad-iNOS therapeutic results



Fishbein, Ilia et al. (2006) Proc. Natl. Acad. Sci. USA 103, 159-164

Adenovirus - Systemic

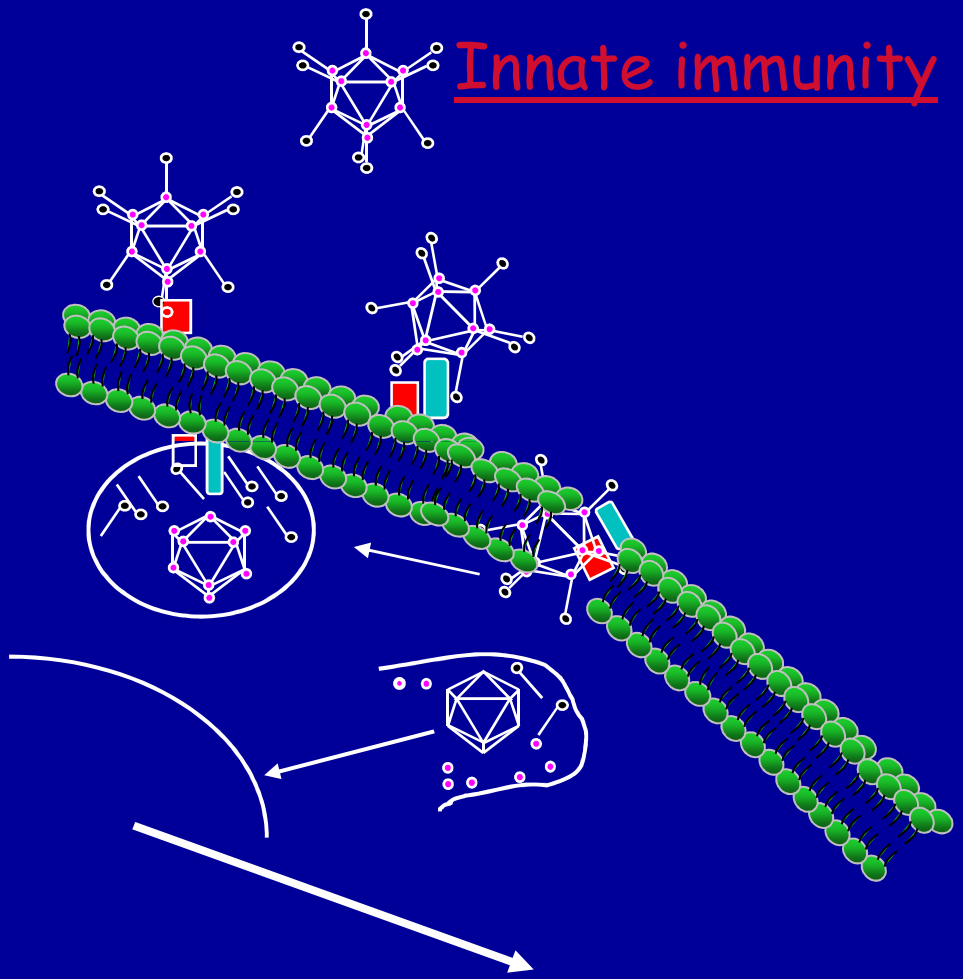



Courtesy of J. Johnson, Bristol

- Liver disorders
- Factory for secreted genes

(e.g. haemophilia)

Adenovirus is highly immunogenic



Innate immunity

Viral
gene expression

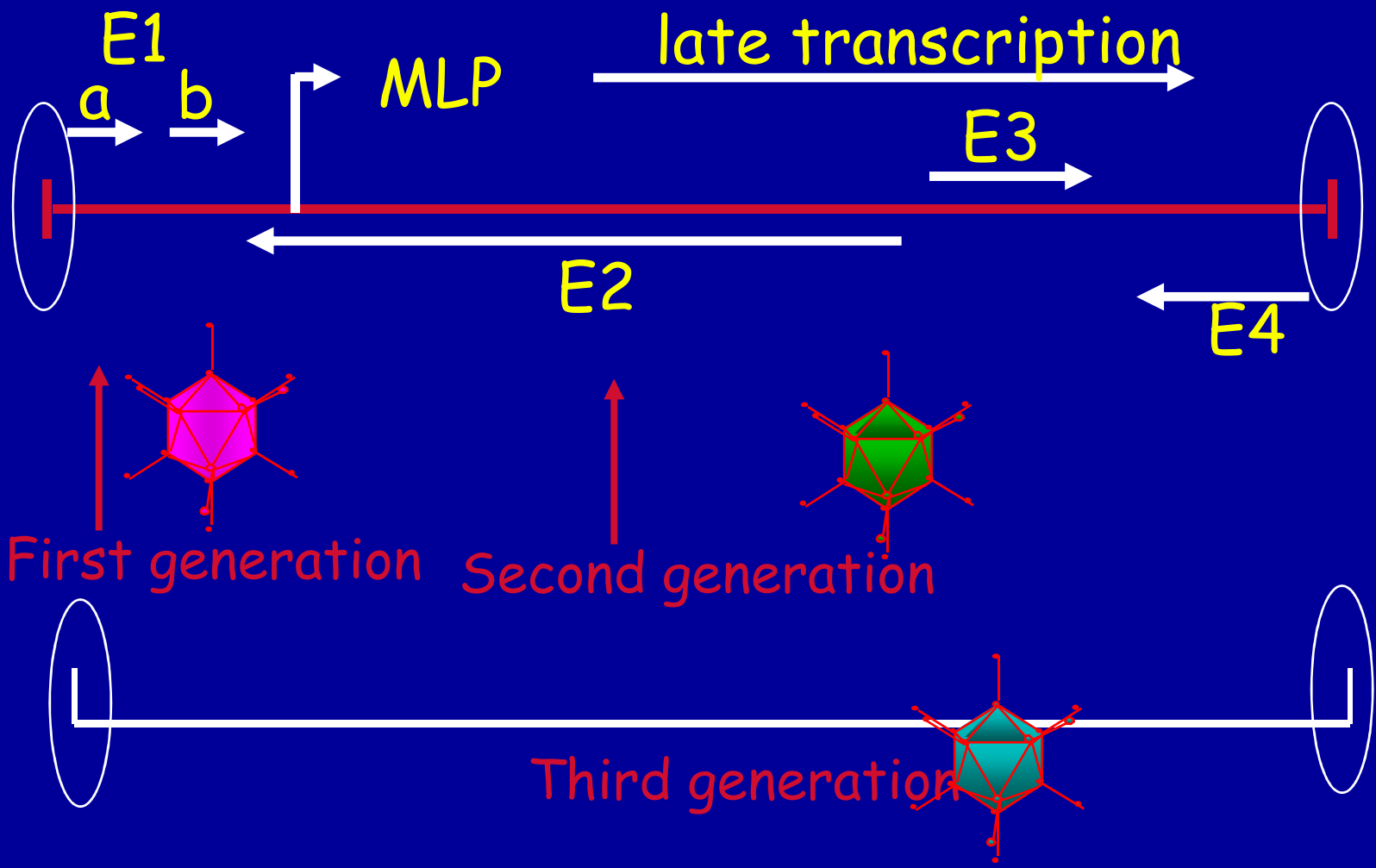
Activates immune response

Cell death

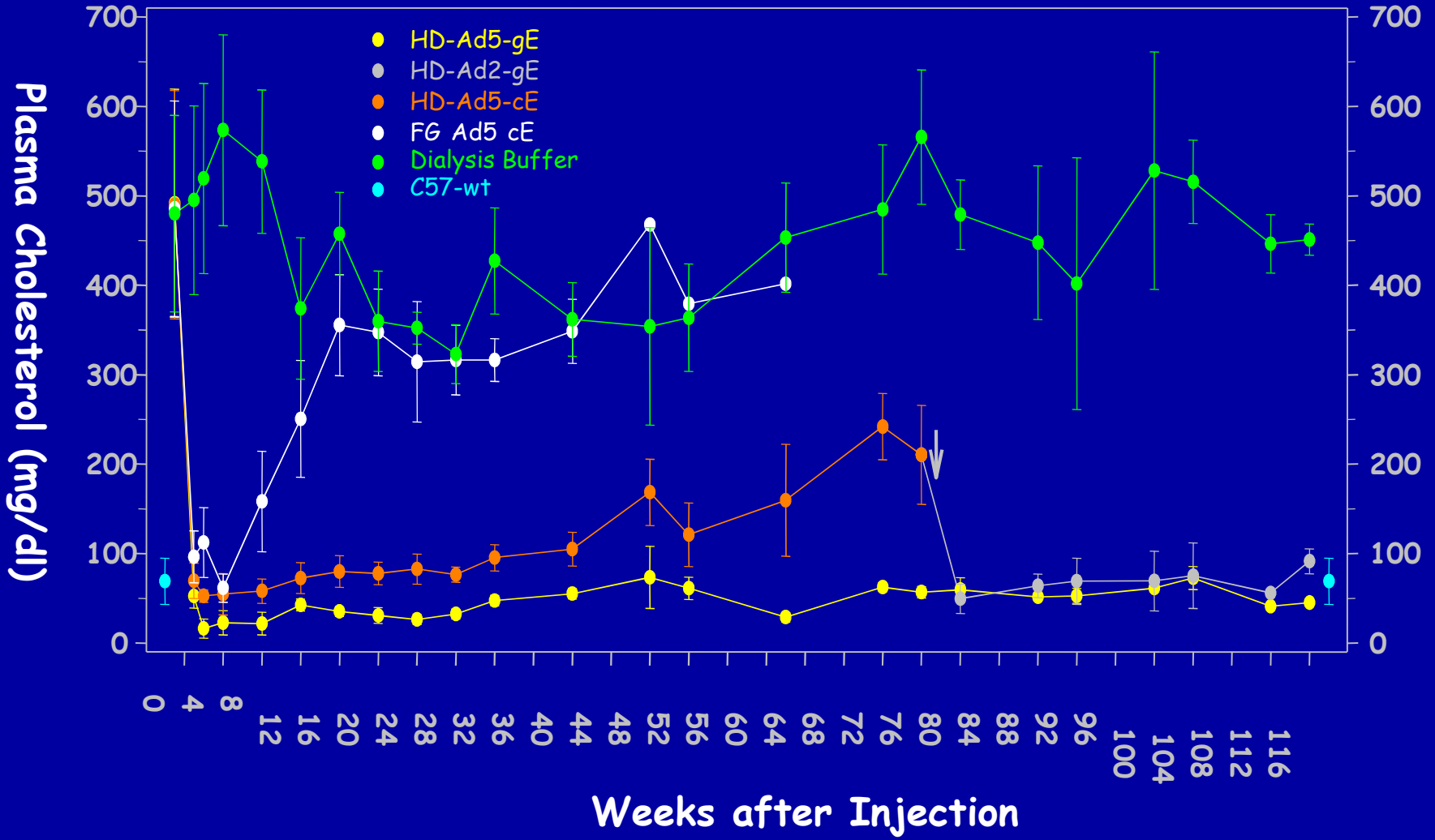
Transient gene expression

Cell-mediated immunity

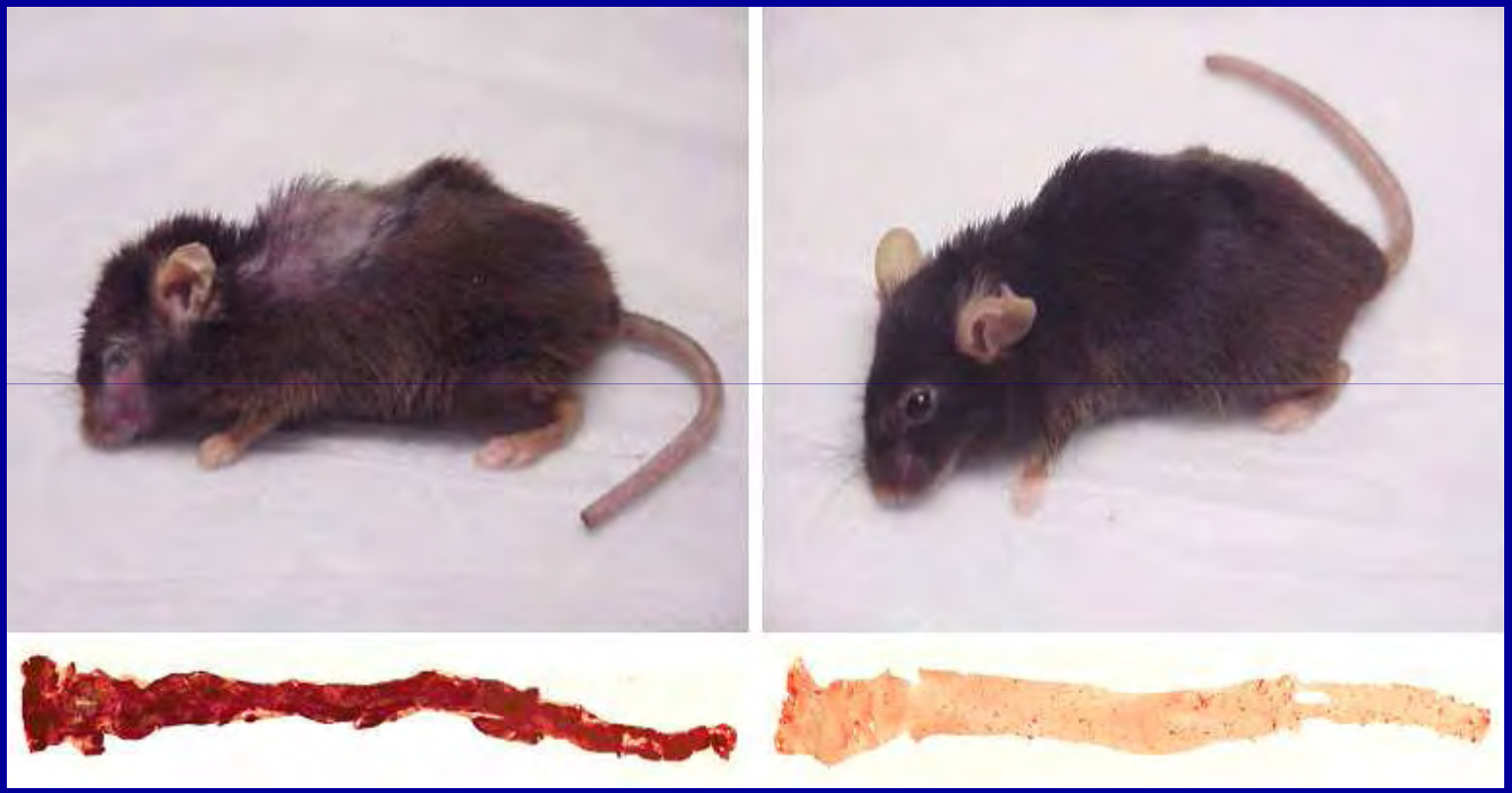
Adenovirus vectors



Lifetime Correction of Hyperlipidemia ApoE-/- Mice by a Single Injection of HD-Ad-ApoE

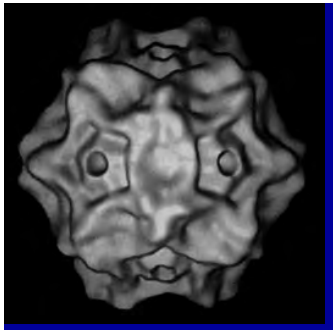


HD-Ad-ApoE Treated and Control ApoE$^{-/-}$ Mice At Age 2.5 Years



Control

Treated

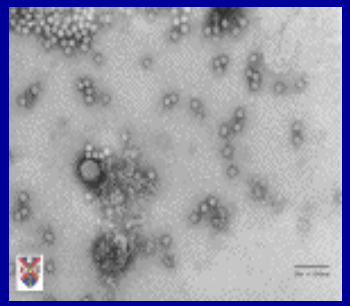


Adeno-associated virus (AAV)

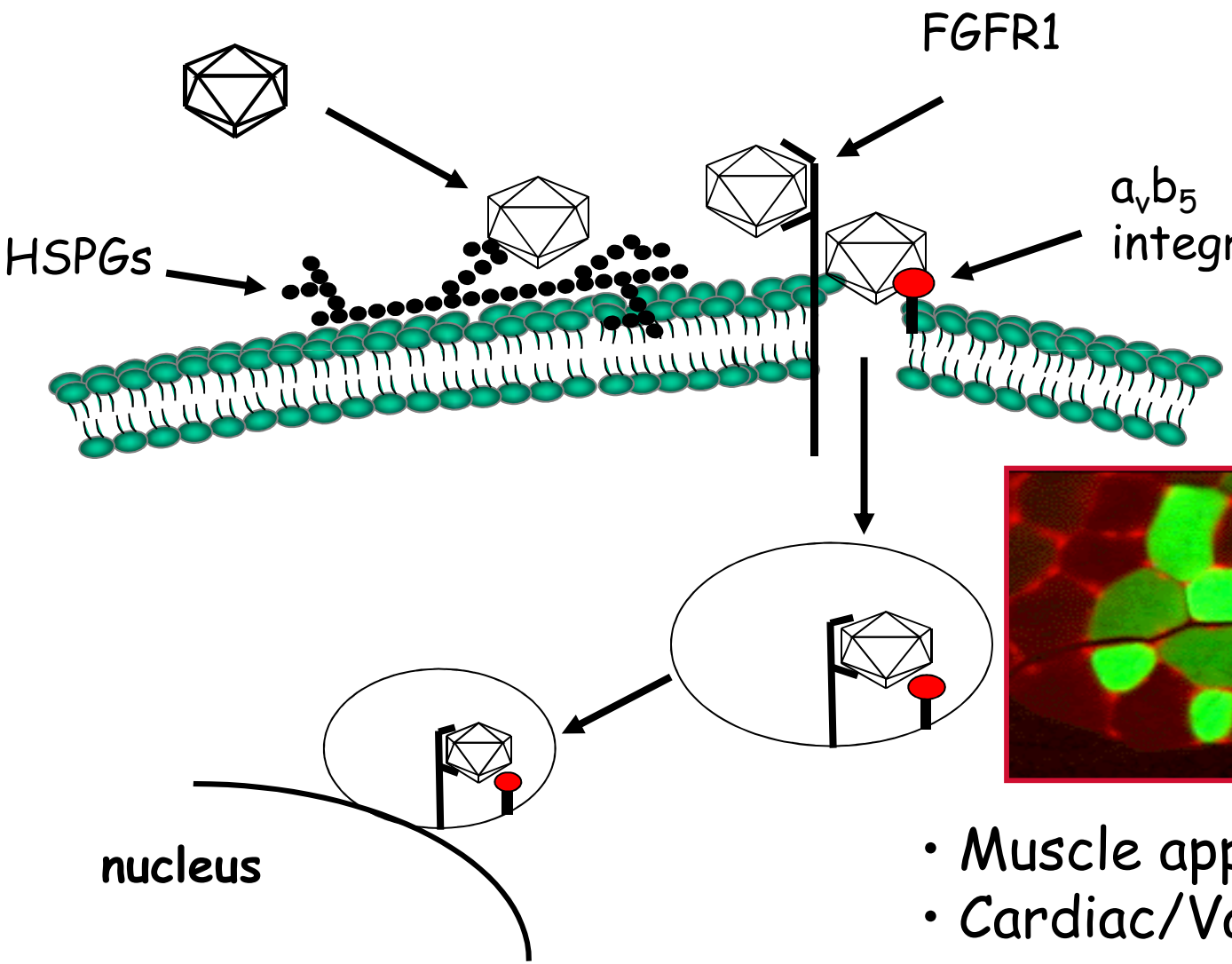
- Parvoviruses - so called dependoviruses
- Small virus - not associated with known human pathogenicity
- rep/cap genes removed and replaced with expression cassette

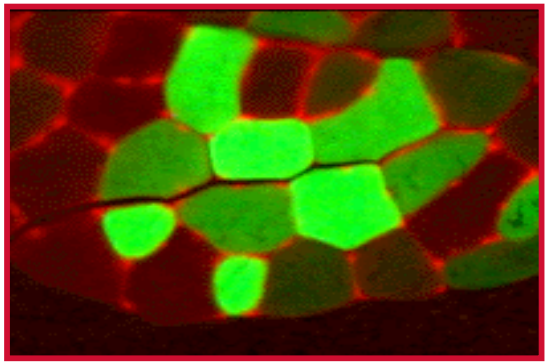
- limited to 4.7 kB
- no viral genes
- less immunogenic
- site specific integration (chr 19)
- SAFE

- long term gene expression
- no very infective for vascular cells
- degraded quickly by the proteasome pathway

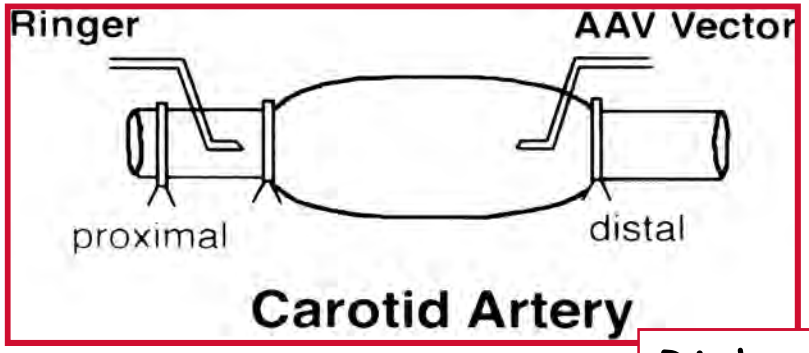


AAV-2 – discovered in early 1980s

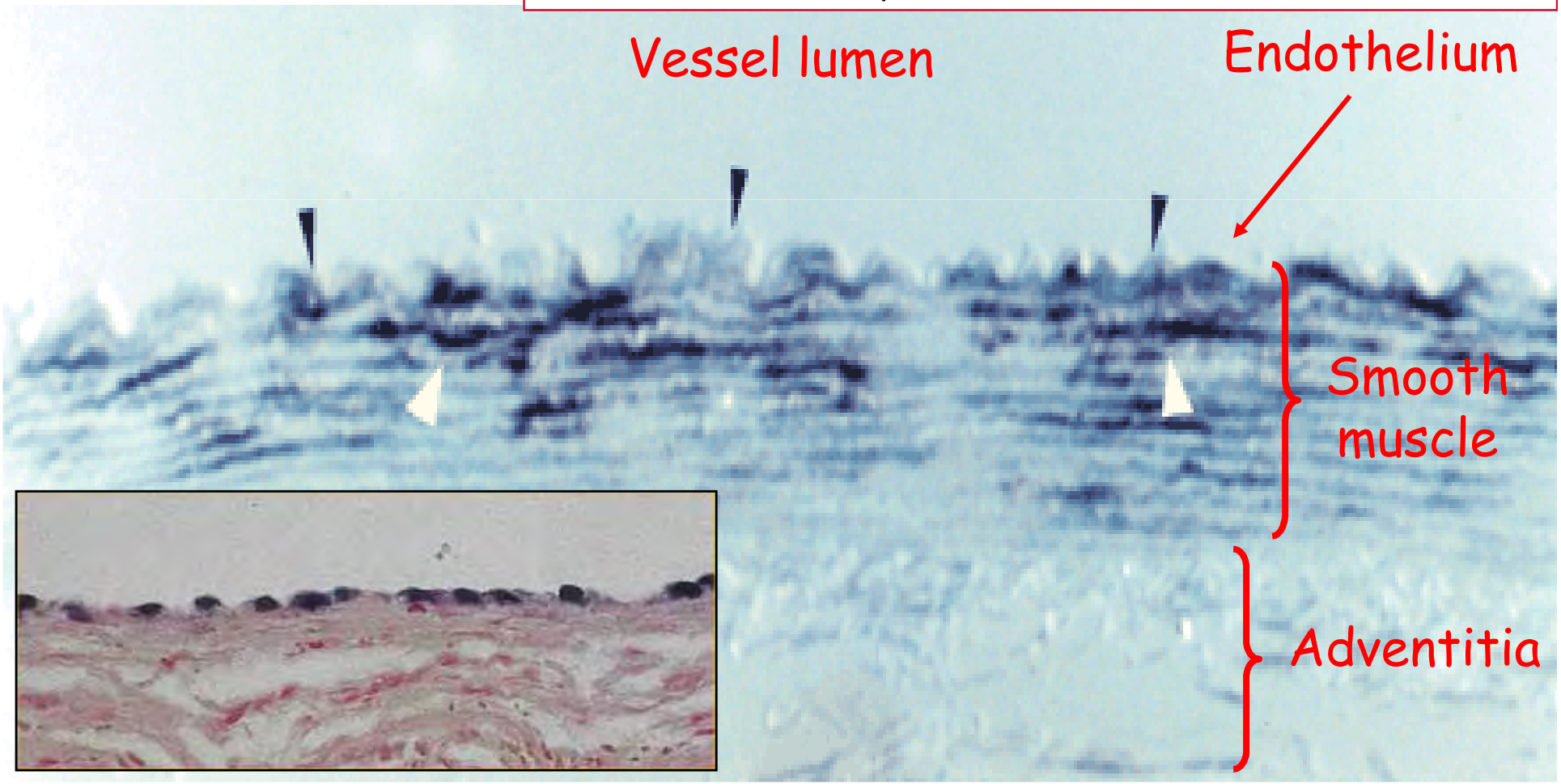




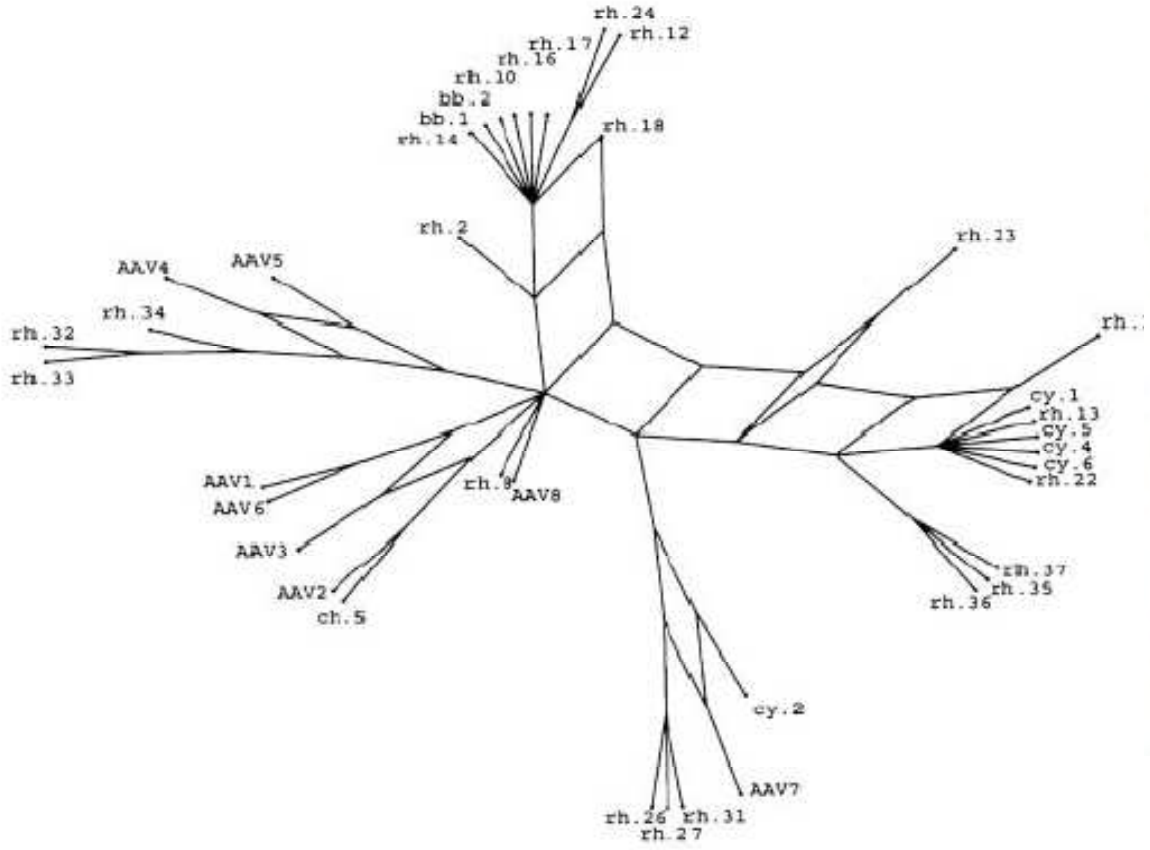
- Muscle applications
- Cardiac/Vascular

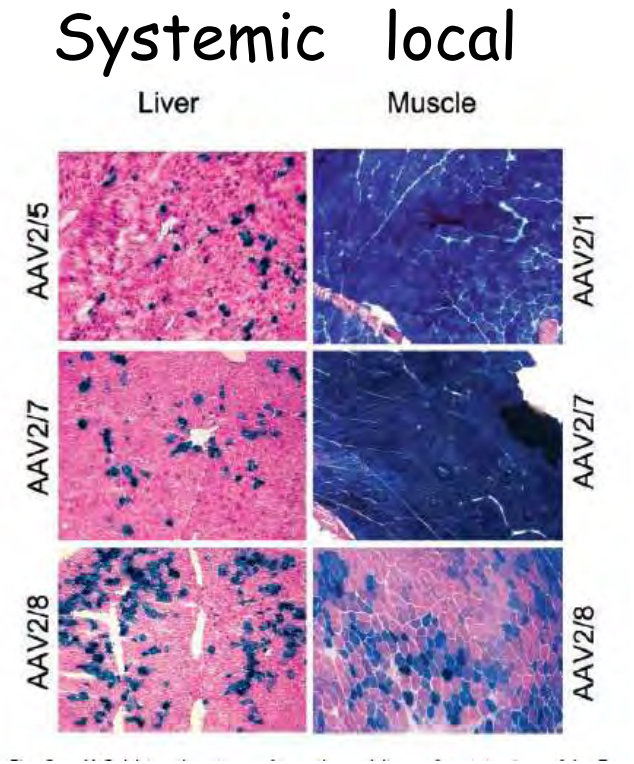


Richter et al., Physiol Genomics 2:117-127 (2000)



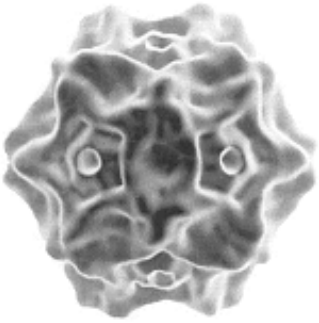
AAV family is now extensive





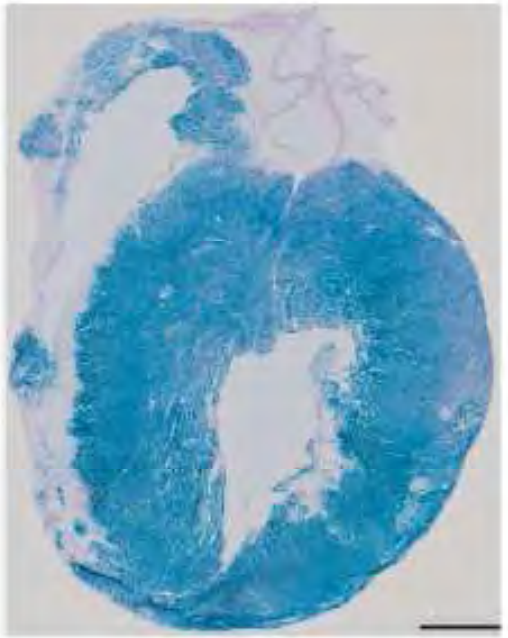
Gao et al., PNAS 100:6081-6086 Gao et al., PNAS 2002 99:11854-11859

AAV cardiac gene delivery



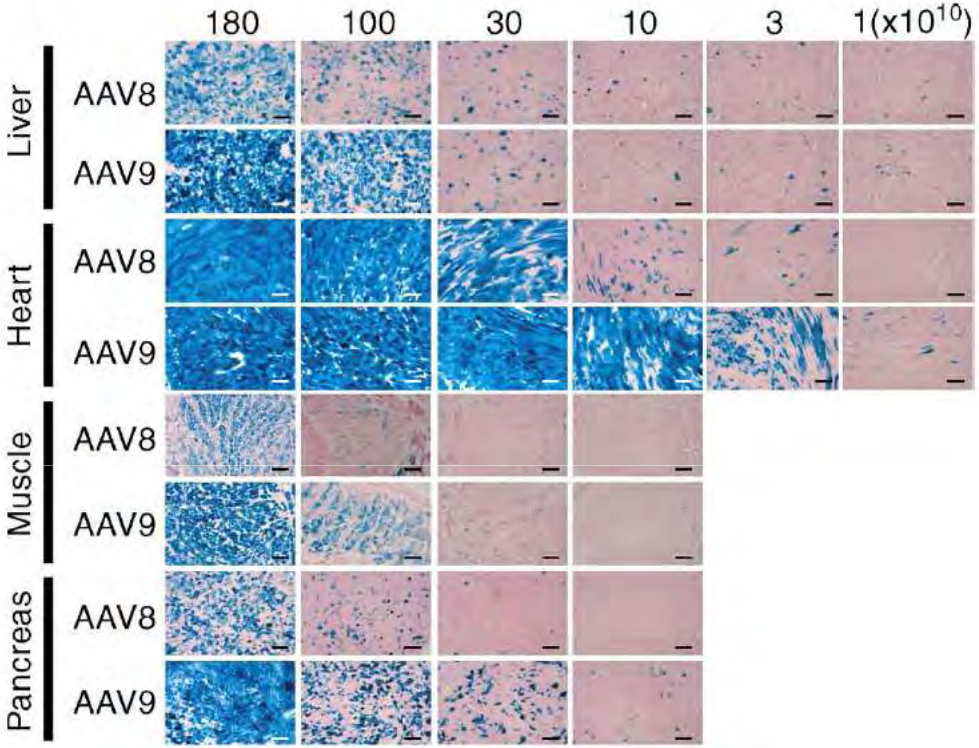
AAV-2

AAV-6/-9

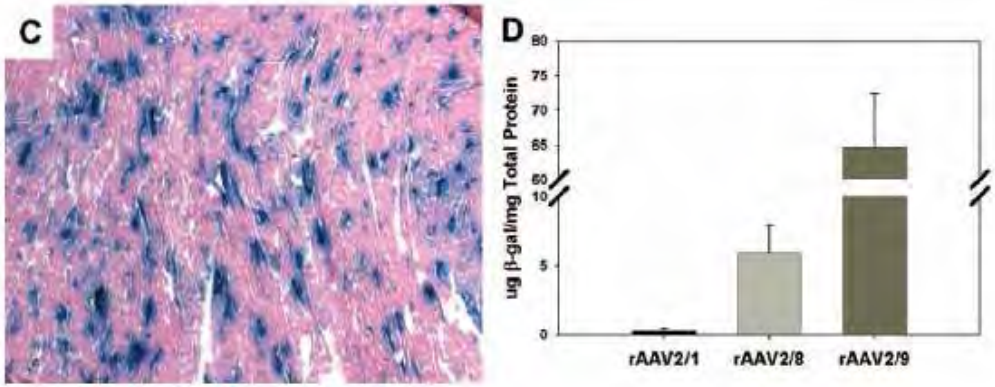


Gregorevic, Nat Med 10: 828-834

AAV-9



Inagaki et al. Molecular Therapy 14: 45-53, 2006



Circ Res Pacak et al. 99 (4): e3.

Next:

3 papers:
Adenovirus and vein grafting
AAV and cardiac disease
Plasmids and peripheral ischaemia

Journal Club.

Further general gene therapy reading:

FOCUS ON GENE-BASED THERAPIES
Nature Reviews Genetics, May 2011.