Cardiac Gene Therapy When, Where and Why?

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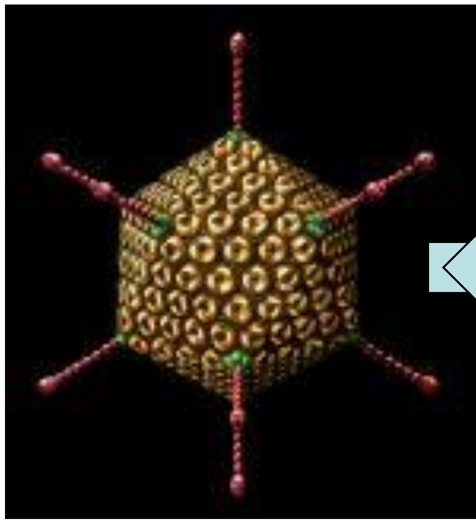
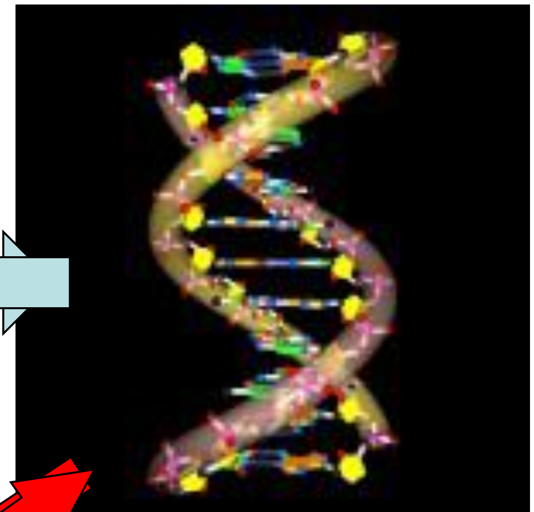
OVERVIEW

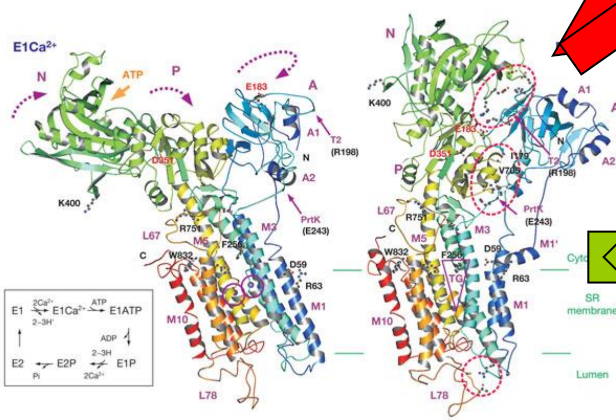
- What is Cardiac Gene Therapy
- Clinical Gene Therapy
- The Past
- Cardiac Gene Therapy
  - Strategy
  - Clinical considerations
  - Gene Therapy Vectors
- The Present
- Candidate genes therapies for CCF
    - SERCA2a gene therapy
    - Development from bench to bedside
    - Arrhythmia Safety Studies
    - Clinical Trials
- The Future

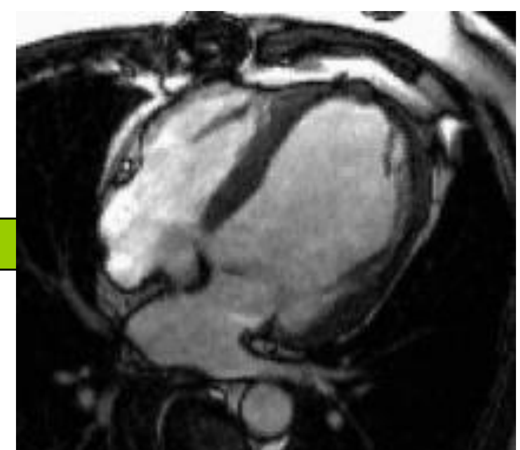
Gene Therapy in Cardiac Failure





SERCA2a protein





Cardiac Gene Therapy

- Myocardial Ischaemia
  - Angioneogenesis
- Myocardial Dysfunction / Heart failure
  - Abnormal Ca^{2+} Cycling
  - Inflammation, cell survival, ECM remodelling

- Monogenic Cardiac Diseases
  - Ion Channelopathies
    - LQTS, SQTS, Brugada, CPVT
  - Familial DCM/HCM/ARVC
- Pulmonary Hypertension
- Atherosclerosis
- Tachyarrhythmias
- Biological pacemakers
- Cardiac Allograft Rejection

Clinical Gene Therapy

- Sceptism in Medical Community
- 20 years of ‘promises’
- Tragedies in 1990s
- Inadequate SAE reporting (37/940!)
- Conflicts of Interest
- Lack of Overt Progress during 1990s
  - Cardiac angioneogenesis trials – VEGF, FGF-4
  - NB adenoviral vector, transgene selection

Cardiac Gene Therapy
The Past

- AGENT trial programme
  - Angioneogenesis for myocardial ischaemia

  Vector Adenovirus

  Gene FGF-4

  Delivery IC infusion

  - AGENT 2 – Phase 2 – 52 pts 2:1 tx vs placebo
    - Non-significant trend to ↓ ischaemia
    - 13/35 pts SAEs – transient ↓ plts, ↑LFTS
  - AGENT 3 – Phase 3 trial – design 450 pts
    - Recruitment stopped after interim analysis 300 pts
    - No benefit

Cardiac Gene Therapy
The Past

- REVASC trial
  - Angioneogenesis for myocardial ischaemia

Vector	Adenovirus
Gene	VEGF121
Delivery	Surgical Intramyocardial injection

  - 77 pts stable angina – 35tx vs 32 placebo
  - Improved symptom scores and time to ST↓
  - No difference on myocardial perfusion scans
  - 1 death (post operative)
  - No vector related SAEs

Cardiac Gene Therapy
The Present

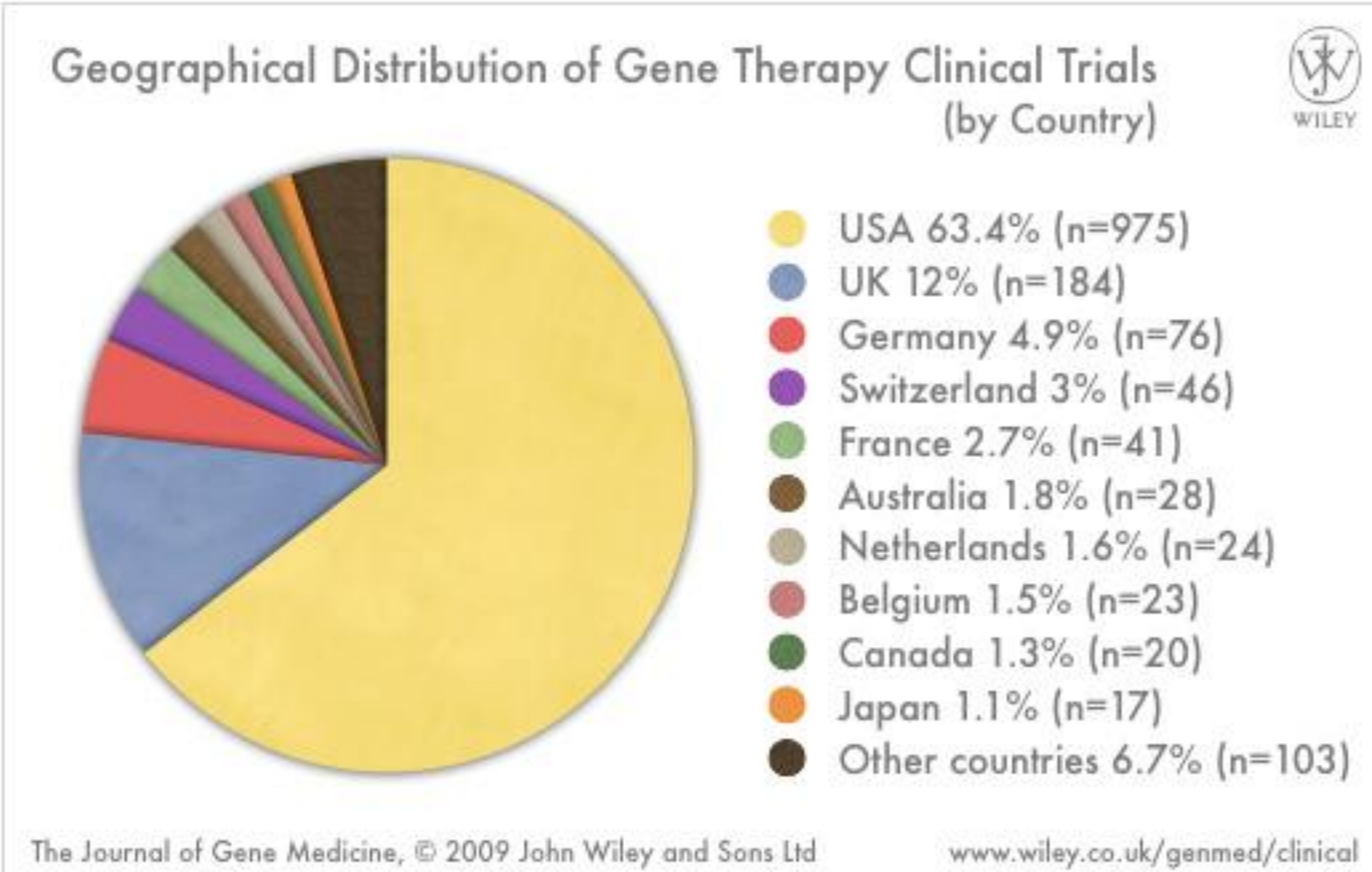
Clinical Gene Therapy
The Present

- New Regulation
  - FDA, EMEA
  - Cell and Gene Therapy Specific National Ethics Committees

- New Vectors

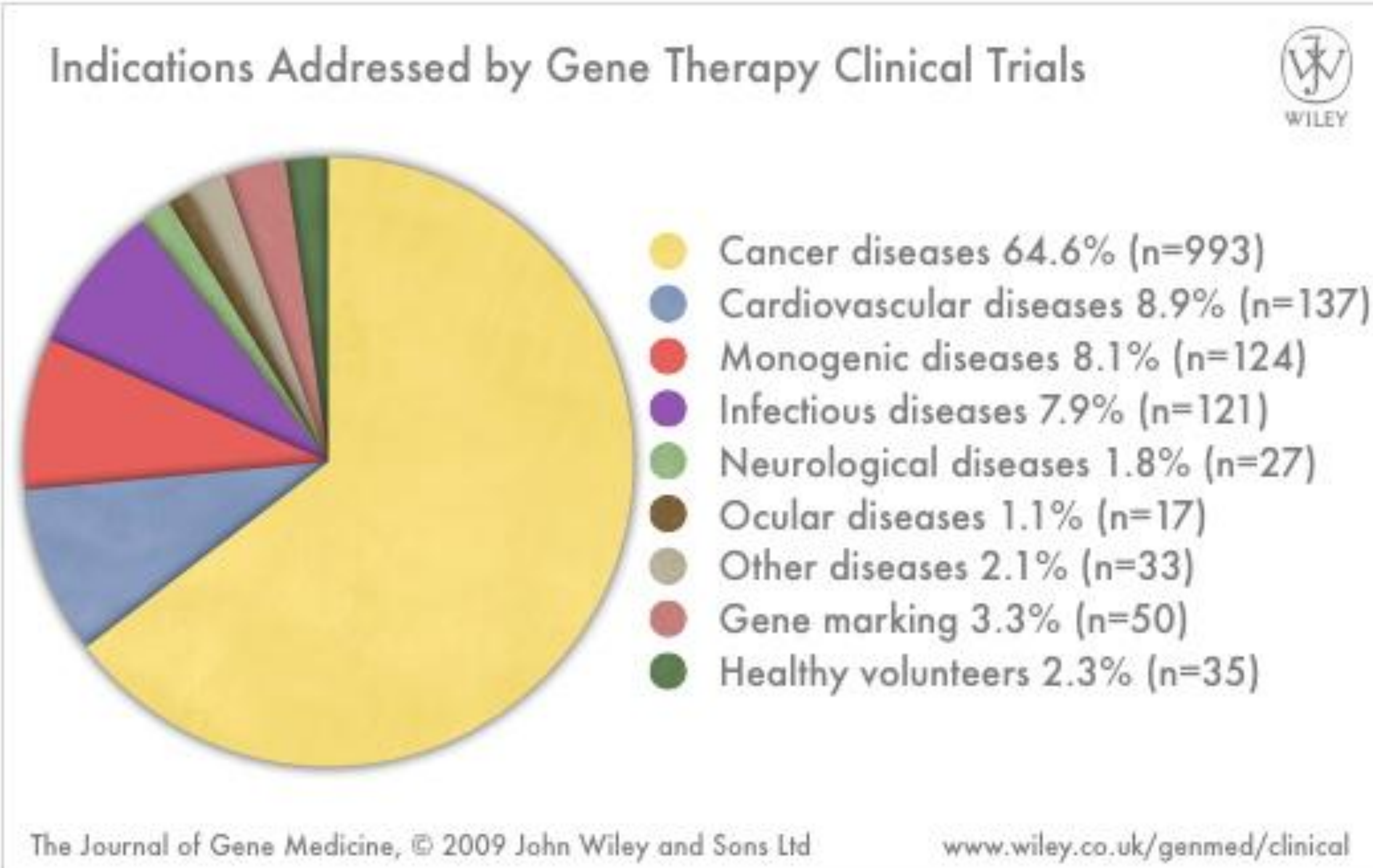
- New Clinical Gene Therapy Successes from 2006-present
  - Metastatic Melanoma
  - Haemophilia
  - Metastatic Breast Carcinoma
  - Leber’s Congenital Amaurosis

Clinical Gene Therapy 2009



1537 patients worldwide
394 pts in Europe (~25%)

Clinical Gene Therapy 2009



Clinical Cardiac Gene Therapy Practical Issues

1. Delivery
2. Therapeutic Efficacy
3. Safety

No different to any other clinical treatment

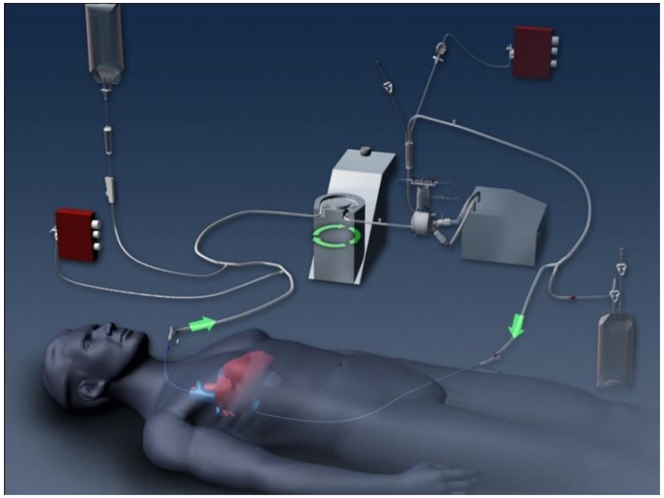
Clinical Cardiac Gene Therapy Practical Issues

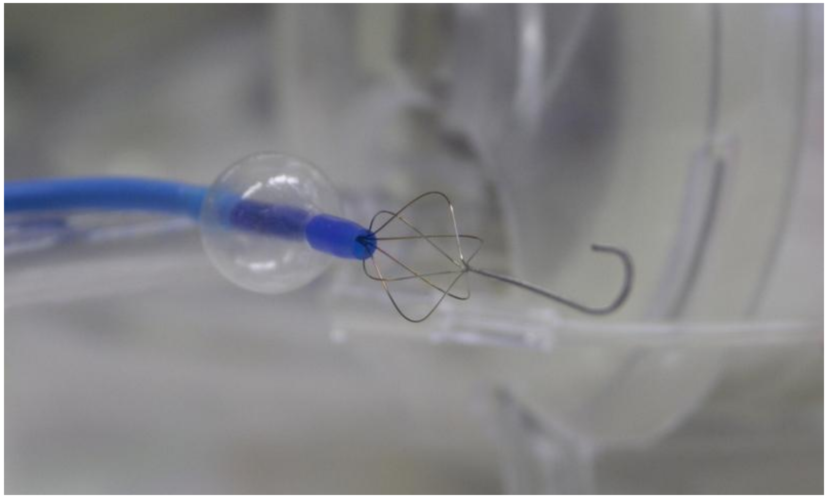
1. Delivery
   - Restrict gene expression to target tissue
     – myocardium
   - Effective gene transfer into cells

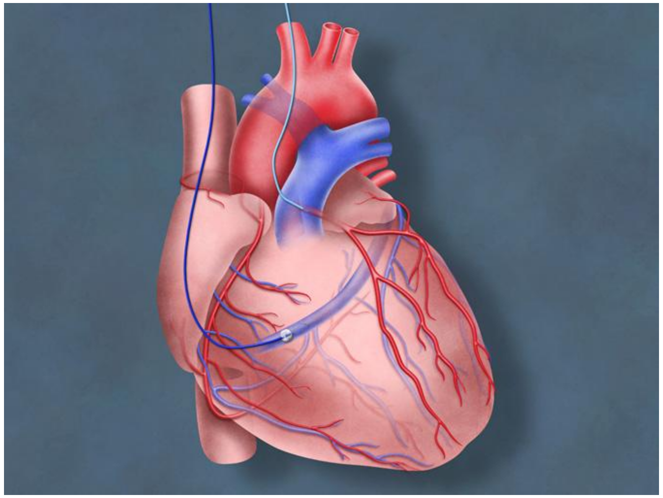
     ~ 10^{10}-10^{11} cardiomyocytes in human left ventricle
   - Effective gene expression
     – Timescale
       » Transient
       » Prolonged
       » Lifelong
     – High levels for therapeutic efficacy

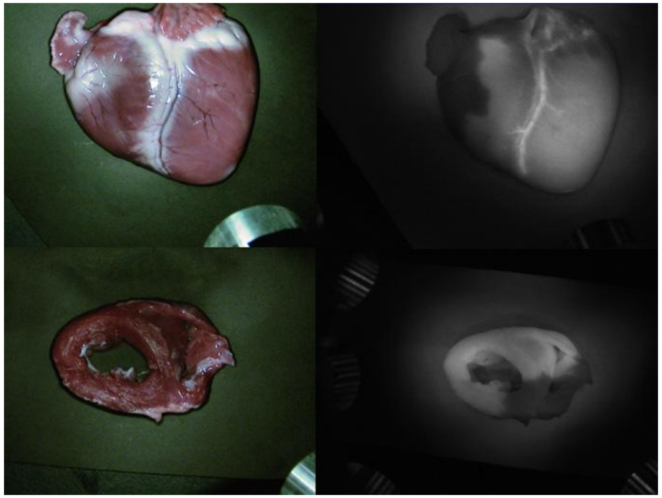
Answer Viral Vectors

New Interventional Technology
V-Focus Recirculating System

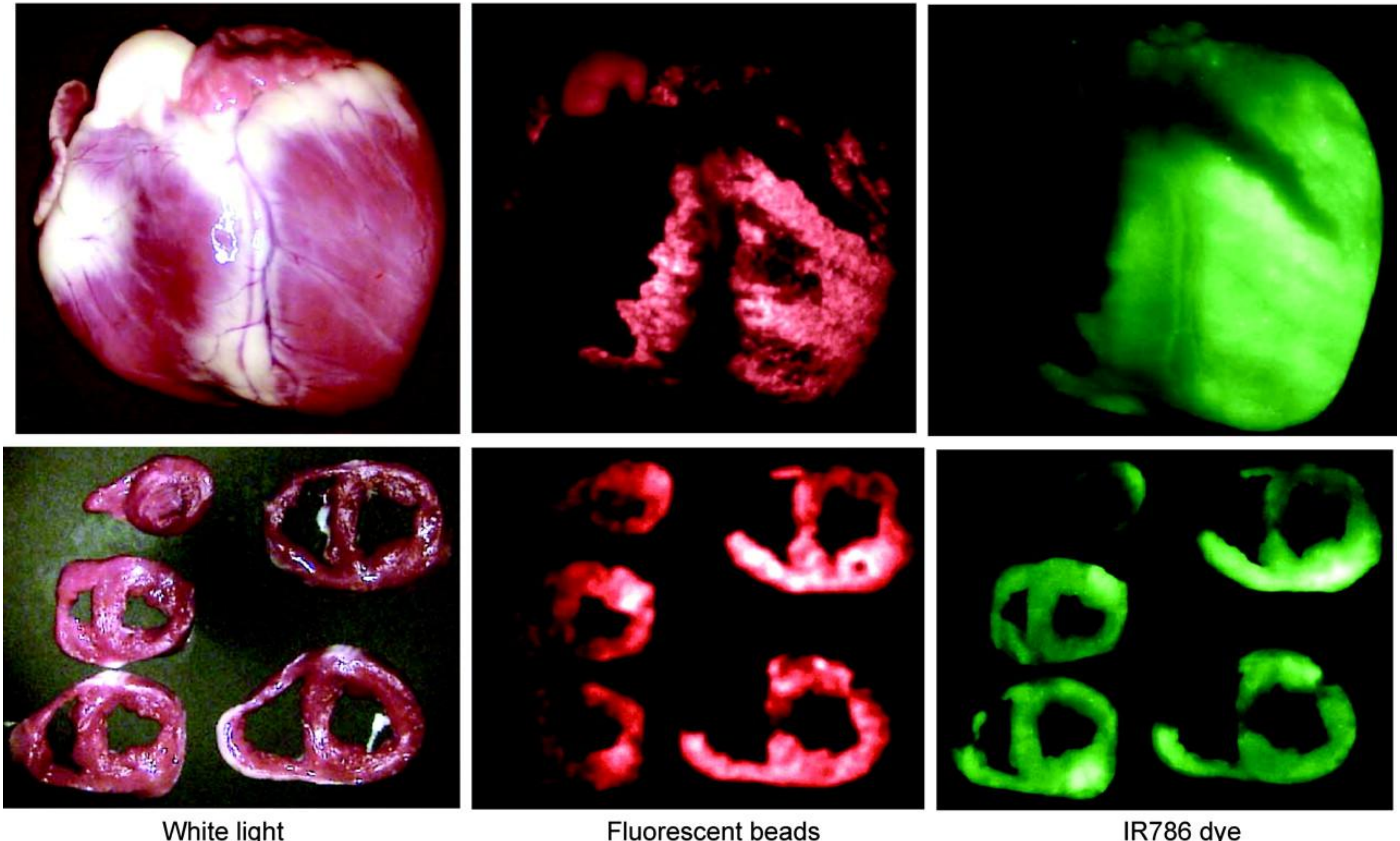








Vector Distribution with Slow Anterograde Left Coronary Infusion



Clinical Cardiac Gene Therapy Practical Issues

2. Therapeutic Efficacy

- Gene selection
- Effective protein production/suppression
- Biological effect
  - Does significant biological effect translate to significant clinical effect?
  - Can this be measured clinically?
- Clinical Outcomes
  - Functional endpoints
    - LVEF
    - LV Remodelling
    - Arrhythmias
    - Neurohormonal markers - BNP
    - QOL/symptoms/exercise tolerance
  - Morbidity and Mortality

Clinical Cardiac Gene Therapy Practical Issues

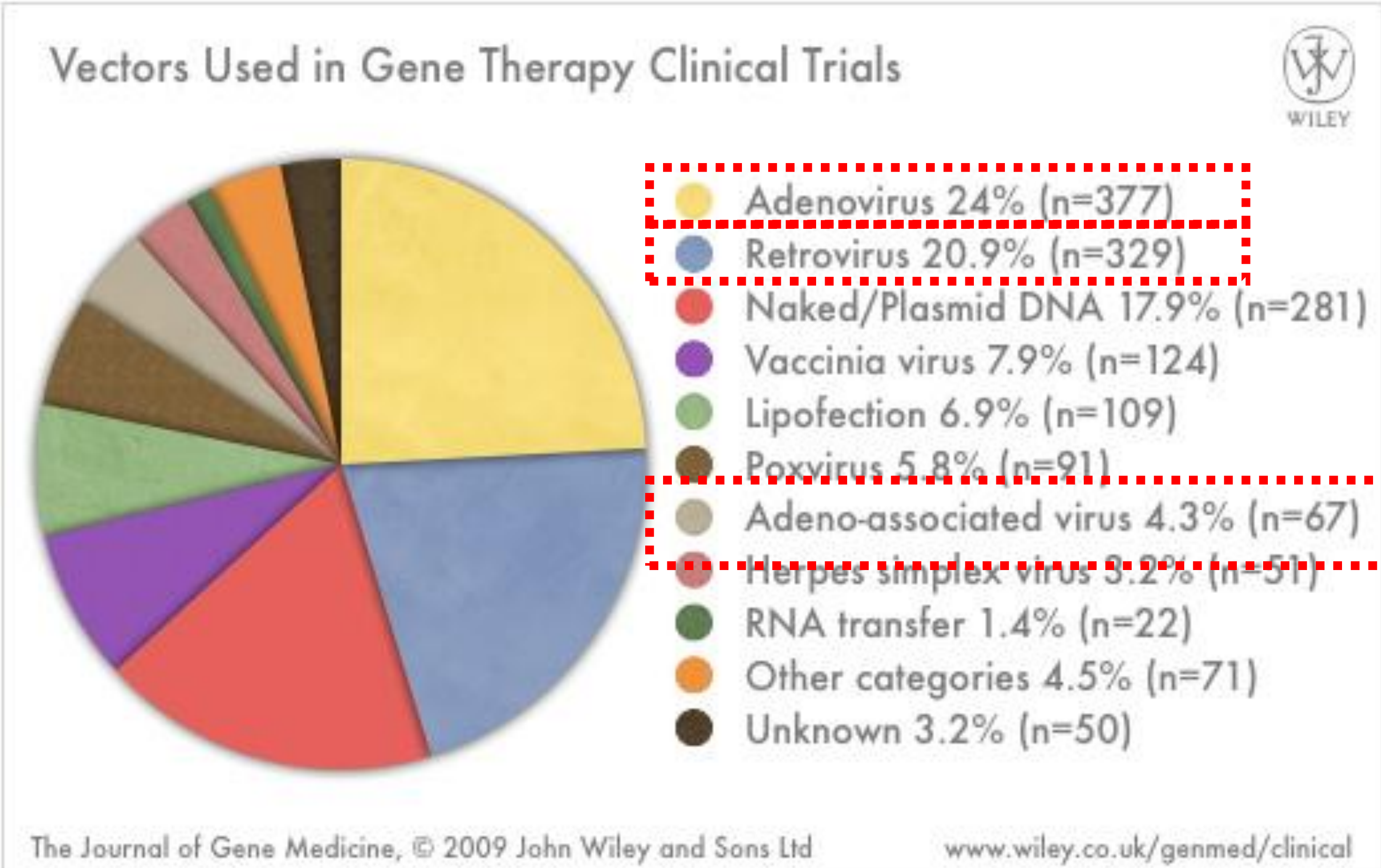
3. Safety

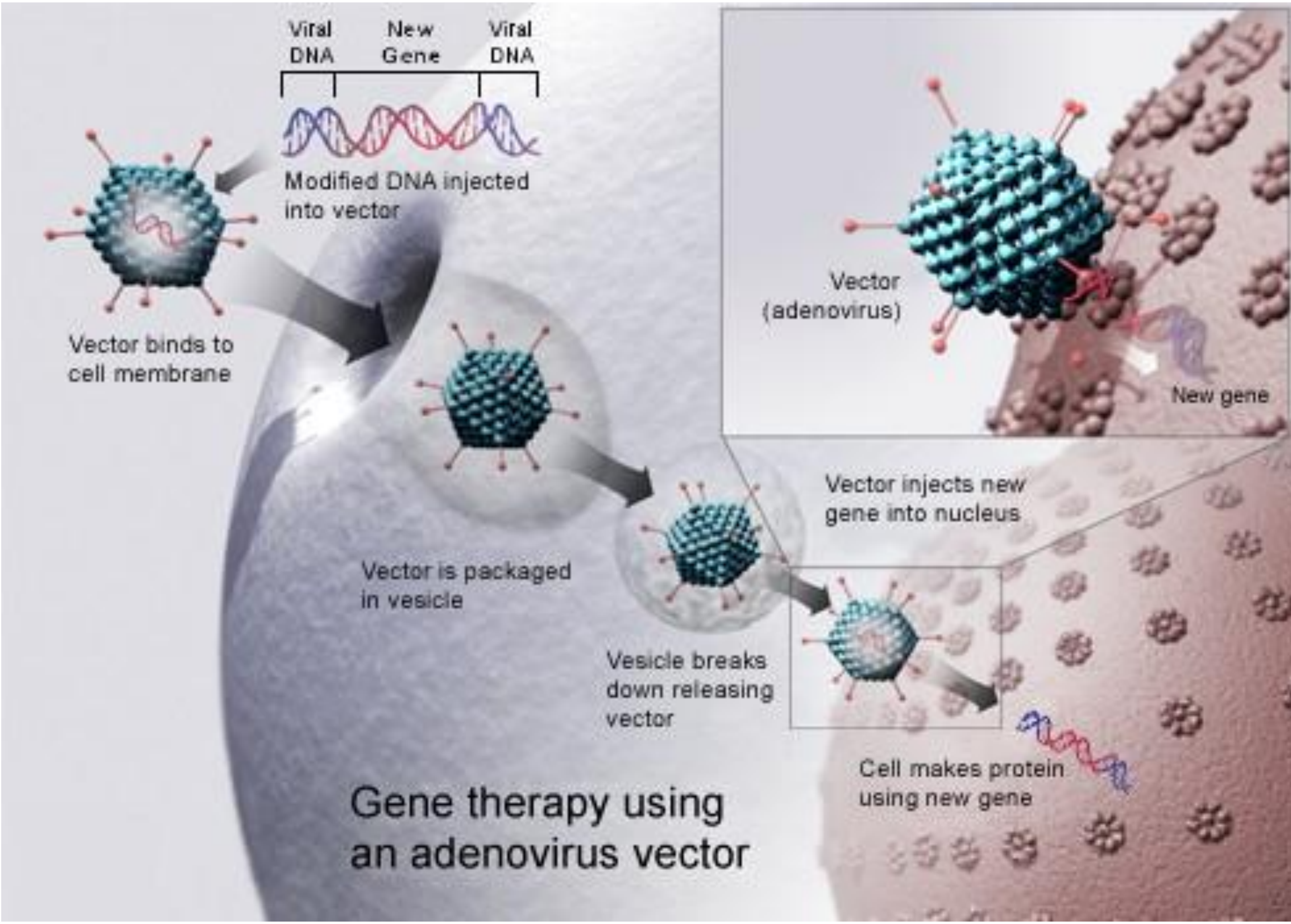
- Who?
  - Patient
  - Close contacts
    - Family
    - Sexual Partners
  - Future Children of Patient
  - Hospital Staff
  - Society
- Why?
  - Cardiac
    - Arrhythmias
    - Myocarditis
    - Increased impairment
  - Systemic
    - Off target effects
    - Inflammatory response syndromes
  - Malignancy
  - Vertical transmission

Viruses

Concerns re Unexpected Problems!

Viral Vectors in Registered Clinical Gene Therapy Trials




Viral DNA
New Gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector is packaged in vesicle
Vesicle breaks down releasing vector
Vector injects new gene into nucleus
Vector (adenovirus)
New gene
Cell makes protein using new gene
Gene therapy using an adenovirus vector

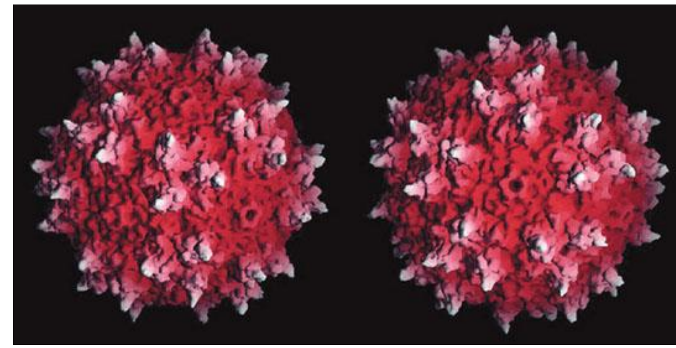
Viral Vectors for Cardiac Gene Therapy Advantages

- High levels of therapeutic gene expression
- Improve tissue targeting with natural tropism
- Produced in high quantities
- Candidates for Myocardial Gene Therapy:
  - Adenoviruses (Ad.)
  - Retroviruses + Lentiviruses (R+L)
  - Adeno-Associated Viruses (AAV)
- All clinical (and research) vectors are recombinant and non-replicating

Viral Vectors for Cardiac Gene Therapy Disadvantages

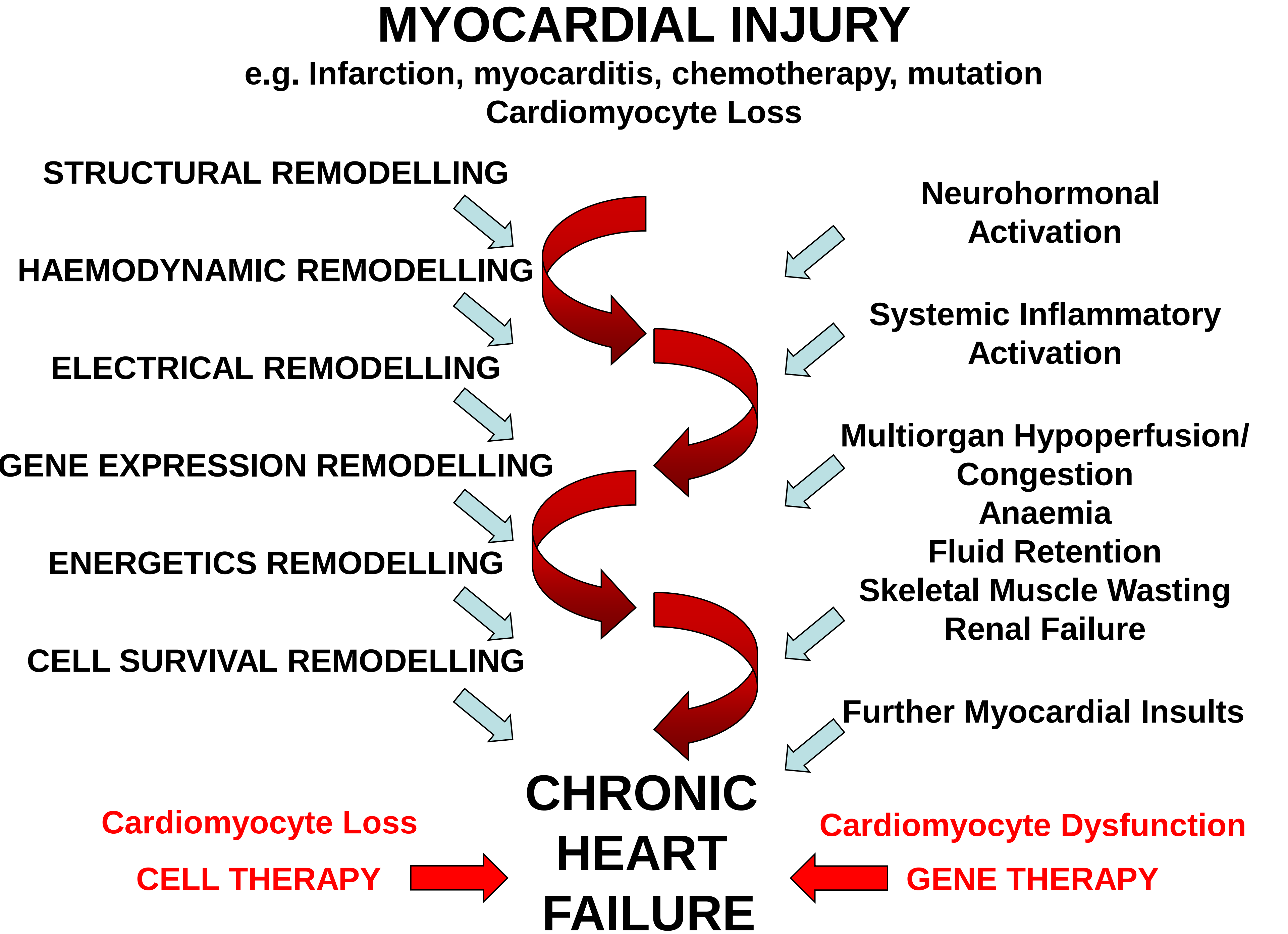
- Provoke immune response (esp. Ad.)
  - Reduces efficacy/duration of gene expression
  - Promotes immune response against host tissue
    - Myocarditis
    - Arrhythmias
  - Neutralising antibodies (all including AAV)
- Lack complete tissue specificity (Ad, R+L)
- Recombination with natural pathogenic viruses
- Cancer Risk
  - DNA integration can promote ‘insertional mutagenesis’ (R+L)
  - SCID children with Retrovirus Gene Therapy ↑Lymphomas
- Smaller viruses limit size of packaged gene (AAV)
- Expensive to produce in GLP/GMP facilities

Cardiac Gene Therapy
The Present
Adeno-Associated Viral Vectors



- Non-pathogenic
- Minimum immunogenicity
- Episomal
- Safety in Humans
  - haemophilia gene therapy trials
- Longterm gene expression in primate muscle studies
- Cardiotropic
  - AAV1
  - AAV6
  - AAV9
- Small
- Safe with immunosuppression in pigs
- NB FIX trial – upper dose ceiling

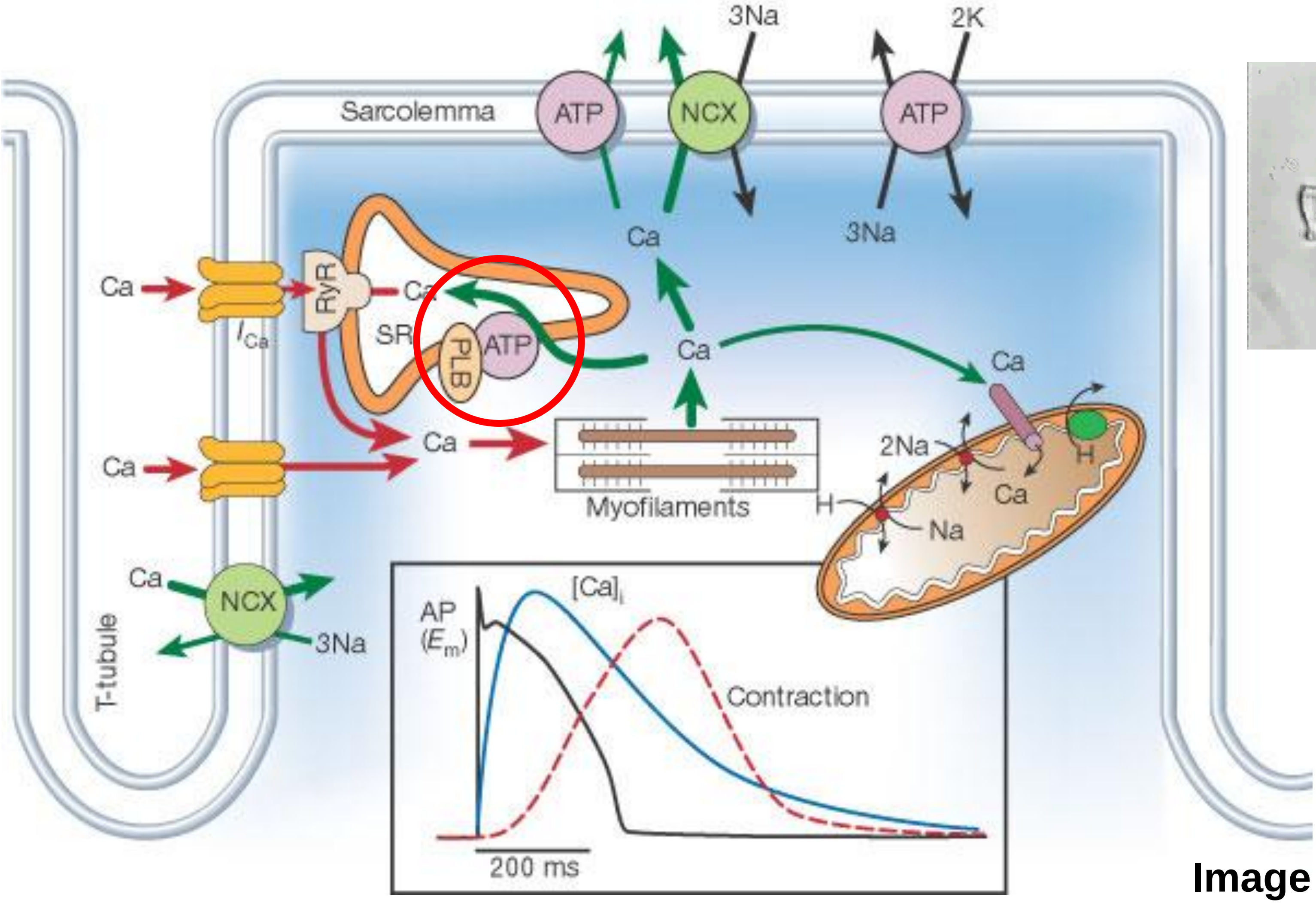
Which Protein/Gene?


MYOCARDIAL INJURY
e.g. Infarction, myocarditis, chemotherapy, mutation
Cardiomyocyte Loss
STRUCTURAL REMODELLING
HAEMODYNAMIC REMODELLING
ELECTRICAL REMODELLING
GENE EXPRESSION REMODELLING
ENERGETICS REMODELLING
CELL SURVIVAL REMODELLING
Neurohormonal Activation
Systemic Inflammatory Activation
Multiorgan Hypoperfusion/ Congestion
Anaemia
Fluid Retention
Skeletal Muscle Wasting
Renal Failure
Further Myocardial Insults
CHRONIC HEART FAILURE
Cardiomyocyte Loss
CELL THERAPY
Cardiomyocyte Dysfunction
GENE THERAPY

Myocardial Gene Therapy
Candidate Genes for Heart Failure

- Cardiomyocyte Ca^{2+} Cycling
  - SERCA2a ★
  - S100A1 ★
  - PLB (dn or as)
  - I-1c ★
- β Adrenoceptor Signalling Pathways
  - βARKct ★
  - Uptake 1
- Anti-inflammatory
  - Soluble TNFα Receptor Fragments
  - ROS Scavengers
    - Haemoxygenase 1
    - Superoxide Dismutase
- Antiapoptotic
  - Cyclin A

Cardiomyocyte Calcium Physiology
Excitation-Contraction Coupling



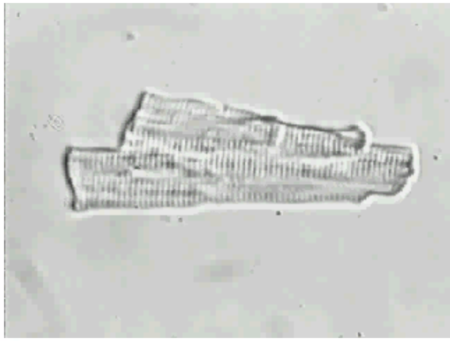


Image from Bers D. Nature 2002 415:198-205

SERCA2a in Heart Failure

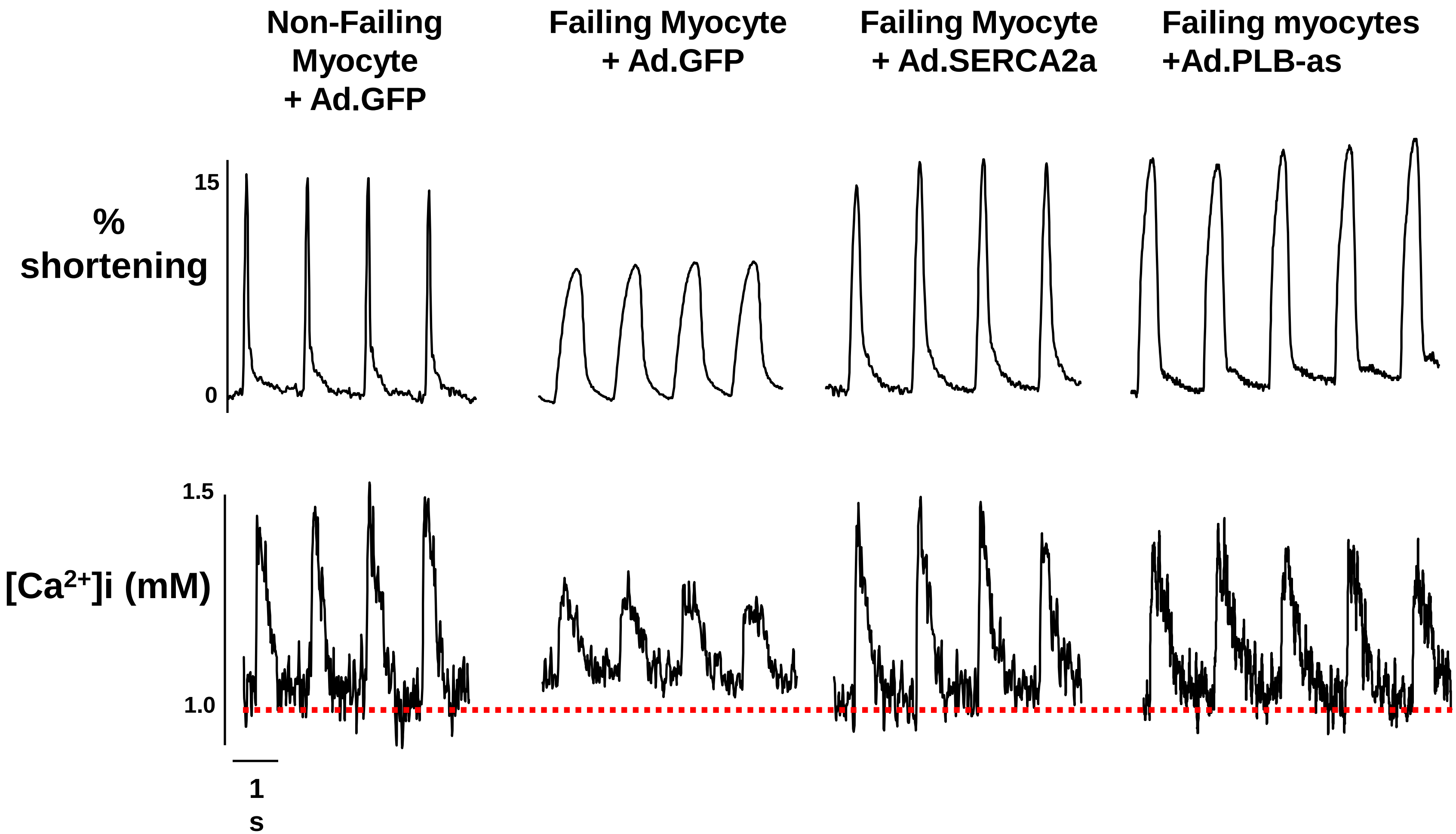
- SERCA2a activity is reduced in Cardiomyocytes from failing hearts – human + animal models
- This causes:
  - Delayed removal of Ca^{2+} in diastole
  - Reduced velocity of diastolic relaxation, leading to increased diastolic chamber stiffness
  - Reduced SR Ca^{2+} stores for systolic release → Reduced inotropism
  - Increased frequency of afterdepolarisations
  - Contributes to action potential prolongation

} VT/VF

SERCA2a Gene Therapy and Heart Failure

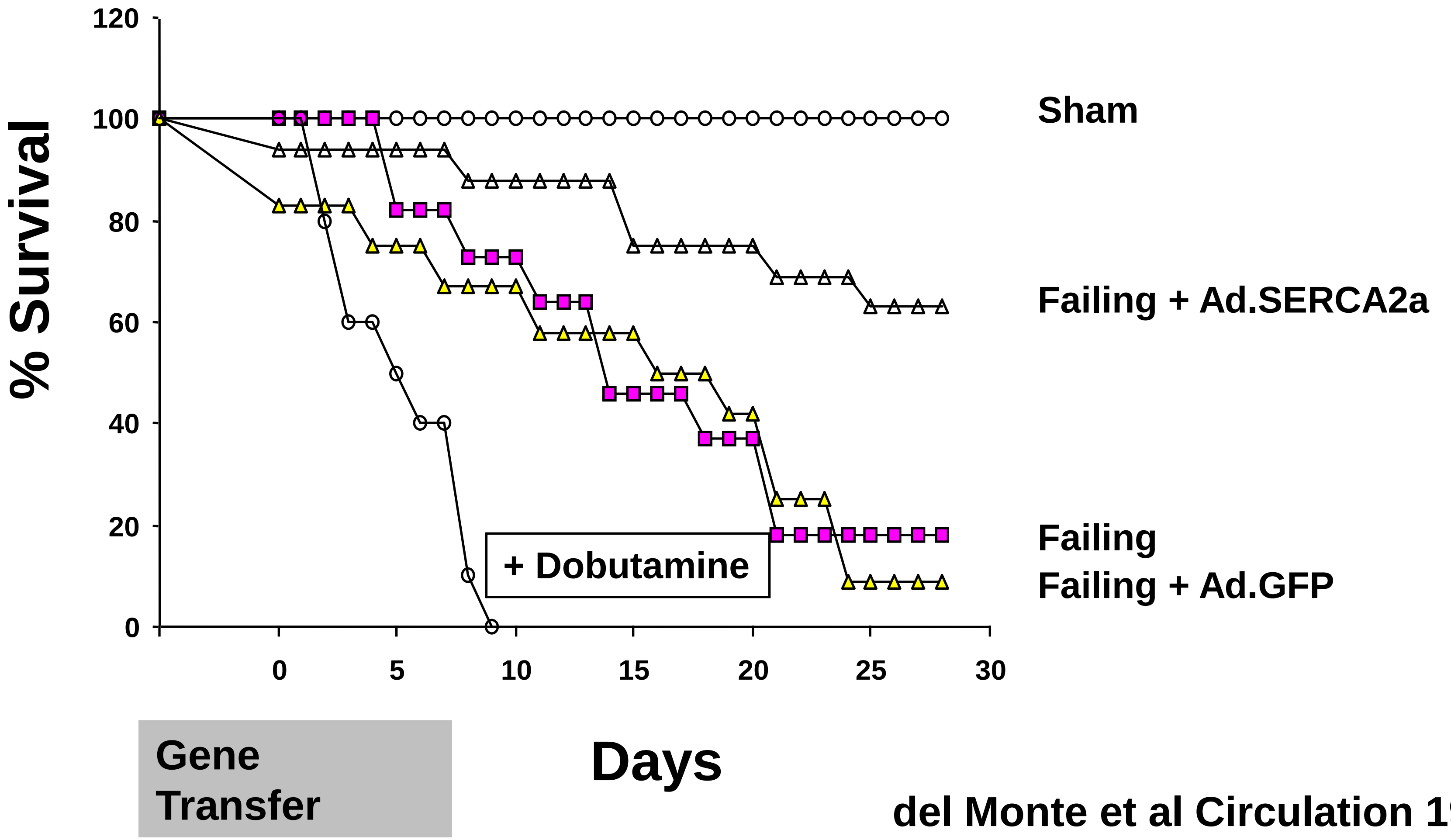
- SERCA2a improves cardiac function
  - Ca^{2+} cycling + SR Ca^{2+} stores
  - Contractility
  - Relaxation
  - Energetics (NB vs dobutamine, milrinone etc)
- Isolated CM – failing human, rat, rabbit
- Animal HF models
    - Rat – Aortic Banding, Acute Post MI, Chronic Post MI HF
    - Mouse – Aortic Banding
    - Pig – Acute and Chronic MR
    - Sheep – Tachypacing HF

In Vitro Adenoviral transfection of myocytes from failing human ventricle



del Monte et al Circulation 1999

Effect of SERCA2a Gene Transfer on Survival in Rats with Pressure-Overload Hypertrophy in Transition to Heart Failure

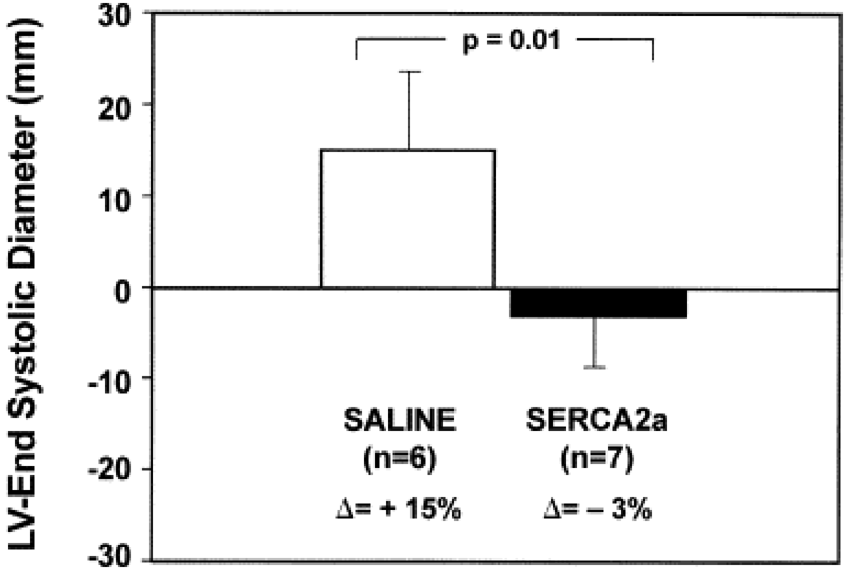


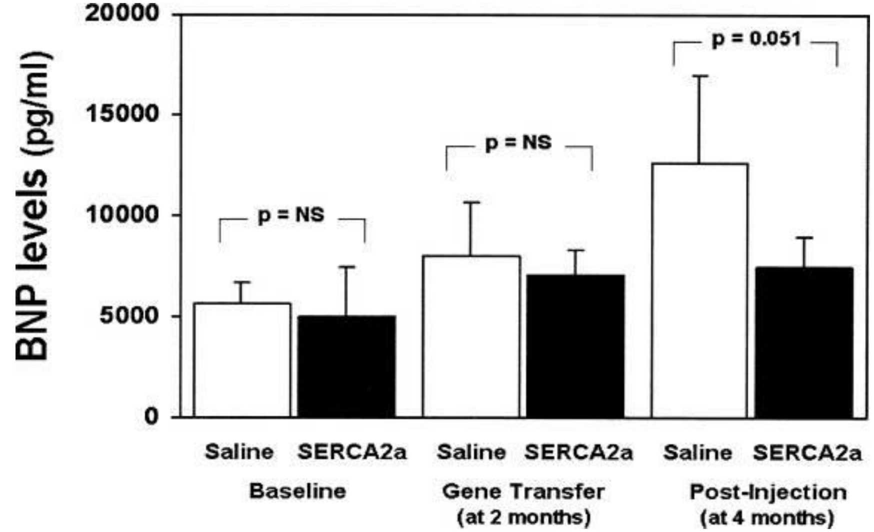
del Monte et al Circulation 1999

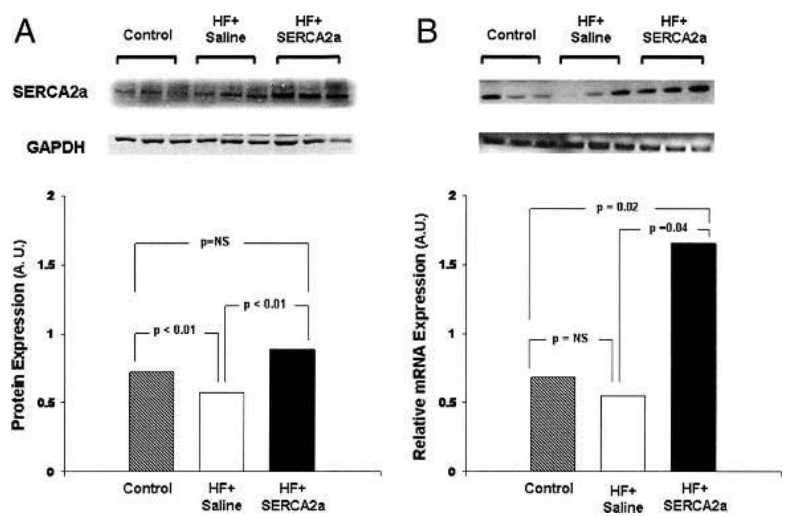
Pig Chronic MR HF Model

AAV1.SERCA2a at 2months post MR

Assessment at Baseline, 2m (PREGENE) and 4m (POSTGENE







Clinical Translation of SERCA2a Gene Therapy for Chronic Heart Failure

Two Clinical Gene Therapy Trials

Both coordinated by Roger Hajjar
(Mount Sinai Hospital, New York)
1. US – Celladon Corporation
AAV1.SERCA2a

2. UK – Imperial College
AAV6.SERCA2a
Prof Sian Harding
Harefield and Papworth
Funded by Fondation Leducq and BHF

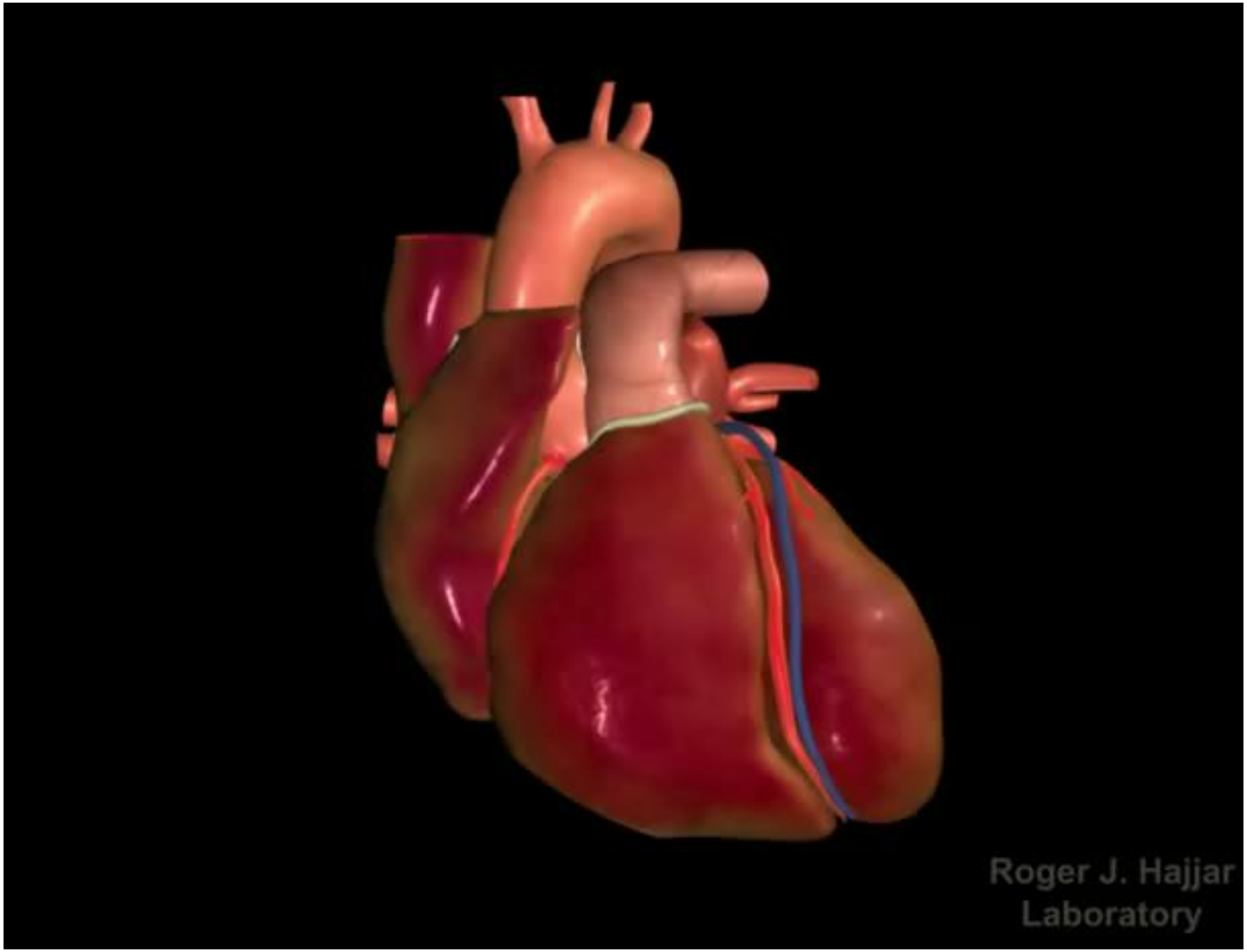
Clinical Translation of SERCA2a Gene Therapy for Chronic Heart Failure

- US Trials (CUPID 1 and 2)
  - Celladon (Biotech)
  - IC infusion of AAV1.SERCA2a
  - NYHA III/IV HF, LVEF <30%, ICD, Heart Tx waiting list
  - First 12 pts – Open label 4 escalating dose tiers
  - Second 39pts – randomised AAV1.SERCA vs placebo (8:8:9:14)
  - Completed recruitment and vector administration 8/09
  - 12m F/U - AHA 2010
- UK Trial
  - Imperial College
    - Harefield and Papworth Hospitals
  - IC infusion of AAV6.SERCA2a
  - Chronic HF, Post LVAD implantation
  - 16 pts – randomised 8 gene tx vs 8 placebo
  - Safety
  - Biological efficacy of gene transfer/expression
  - ETA Q3 2011

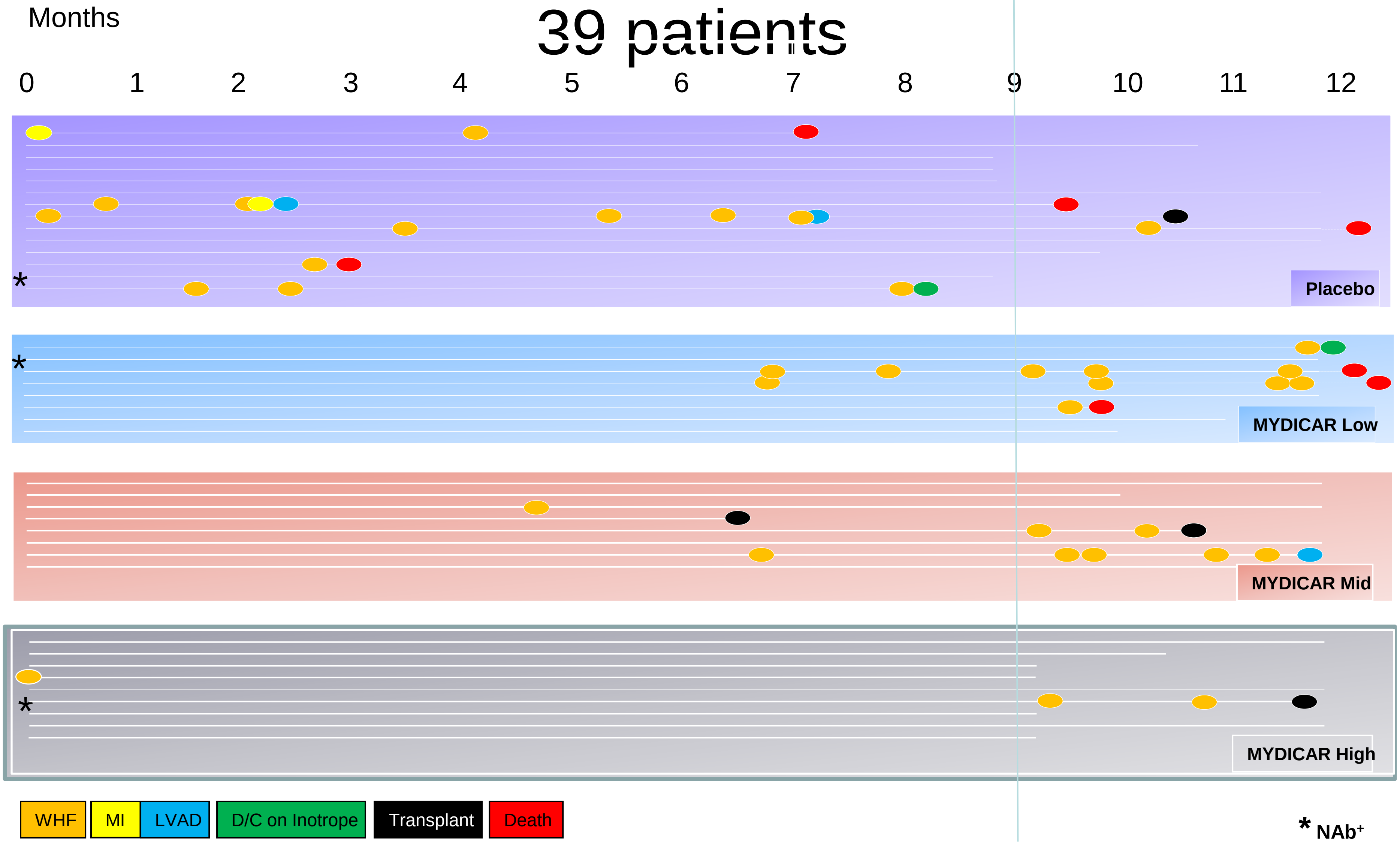
CUPID Trial Population
Severe Chronic Heart Failure

- Events in pre-screened patients (n=102)
  - Worsening Heart Failure n=11 (11%)
    - Transplant n=9 (9%)
    - Implanted LVAD n=2 (2%)
  - Other CV hospitalization=7 (7%)
  - All-Cause Death n=4 (4%)
- Total n=22 (22%) in 14 months

Clinical AAV SERCA2a Gene Therapy

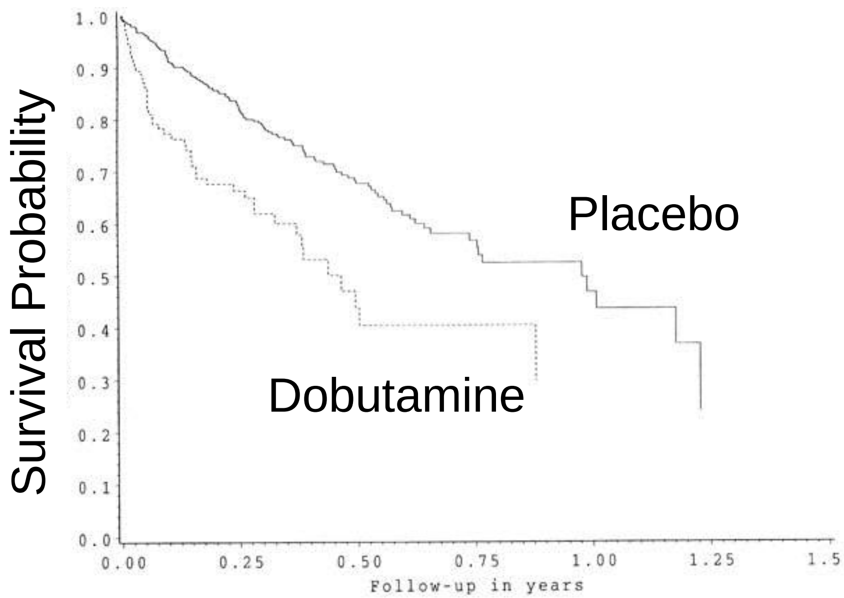


CUPID Phase 2 SERCA2a Gene Therapy Trial
Time to Multiple Clinical Events
39 patients



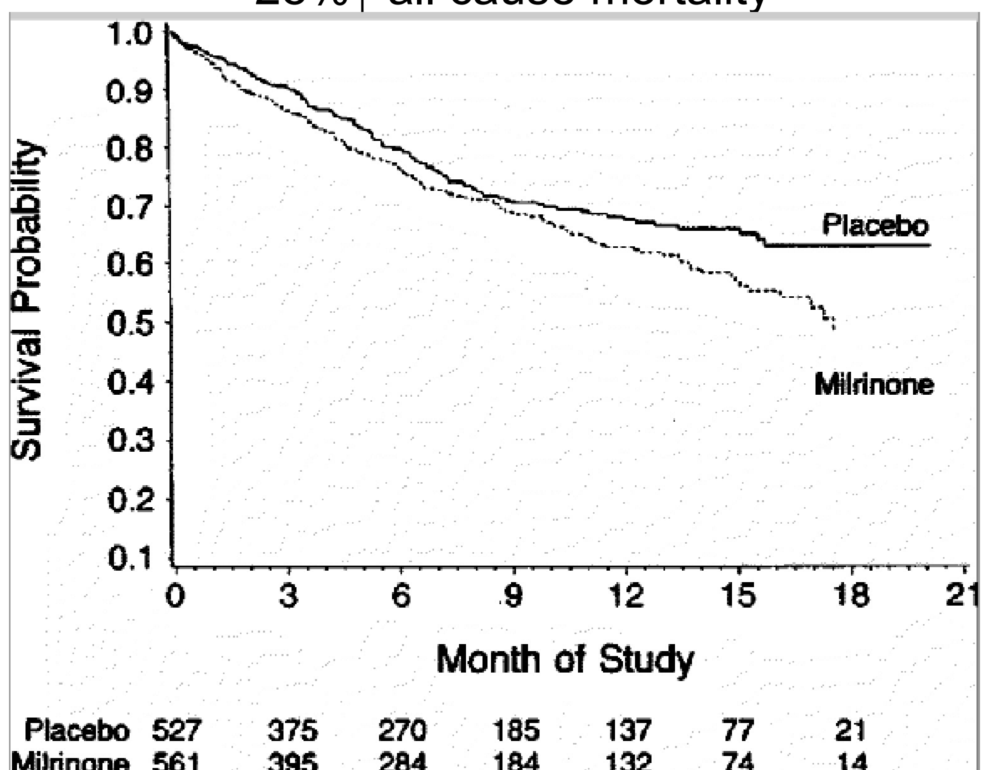
Current Positive Inotropes Increase Mortality

- Flolan Study Group: FIRST Trial
  - Dobutamine vs No dobutamine
  - Mortality 70.5% vs 37.1%



AHJ 1999 138 78-86

- PROMISE study (NEJM 1991)
  - 1081 pts with NYHA III/IV HF
  - 40mg po milrinone vs placebo
  - 28%↑ all cause mortality



SERCA2a Gene Therapy for Heart Failure
Suspected and Unexpected Surprises

Best Penalty ever Awana Diab (UAE) vs Lebanon via backheel.mp4

Could SERCA2a gene therapy in the failing ventricle be proarrhythmic?
Cellular and Subcellular Hypotheses

1. Cardiomyocyte Ca^{2+} cycling and arrhythmias
   - SR calcium leak arrhythmogenic
     - Ca^{2+} sparks → Ca^{2+} waves → DADs
     - DADs → Ventricular ectopics (trigger) + (?) Sustained VT
   - SR calcium overload → ↑ leak
   - ?↑SERCA2a →↑SR [Ca^{2+}] → ↑SR Ca^{2+} leak→↑ DADs

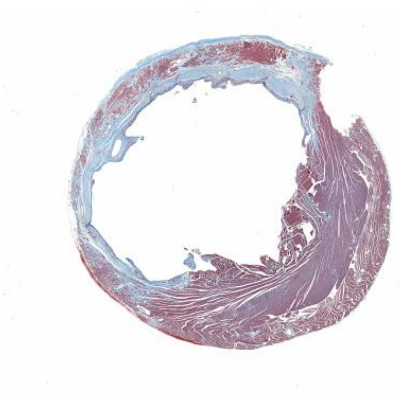
2. These arrhythmic mechanisms are potentially amplified with β1AR + β2AR activation
   - PKA – PLB, RyR2, I-1
   - CaMKII – RyR2, PLB

Could SERCA2a gene therapy in the failing ventricle be proarrhythmic? Multicellular, Tissue and Intact Organ Levels

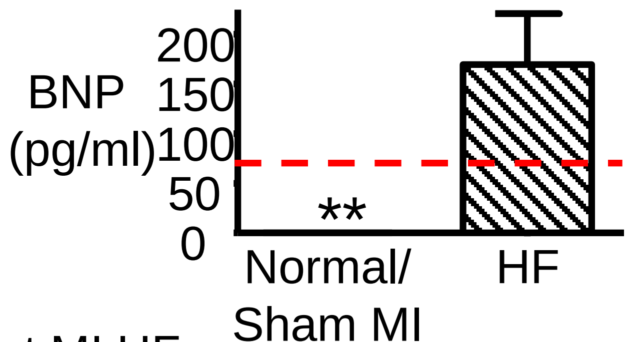
3. APD heterogeneity – arrhythmogenic – local reentry
   - Reduced Ca^{2+} transients contributes to APD prolongation
   - SERCA2a expression shortens APD
   - Patchy SERCA2a transfection - ? ↑heterogeneity of APD

4. T wave and APD alternans arrhythmogenic
   - Secondary to discordant calcium alternans
   - Gradients maximal where SERCA2a expression lowest

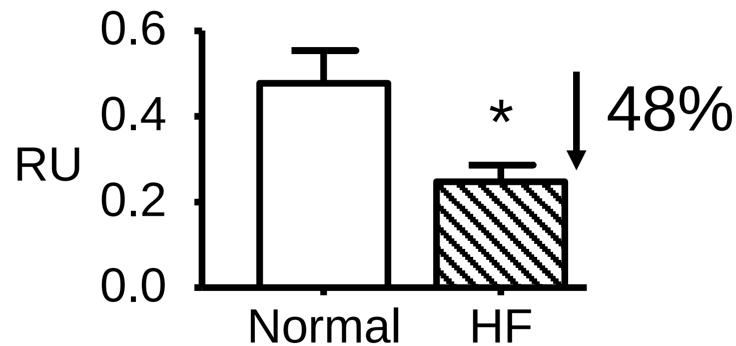
HF Model

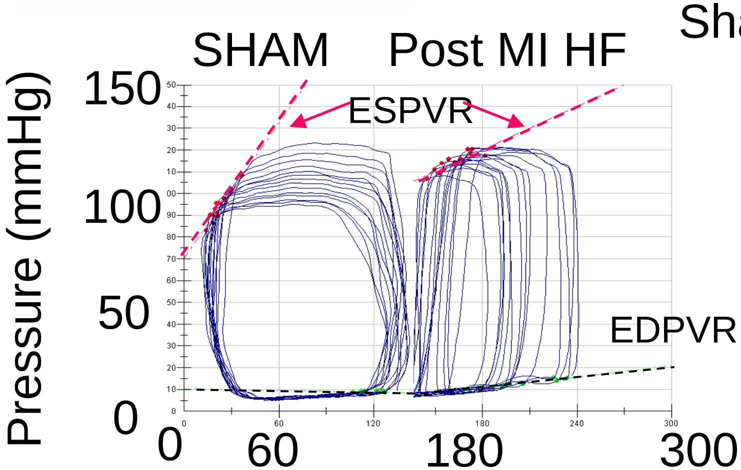


Serum BNP

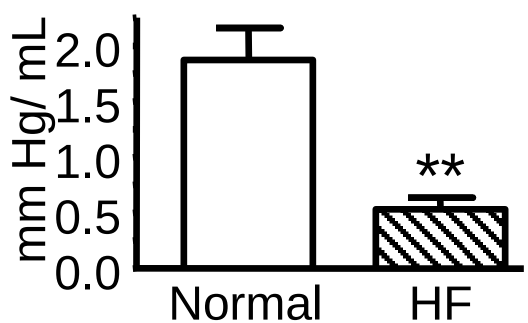


Myocardial SERCA2a

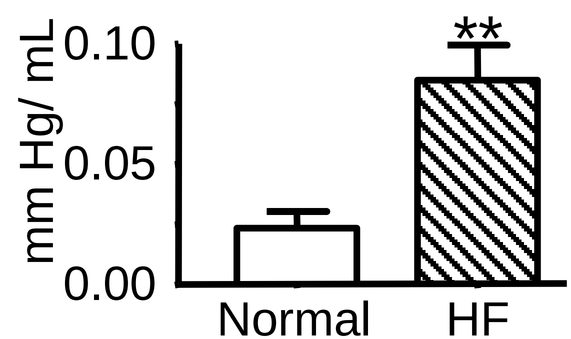




ESPVR



EDPVR

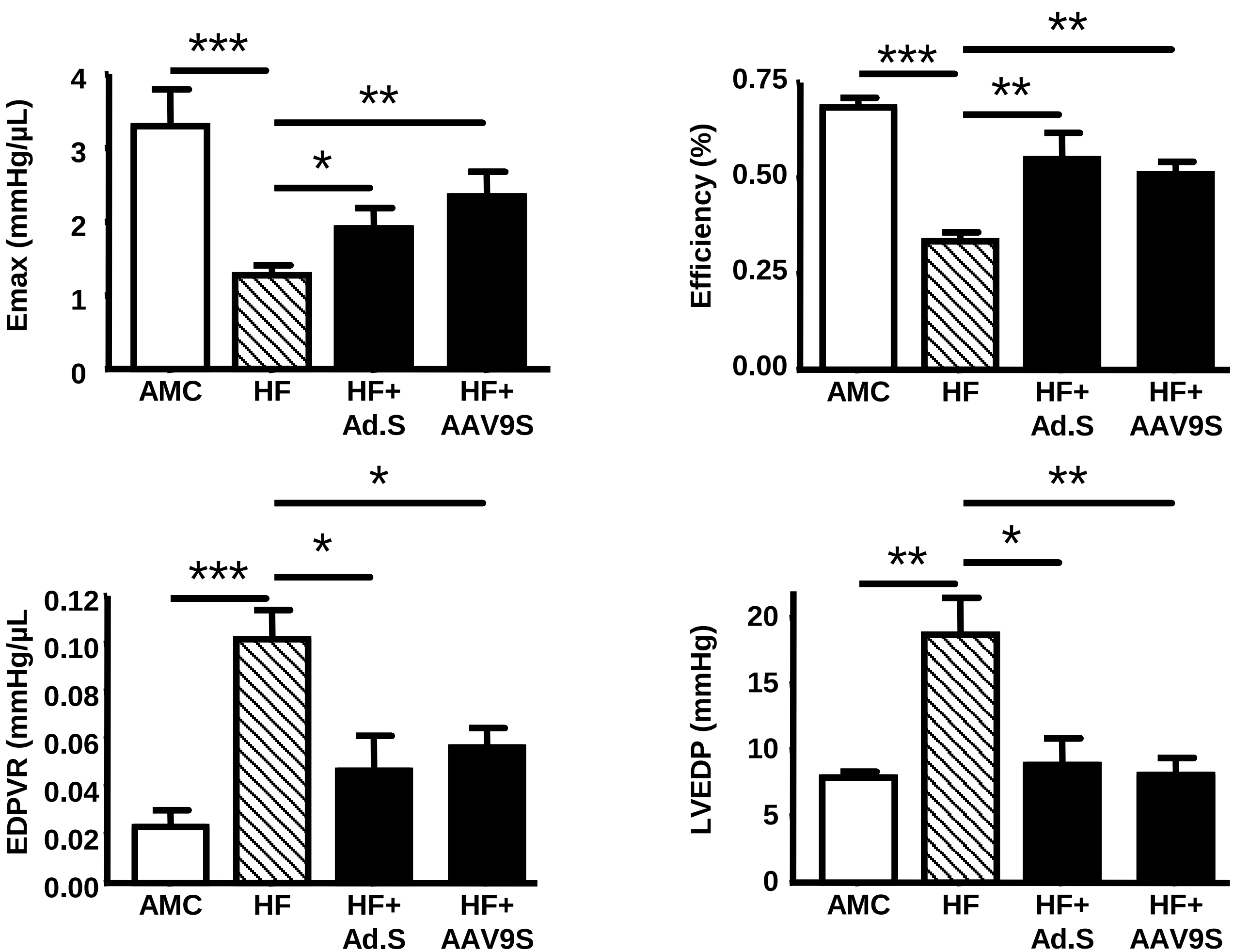


Lyon AR et al PNAS 2009 106 (16) 6854-59.

SERCA2a Gene Therapy Arrhythmia Studies

MI	*In vivo* 24 hour ECG recording + ISO challenge (PREGENE)	*In vivo* Intramyocardial Ad.SERCA2a.GFP Gene Injection x3	*In vivo* 24 hour ECG recording + ISO challenge (POSTGENE)	*Ex vivo* Langendorff: PES Challenge Western Blotting PV Loops
Wk1	**15**	**16**	**16+6/17**	**17+1**

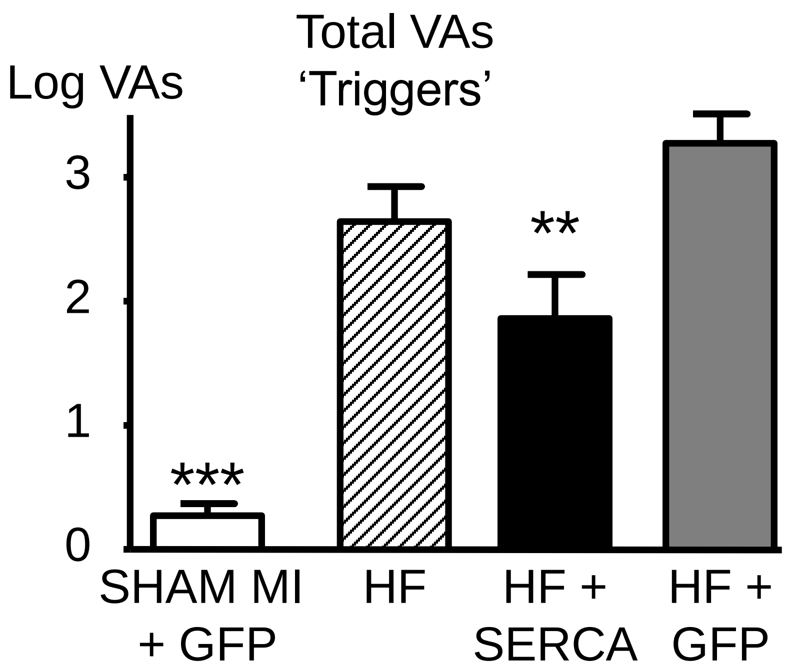
SERCA2a Gene Therapy is a Positively Inotropic and Lusitropic in the Chronic Post MI HF Model

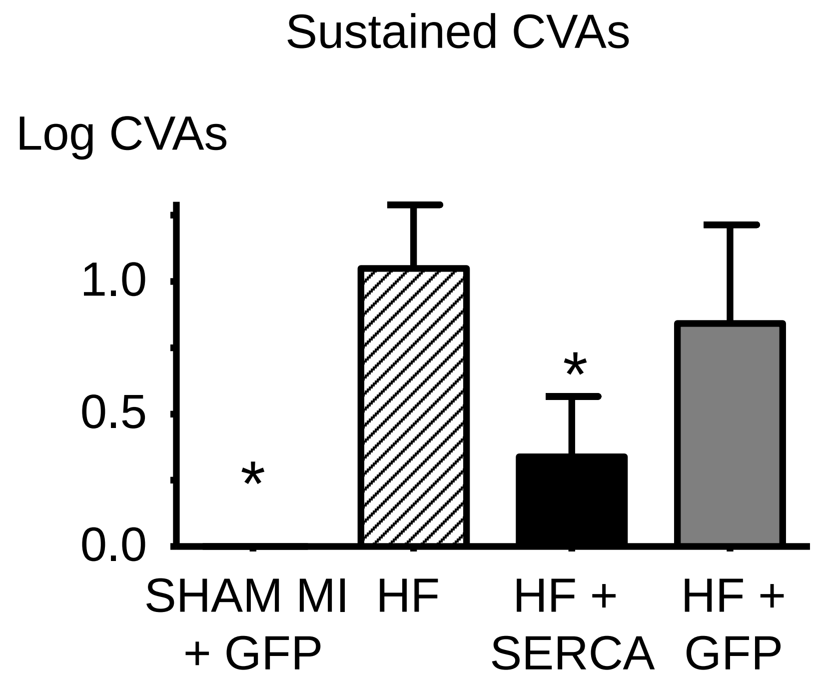


* p<0.05
**p<0.01
***p<0.001
vs HF

Lyon AR et al Circulation Arrhythmia Electrophysiology (in press)

In Vivo Arrhythmia Assessment
Spontaneous Ventricular Arrhythmias





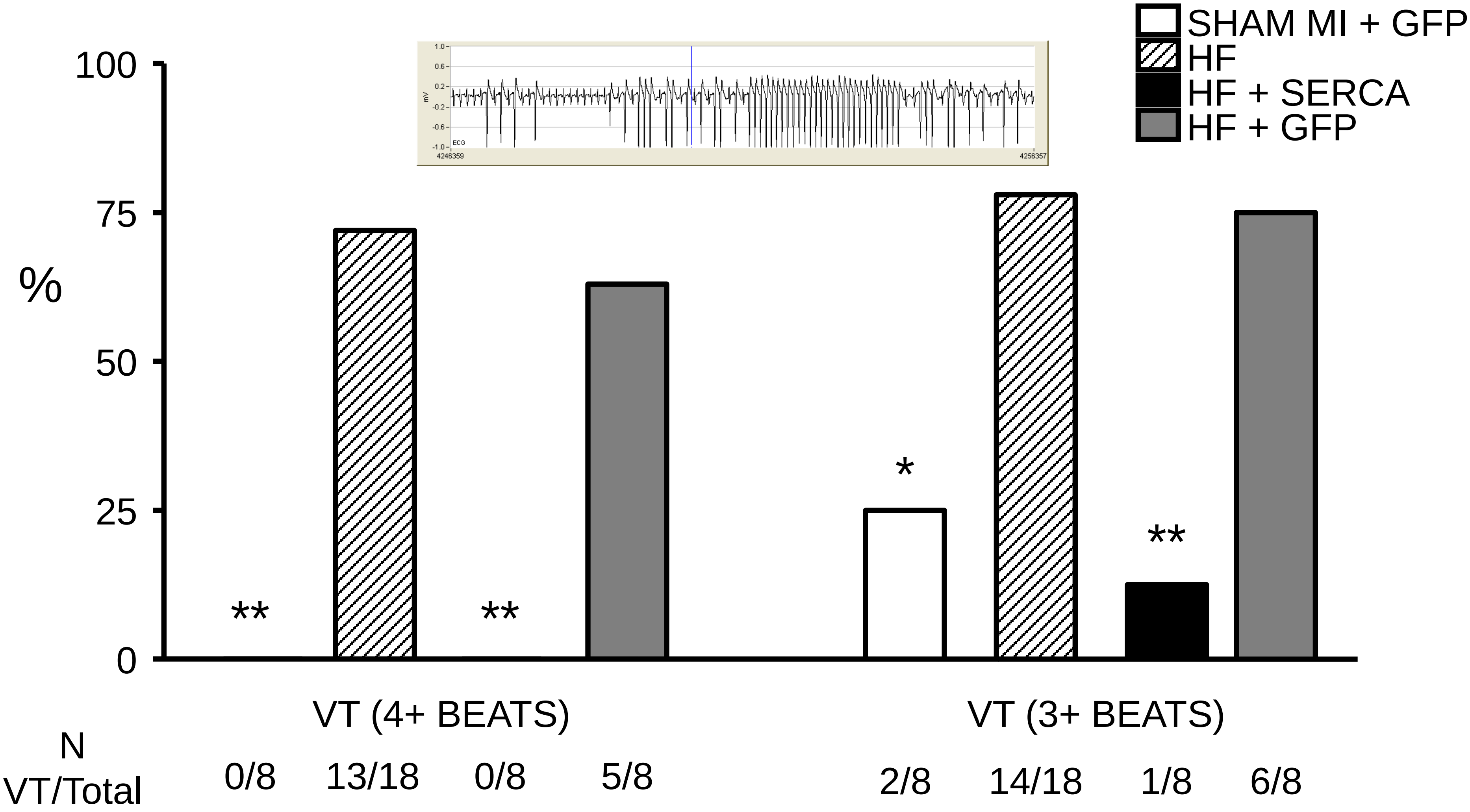
CVAs = Couplets, Triplets, VT and VF

n=8 per study arm *p<0.05, **p<0.01 and ***p<0.001 vs HF and HF + GFP

Lyon AR et al Circulation Arrhythmia Electrophysiology (in press)

In Vivo Arrhythmia Assessment
ISO-Induced Ventricular Tachycardia

*p<0.05 and **p<0.01 vs HF



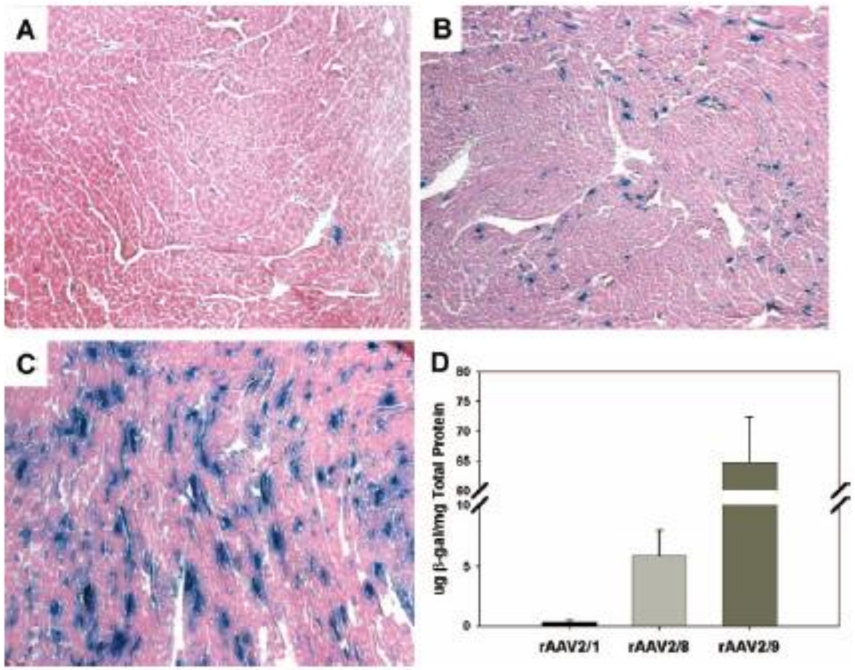
Lyon AR et al Circulation Arrhythmia Electrophysiology (in press)

What are the mechanisms underlying the antiarrhythmic effect of SERCA2a gene therapy?

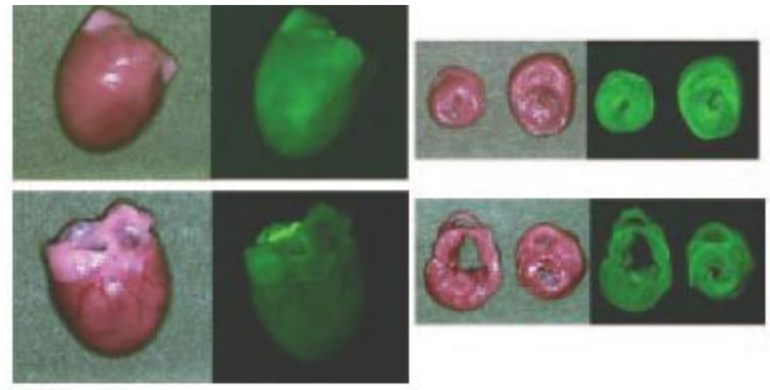
Mechanisms of Arrhythmogenesis in Chronic Heart Failure

- Subcellular
- Cellular
- Intact heart

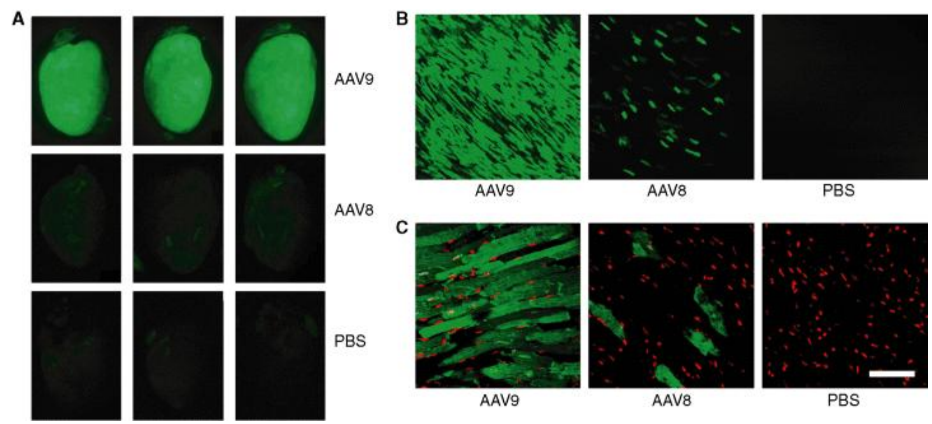
Cardiac Gene Transduction by AAV9 Vectors



Pacak et al Circ Res 2006 <u>99</u> 3-9

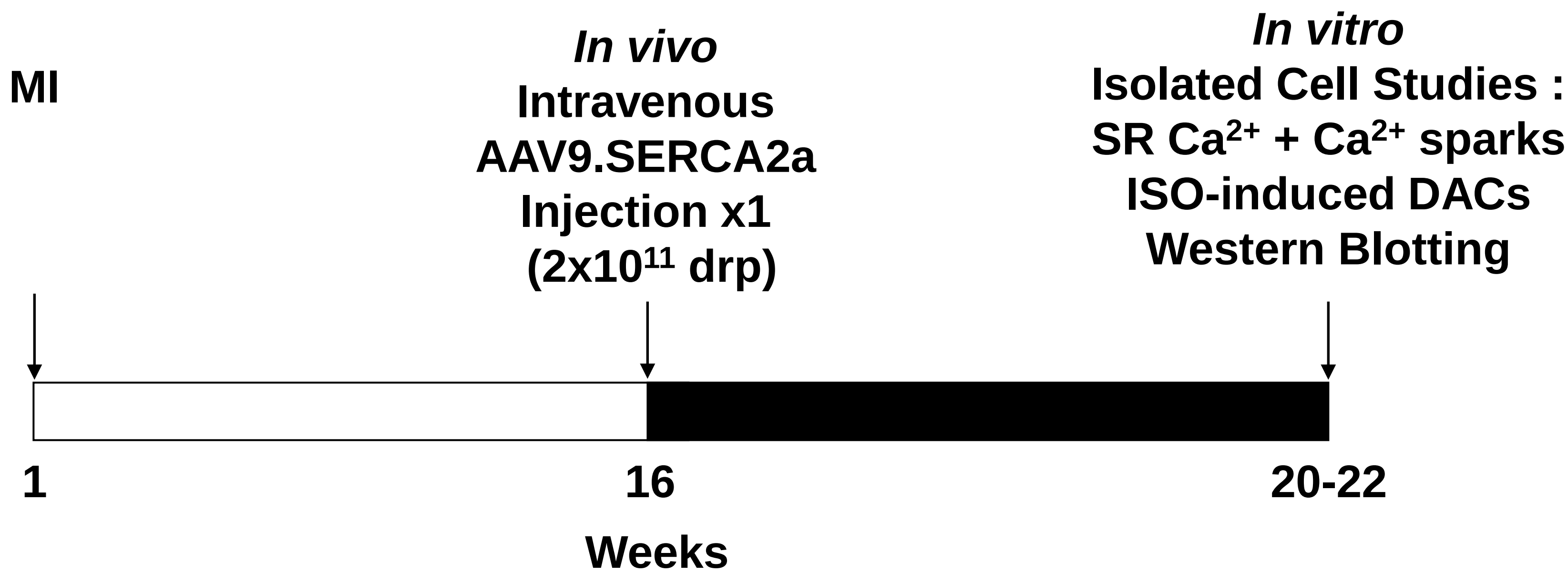


Suckau et al Circ 2009 <u>119</u> 1241-52

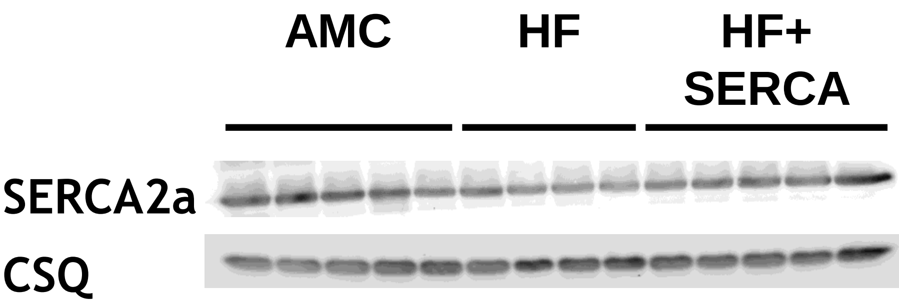


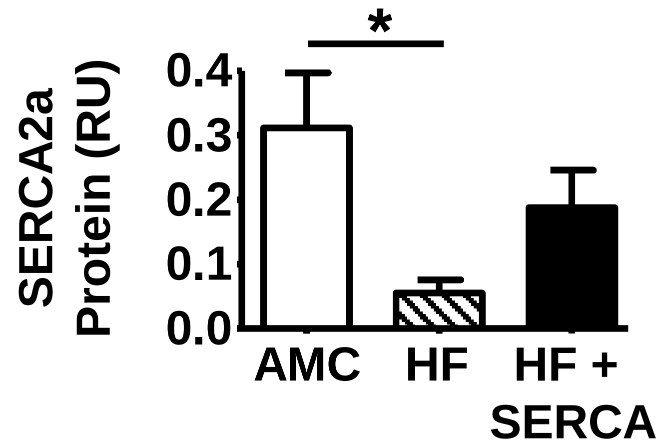
T . Vandendriessche et al. J. Thromb. Haem. <u>5</u> 16-24

Intravenous SERCA2a Gene Therapy

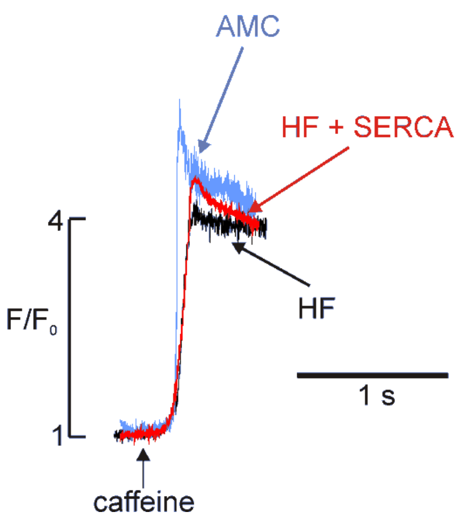


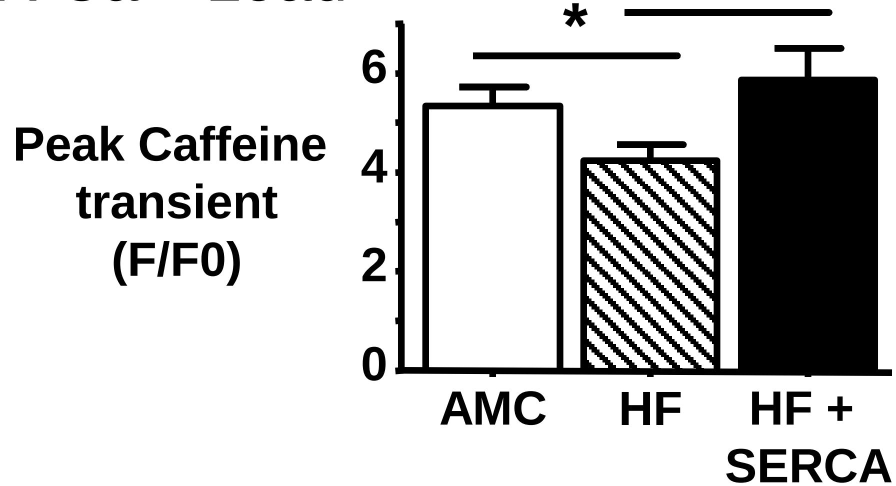
AAV9.SERCA2a gene therapy normalises SR Ca^{2+} content





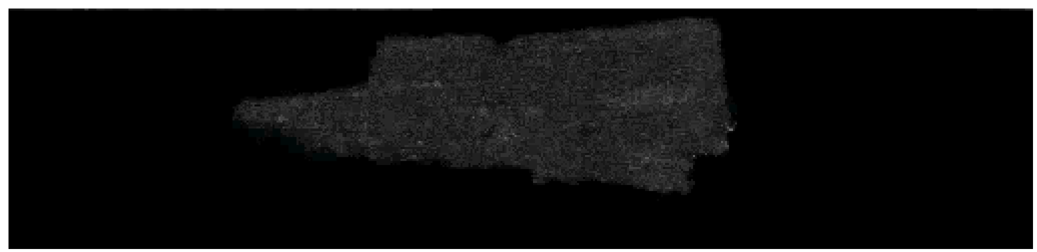
SR Ca^{2+} Load



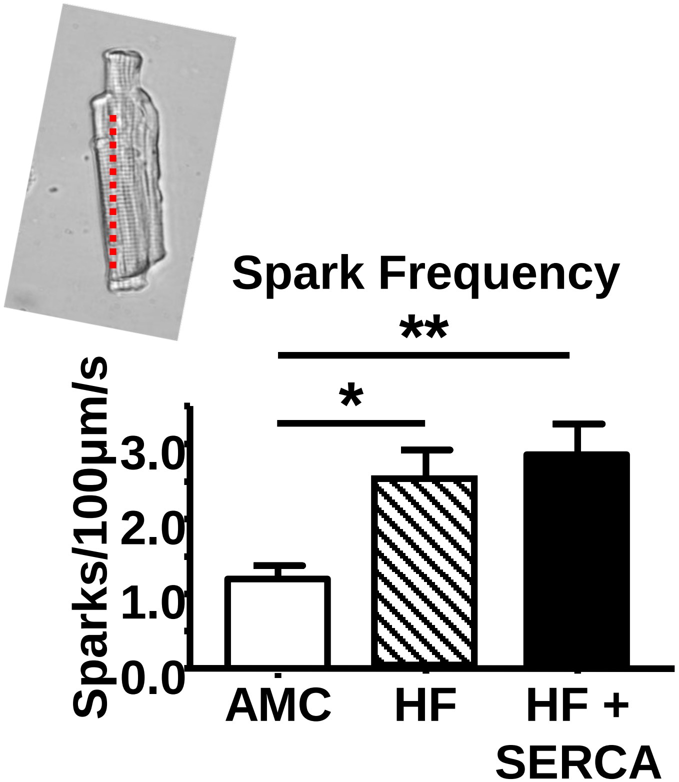


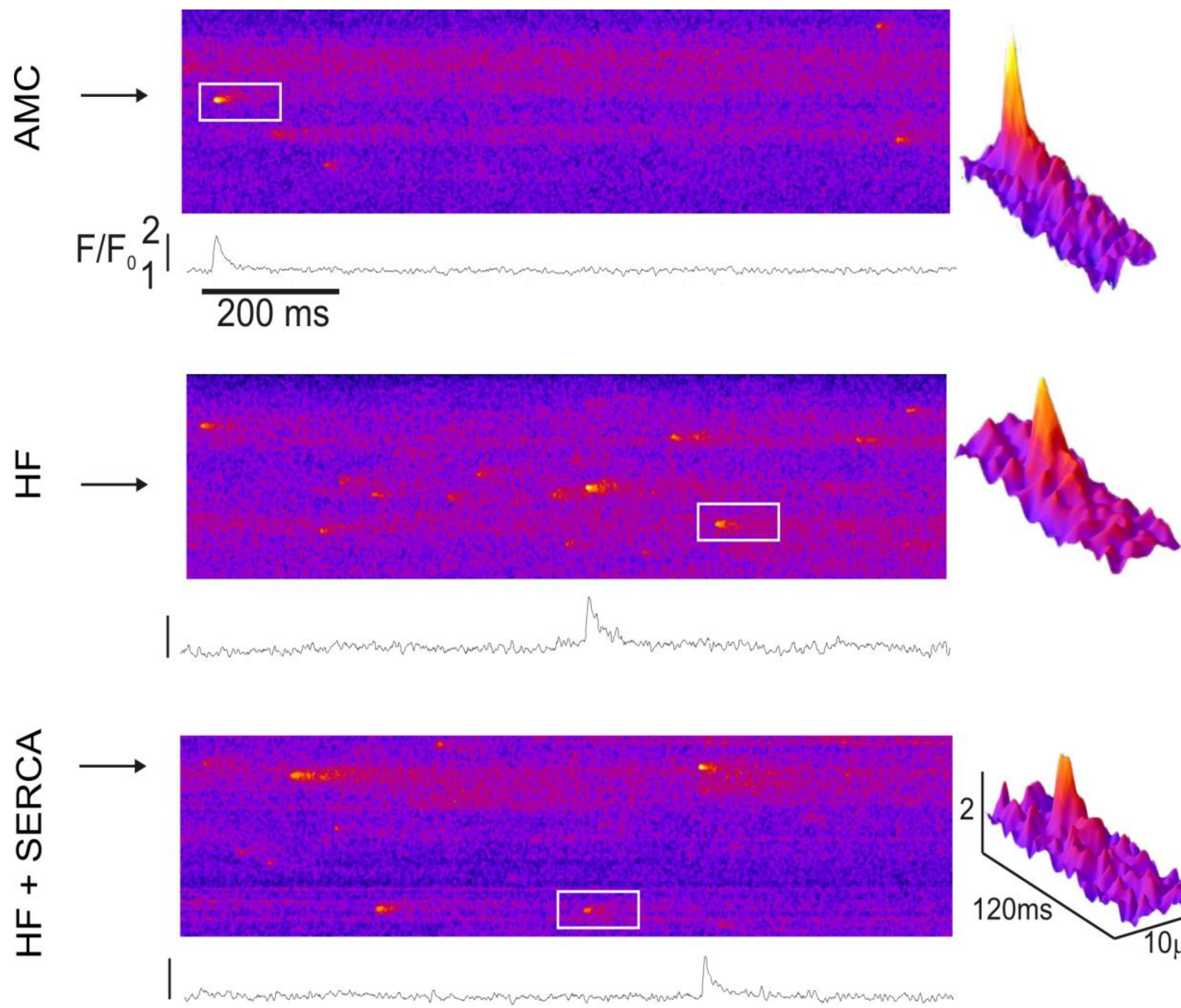
AMC n=8, HF n=12, HF+SERCA n=9 * $p<0.05$

Sparkology



Spontaneous SR Ca^{2+} spark frequency unchanged in failing myocytes after AAV9.SERCA2a gene transfer

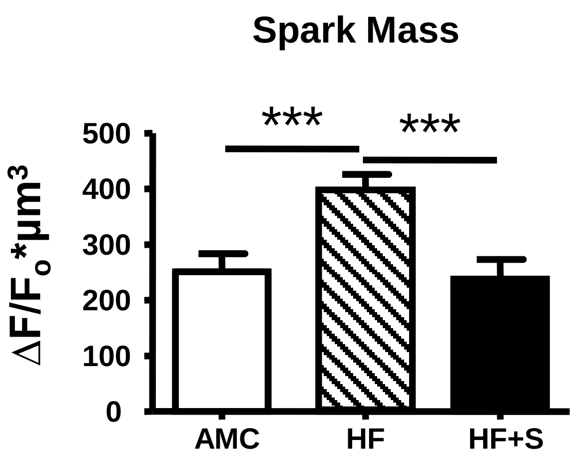


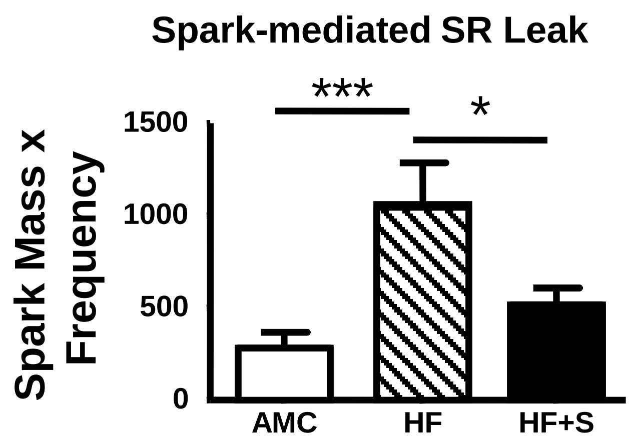


Number of sparks/cells/hearts:
AMC=247/19/7
HF=853/30/11
HF+SERCA=445/10/5
$*p<0.05$, $**p<0.01$

Lyon AR et al Circulation Arrhythmia Electrophysiology (in press)

SERCA2a Gene Therapy Reduces Spark-mediated SR Calcium Leak

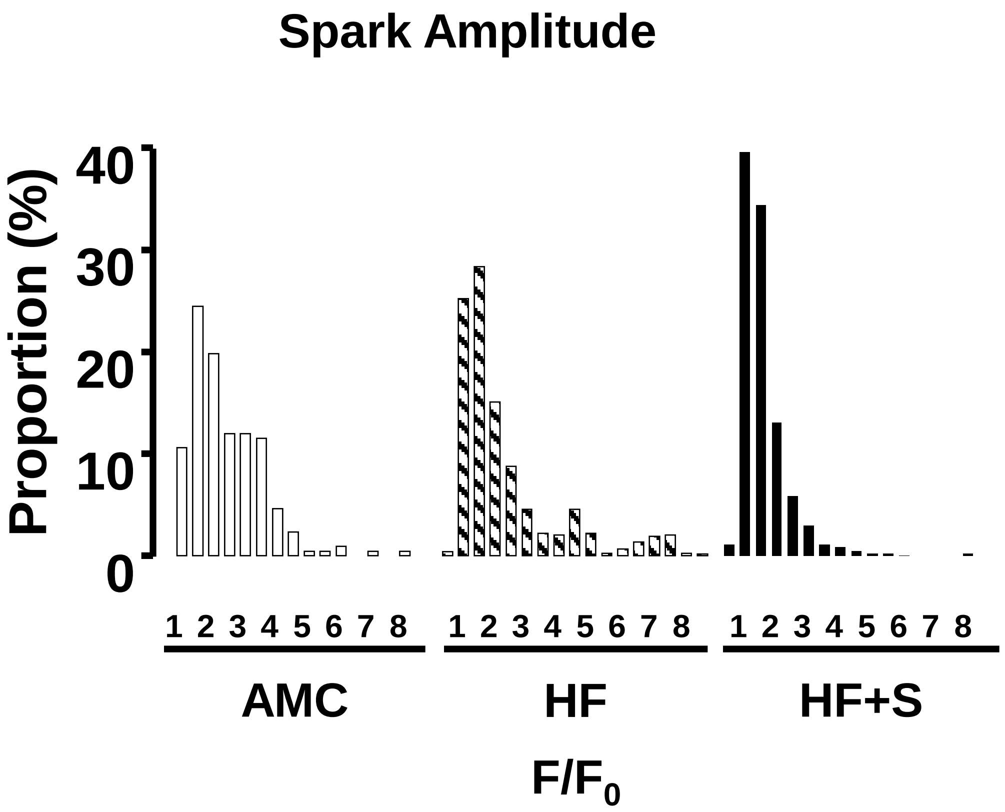


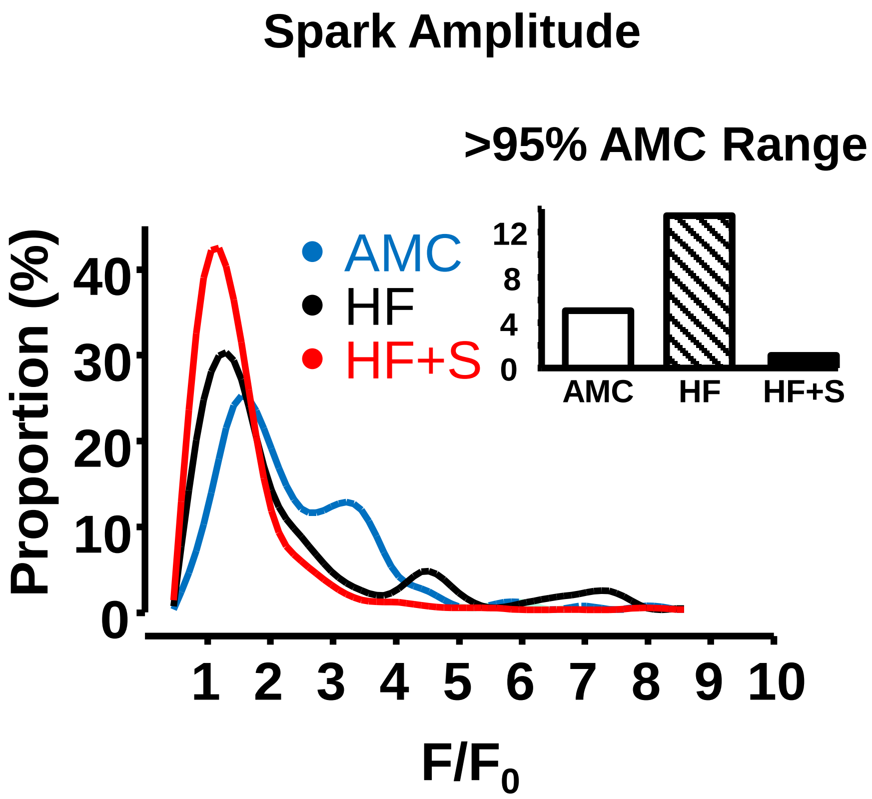


Number of sparks cells/hearts:
AMC=247/19/7
HF=853/30/11
HF+SERCA=445/1
$^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

Lyon AR et al Circulation Arrhythmia Electrophysiology (in press)

Modification of spark properties in failing myocytes after SERCA2a gene transfer

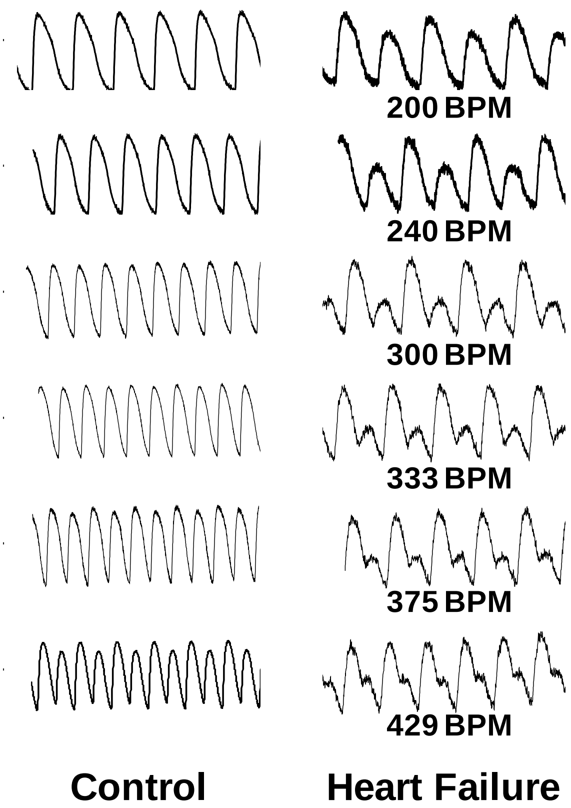




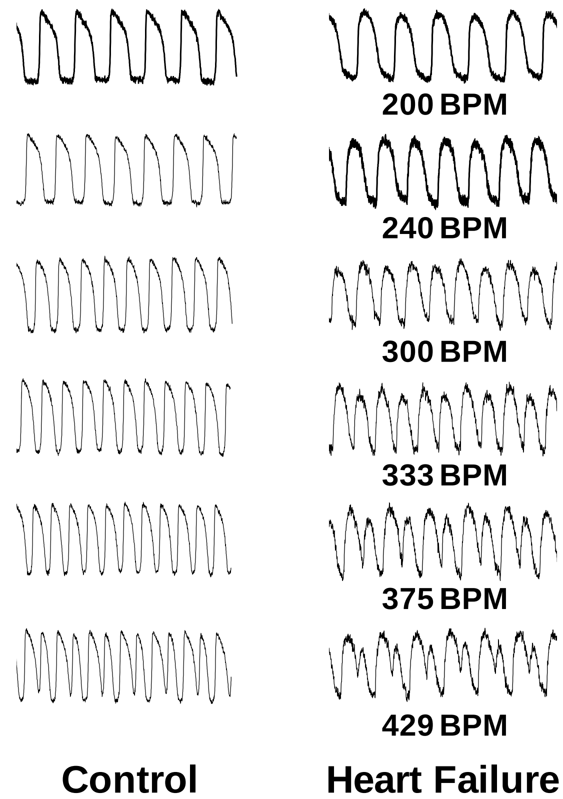
Number of sparks studied: AMC=247, HF=853, HF+SERCA=445.
$p<0.001$ for HF vs HF+S (Kruskal-Wallis test).

SERCA2a Overexpression Suppresses Ca^{2+} and APD Alternans in the Heart Failure
Guinea Pig HF Model +/- AAV9.SERCA2a

Ca^{2+} Alternans



APD Alternans



Courtesy of Cutler M, Hajjar RJ, Rosenbaum DS (Submitted)

SERCA2a Gene Therapy

- Positive Inotrope
- Positive Lusitrope
- Antiarrhythmic
- Improves Myocardial Energetics

- Benefits in small and large animal HF models

- Await outcome of the 2 clinical trials
  - Clinical endpoints
  - Biological success

Cardiac Gene Therapy
The Future

Cardiac Gene Therapy
The Future
Which Gene?

1. SERCA2a Gene Therapy
   - CUPID II and III
   - HF with Normal EF
   - HF with frequent VT/VF/ICD shocks
   - Instent Restenosis
2. Other genes
   - S100A1, Inhibitor 1c
   - Master regulatory genes re fetal vs adult gene programme
   - Pharmacogenomics
   - Gene replacement
     - LQTS
     - Familial DCM, HCM, Fabry’s CM

Cardiac Gene Therapy
The Future
Which Gene?

- siRNA

Long-Term Cardiac-Targeted RNA Interference for the Treatment of Heart Failure Restores Cardiac Function and Reduces Pathological Hypertrophy

Suckau et al Circulation 2009 <u>119</u> 1241-52

- MicroRNA

Silencing of microRNAs *in vivo* with 'antagomirs'

Jan Krützfeldt[1], Nikolaus Rajewsky[3], Ravi Braich[4], Kallanthottathil G. Rajeev[4], Thomas Tuschl[2], Muthiah Manoharan[4] & Markus Stoffel[1]

Krutzfeldt et al Nature 2005 <u>438</u> 685-689

Cardiac Gene Therapy
The Future

Novel Gene Expression Control Systems

- Tissue specific
  - Promoters
  - Activation
- Drug Inducible

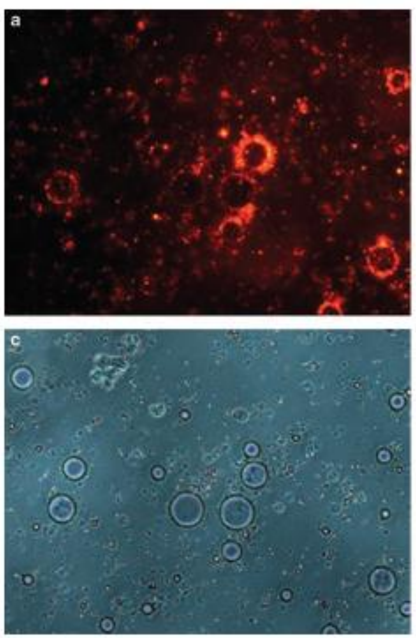
  ‘Physician control’

  Organ targeting
- Negative feedback eg BNP

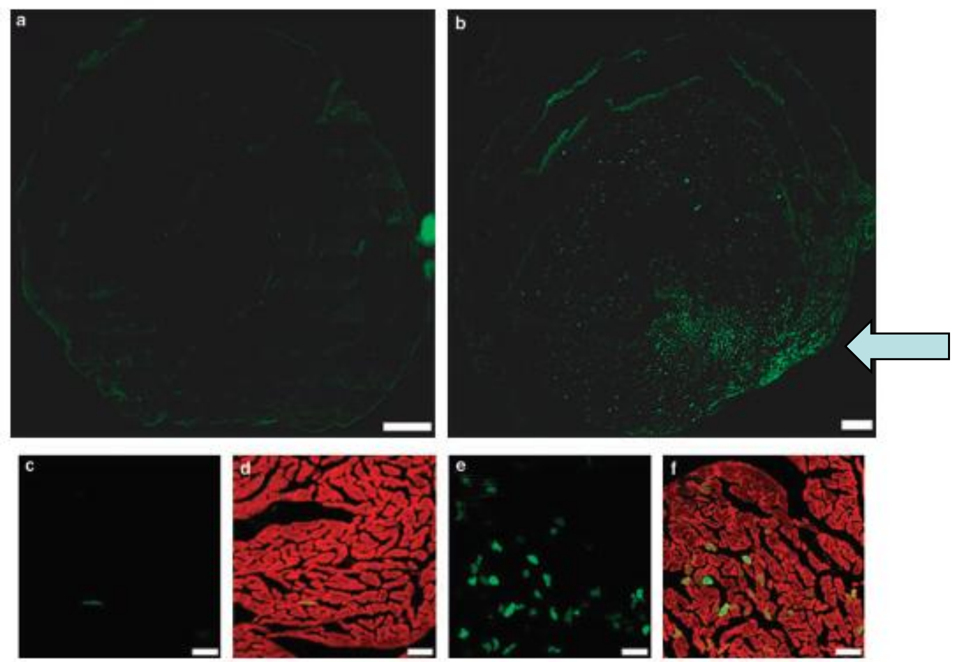
  ‘Disease control’

Cardiac Gene Therapy
The Future

- Novel Delivery Techniques
  - Physical Targeting
  - Microbubbles



Control USS + Microbubble

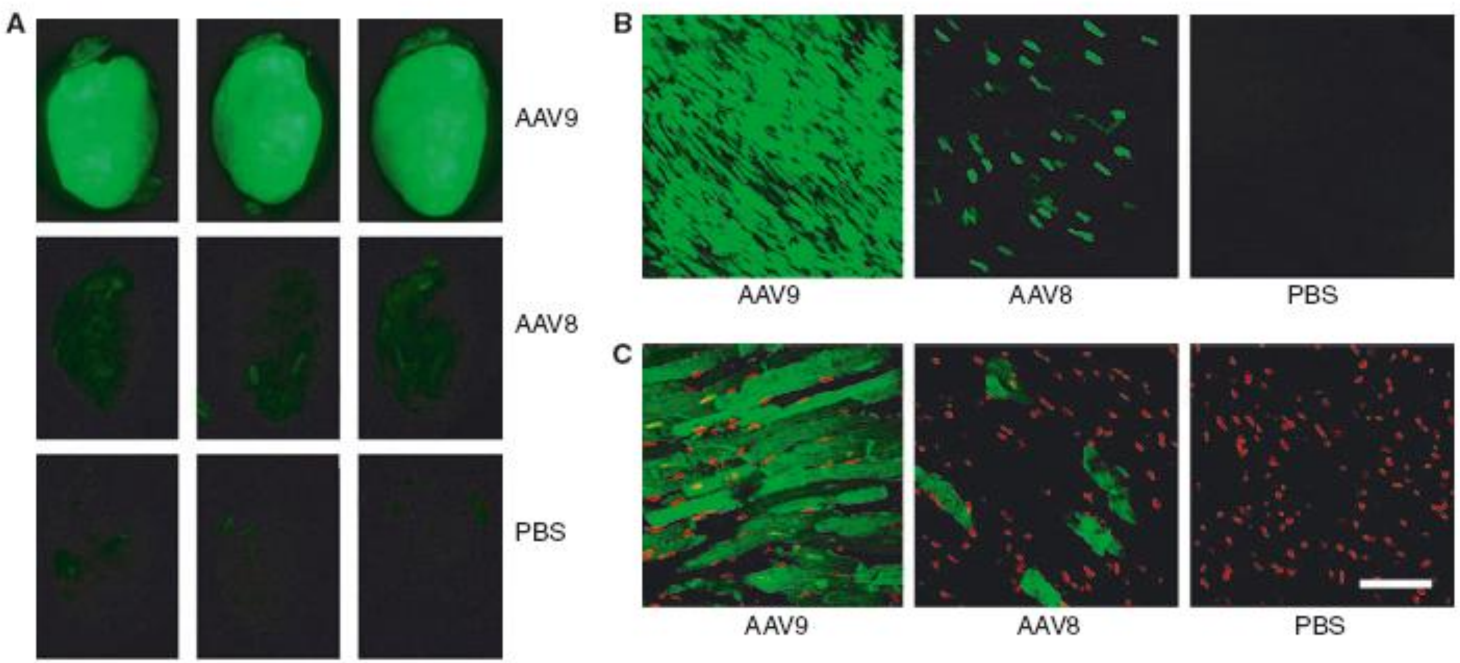


Muller et al Gene Therapy 2008 <u>15</u> 1558-65

Cardiac Gene Therapy
The Future

Novel Vectors

AAV9



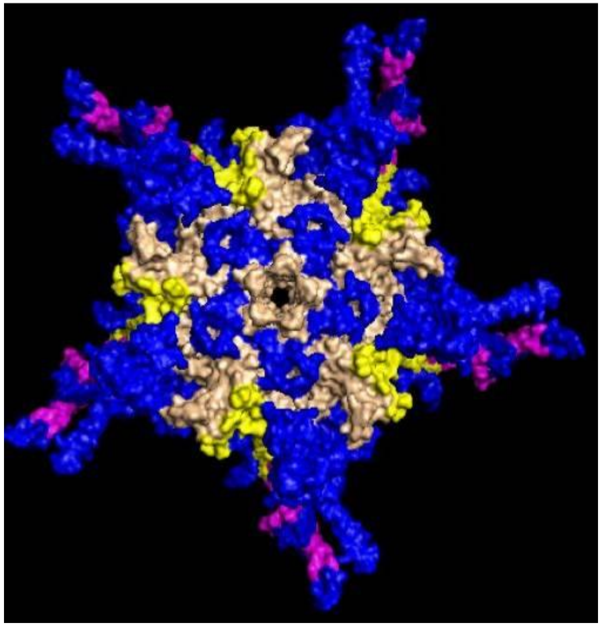
Cardiac Gene Therapy
The Future

Novel vectors

- Bioengineered AAVs
  - Reduced immunogenicity, neutralizing Abs
  - Improve purification
  - Increase cardiac specificity

- Subcutaneous ‘biopumps’

Chimeric rAAV



Courtesy of Dr. Jude Samulski

Combined Gene and Cell Therapy Strategies

- Improve Quality of Stem Cell Therapies
  - Delivery
  - Survival
  - Efficacy
  - Cardiomyogenesis
- Improve function of failing CM
  - Paracrine effect
- Inducible Pluripotent Stem Cells (IPCs)
- Biological Pacemakers (I_f)

Gene Therapy for Heart Failure Summary

- Need for New Therapeutic Strategies for Heart Failure
- Cardiac Gene Therapy
  - Developed on a base of >20 years myocardial biology
  - Regulation stringent
  - New Viral Vectors - AAV
    - safe
    - higher transfection efficiency
    - long lasting expression
- SERCA2a Gene Therapy
  - Positive Inotrope
  - Positive Lusitrope
  - Antiarrhythmic
  - Improves Myocardial Energetics
- Phase 2 SERCA2a trial in US
  - Safe
  - Efficacy
- UK Trial 2011
- FDA approved Phase 3 Trial
- Judge on Clinical Outcomes