

Gene Transfer During LVAD Support

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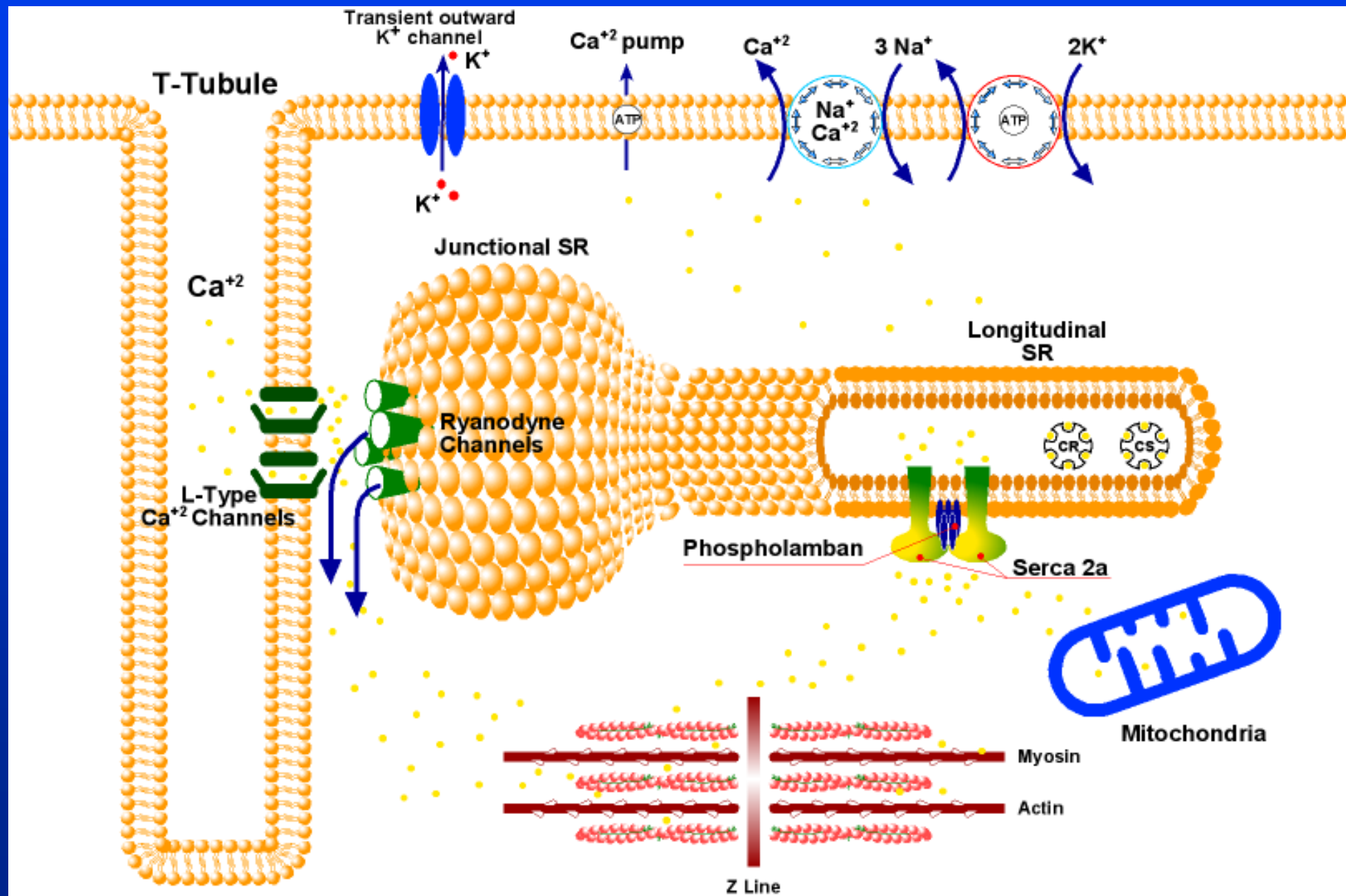


Heart Failure

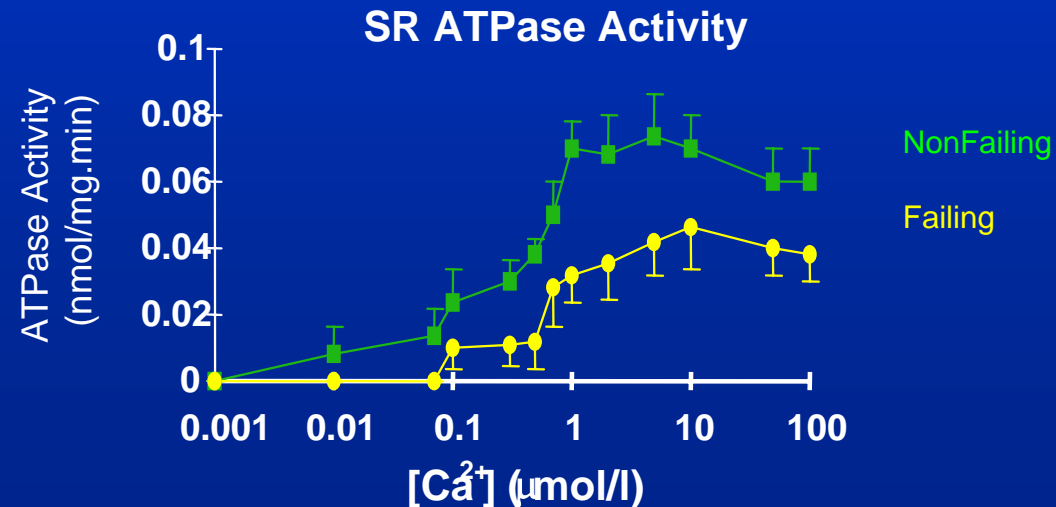
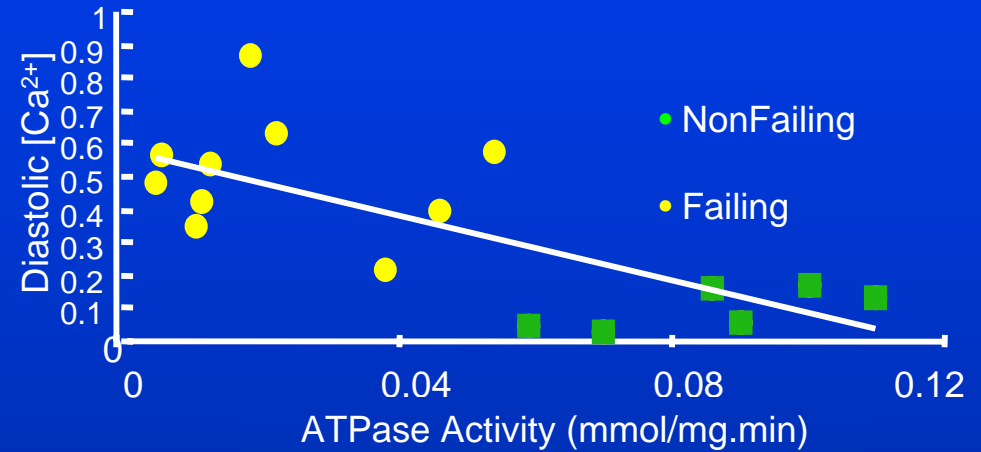
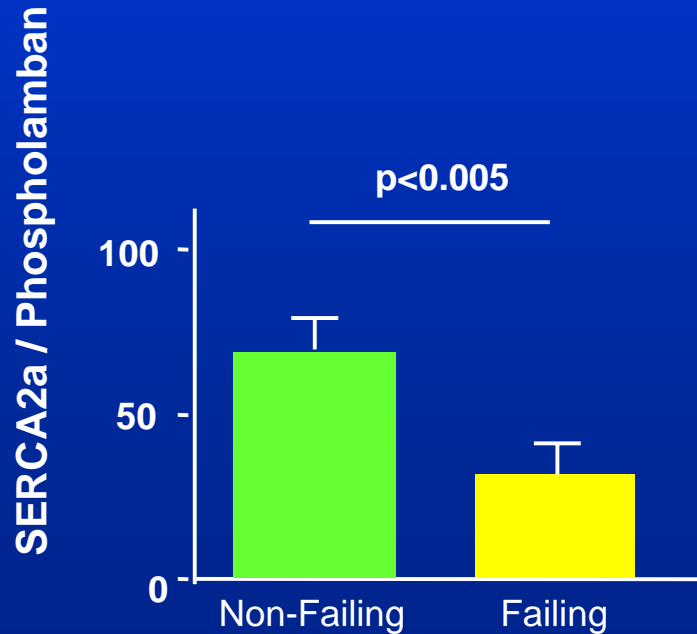
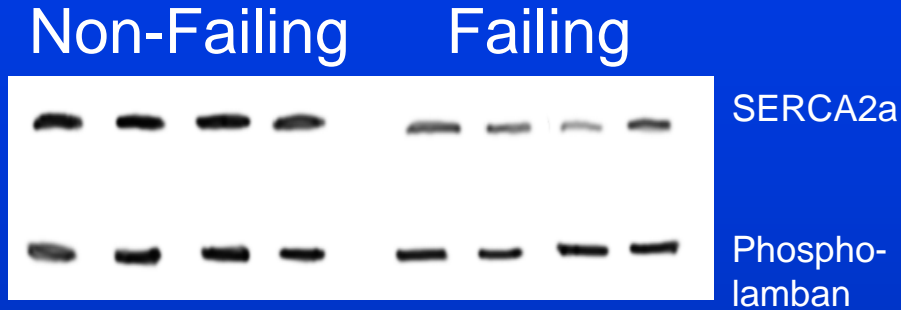
- Major cause of morbidity and mortality
- In the United States each year, more than 1,000,000 hospitalizations and 500,000 deaths
- Ischemic versus non-ischemic etiology
- Mortality remains high despite recent advances, including β -blockers, ACE inhibitors, ICDs, BiV Pacers, Ventricular Assist Devices (VADs), Transplants

Calcium Handling in Heart Failure

- Smaller, delayed calcium transients
- Decreased amount and activity of the Sarcoplasmic Reticulum Calcium ATPase (SERCA2a)
- Altered ratio of SERCA2a and phospholamban



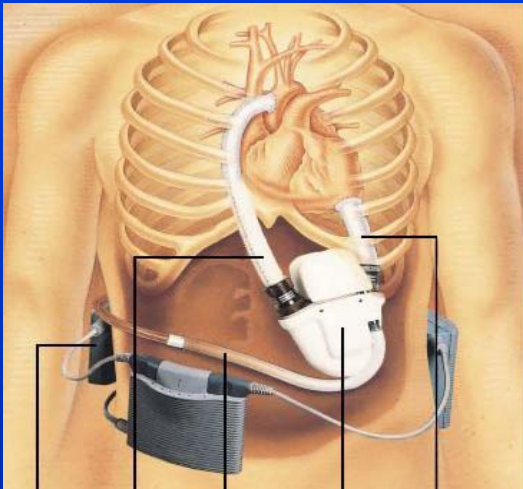
SERCA2a Expression & Activity in Human Failing Hearts



Ventricular Assist Devices

- LVADs, RVADs, BiVADs
- Morbidity from infection, bleeding, clotting, device failure
- Most patients go to transplant, but some recover
- Ideal to study novel heart failure treatments
- More than 50 heart transplants & 50 LVADs/year at the University of Pittsburgh & Massachusetts General Hospital

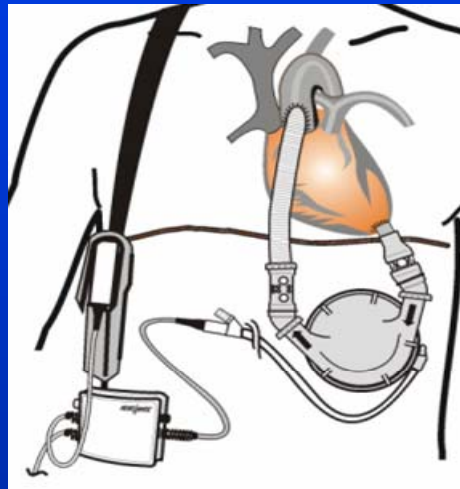
Methods: Devices



Novacor® LVAS

Electrically powered

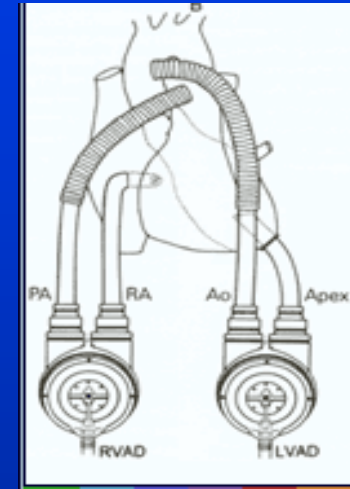
10 L/min



Heartmate® LVAS VE

Electrically powered

10 L/min



Thoratec® VAD

Pneumatically powered

7.2 L/min

VAD Implantations 1996-2002

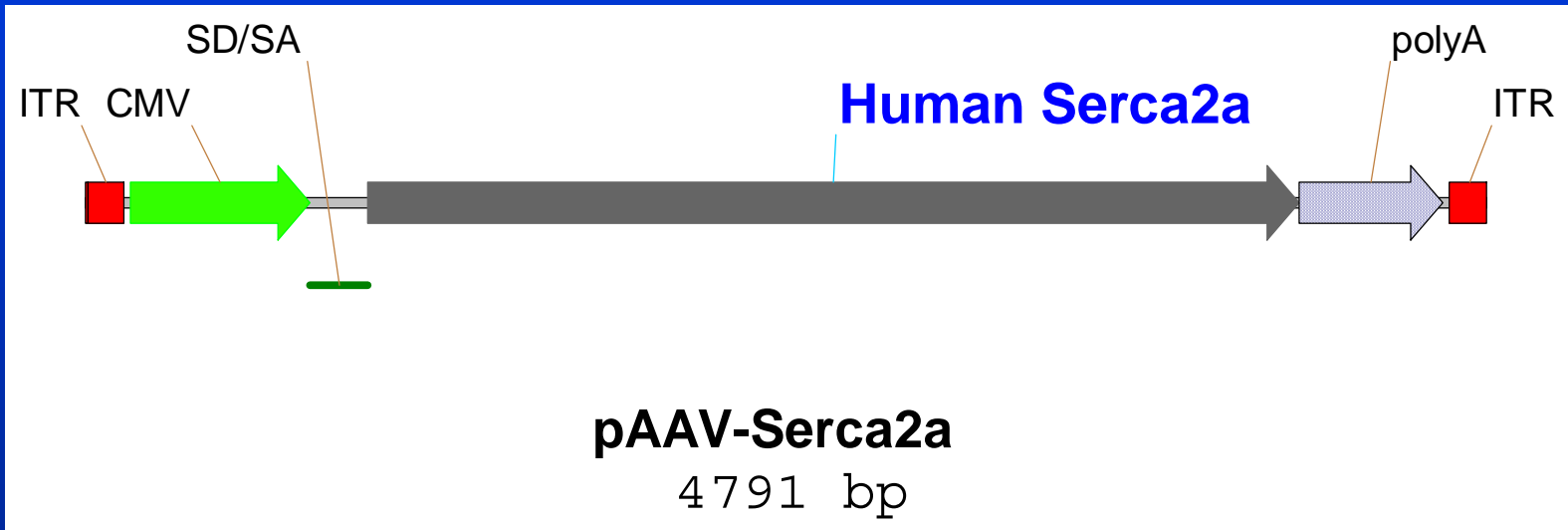
(n=125)

Ischemics	58	(46%)
Non-Ischemics	67	(54%)
Transplanted	92	(74%)
Died on Device	25	(20%)
Weaned	8	(6%)
Addition of RVAD	13	(10%)
Pump Replacements	7	(6%)
Mean Time to Transplant	118 days	(2 – 548)

Hypothesis

- In patients on Ventricular Assist Devices (LVADs) with end-stage heart failure, gene delivery of SERCA2a by direct intracardiac injection of AAV6-CMV-SERCA2a can safely:
 - restore SERCA2a protein levels and activity to normal
 - improve local molecular and cellular indices of cardiac function
 - improve global indices cardiac function and ability to wean off VAD support

AAV6-CMV-SERCA2a



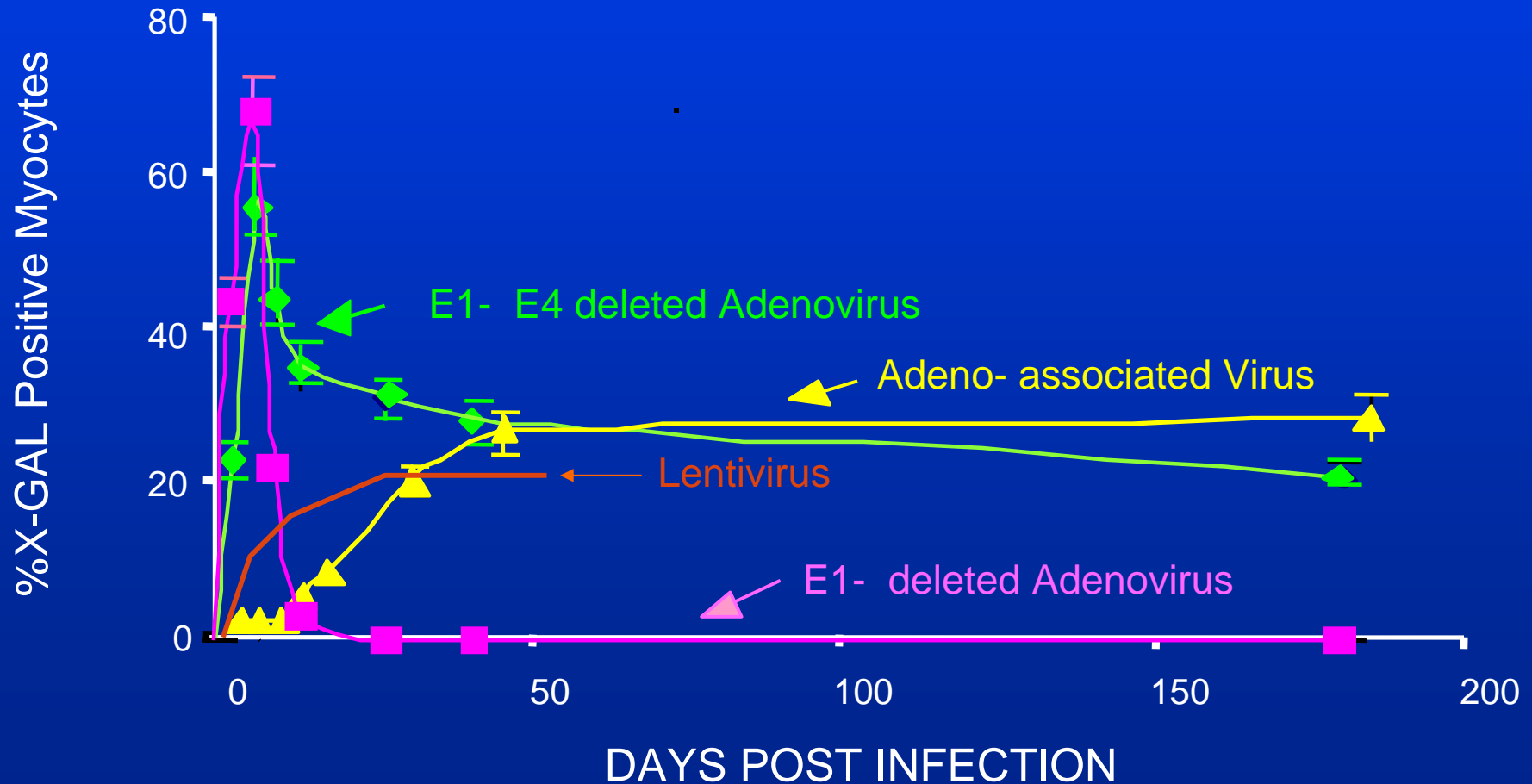
AAV6-CMV-SERCA2a

- Long-term expression of gene product, driven by CMV promoter
- Produced by an adenovirus-free, triple plasmid transfection method
- Purified by a heparin affinity FPLC

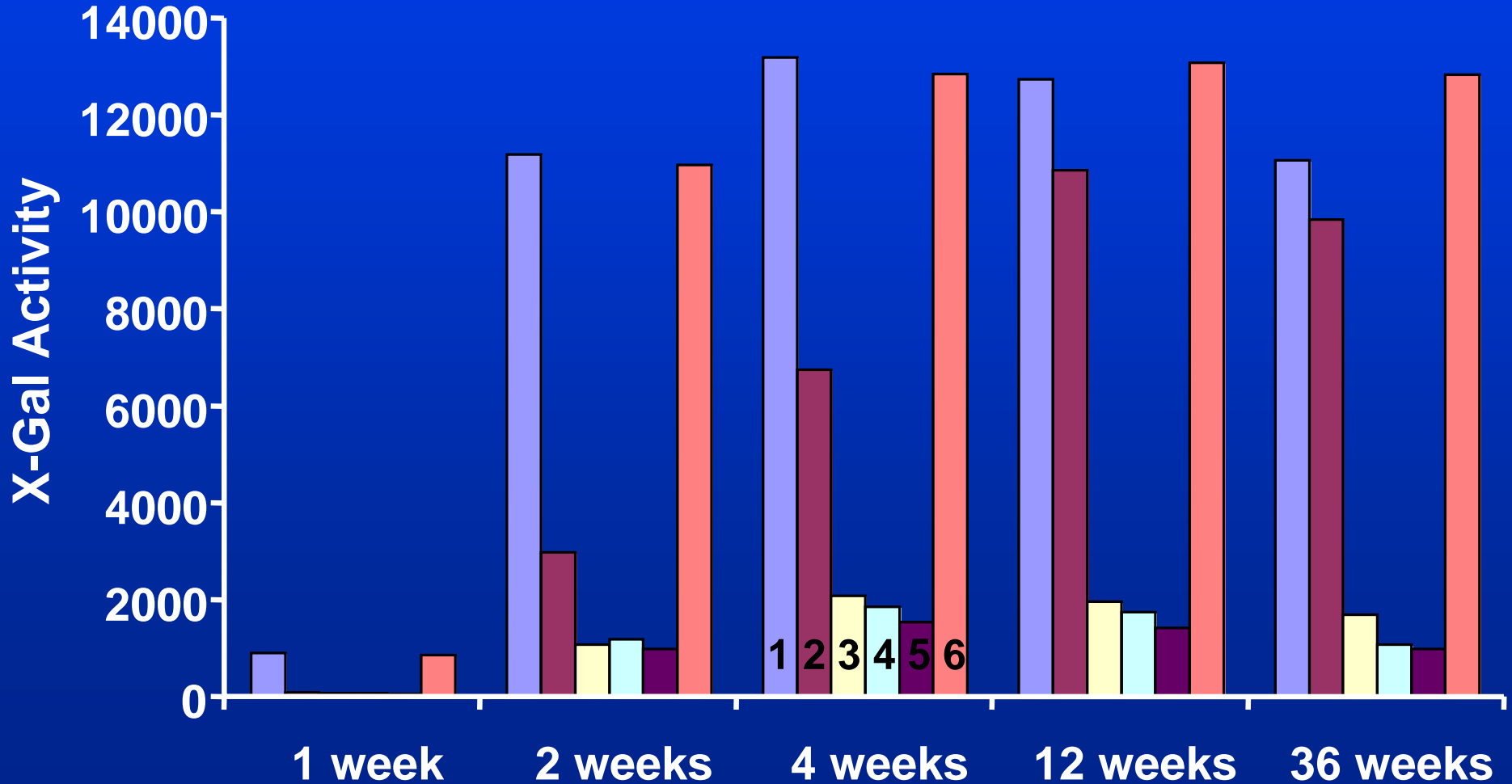
Assaying AAV6-CMV-SERCA2a

- Titters: Dot blot (v.g./ml); Infectious center assay; immunostaining; SDS PAGE
- Sterility: inoculation in tissue culture medium
- Human genomic DNA
- Wild-type or wild-type-like AAV
- Other proteins by Western blot

Gene Transfer to Myocardium using Different Viral Vectors



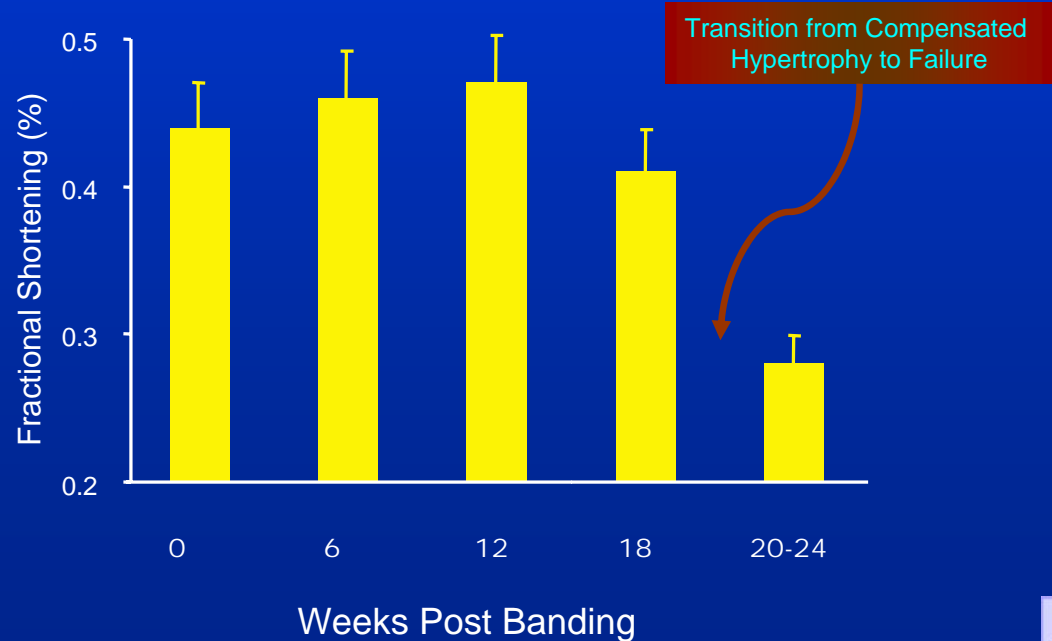
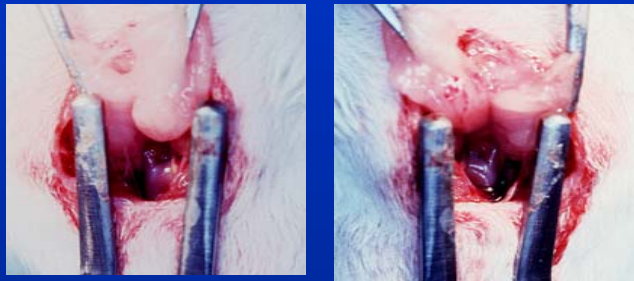
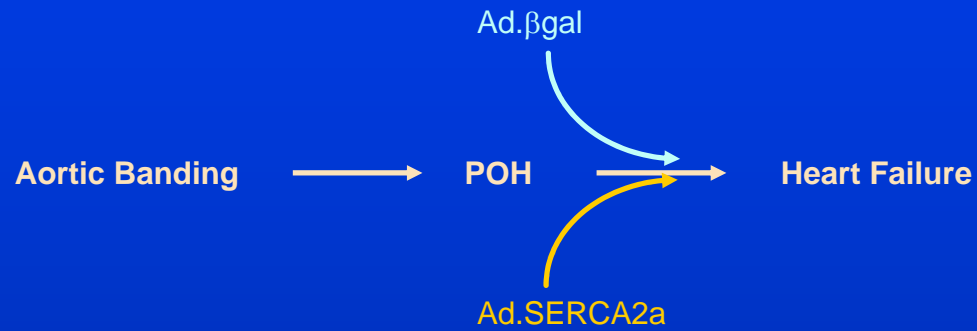
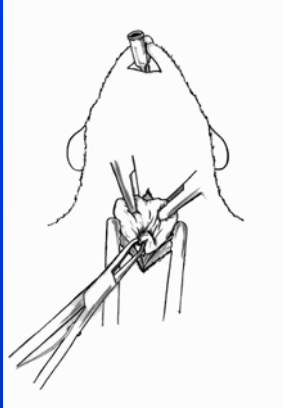
Gene Transfer into Rat Myocardium: Superior Efficiency with AAV6



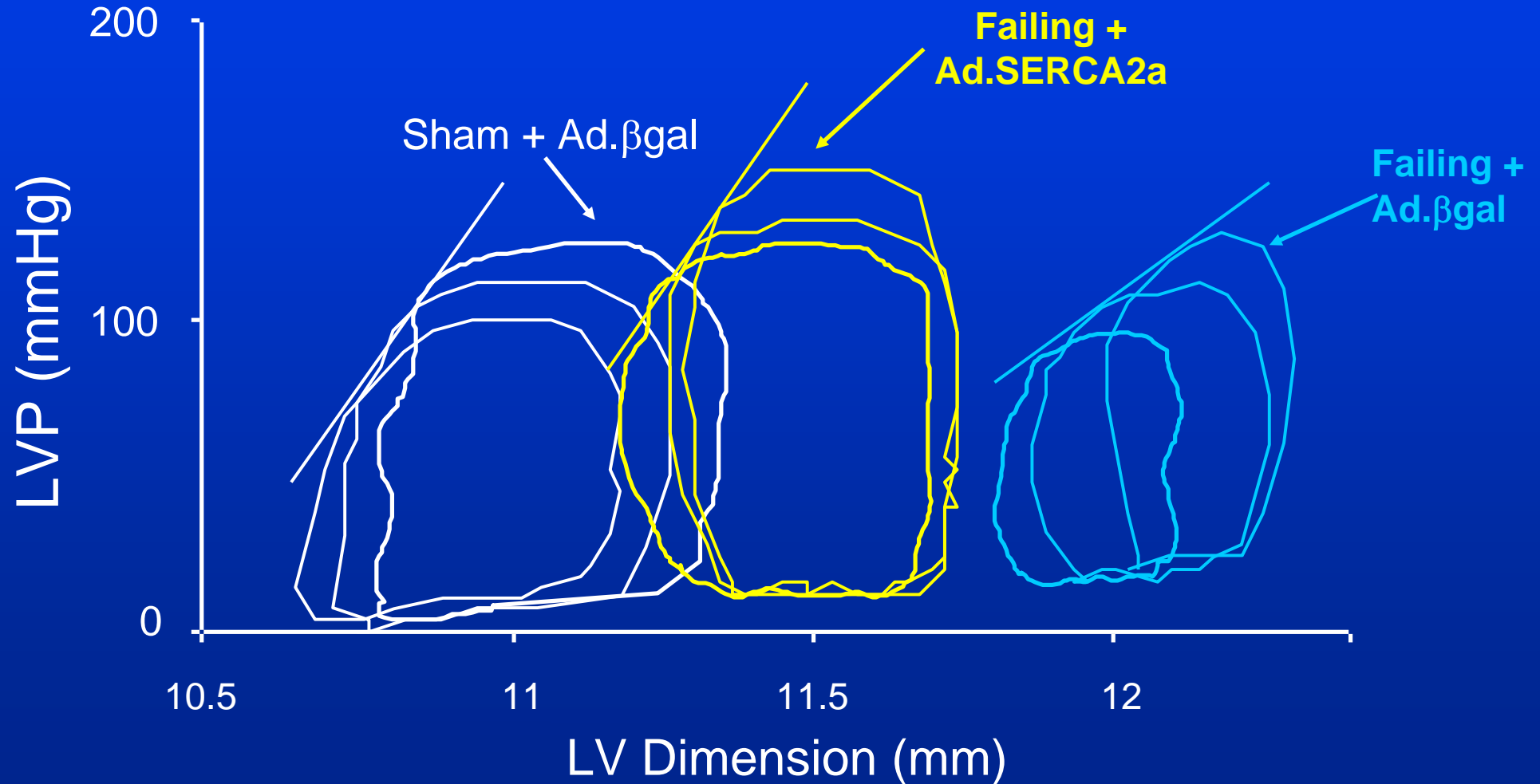
SERCA2a Gene Transfer: Efficacy in Heart Failure

- Ad-CMV-SERCA2a improves contractility in human myocytes isolated from failing hearts
- Ad-CMV-SERCA2a improves function in a rat aortic-banded model of heart failure
- Ad-CMV-SERCA2a and AAV6-CMV-SERCA2a improve function in a porcine mitral regurgitation model of heart failure

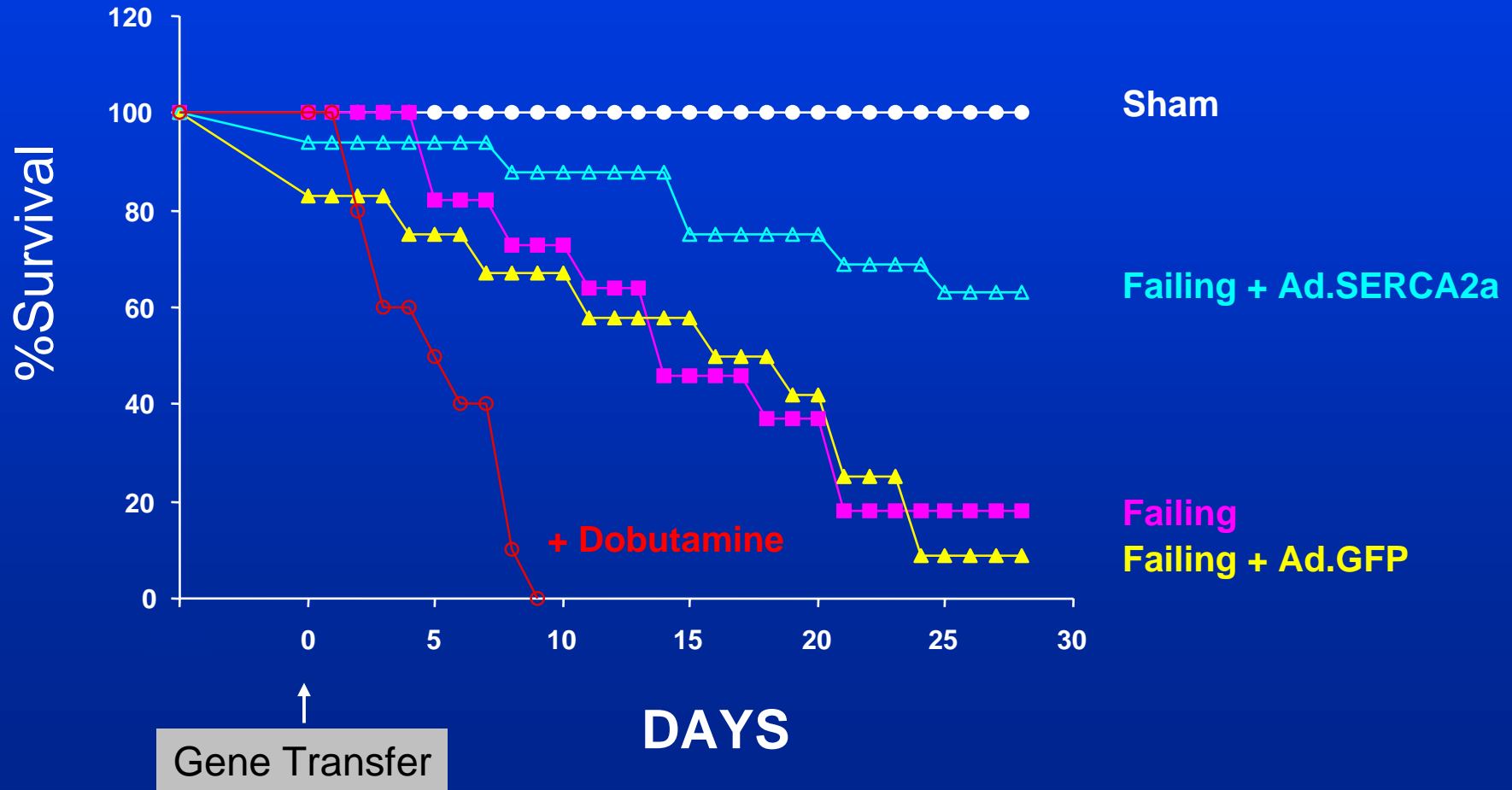
Adenoviral Gene Transfer of SERCA2a in a Rat Pressure-Overload Model



SERCA2a Overexpression Improves the Pressure-Dimension Relationship



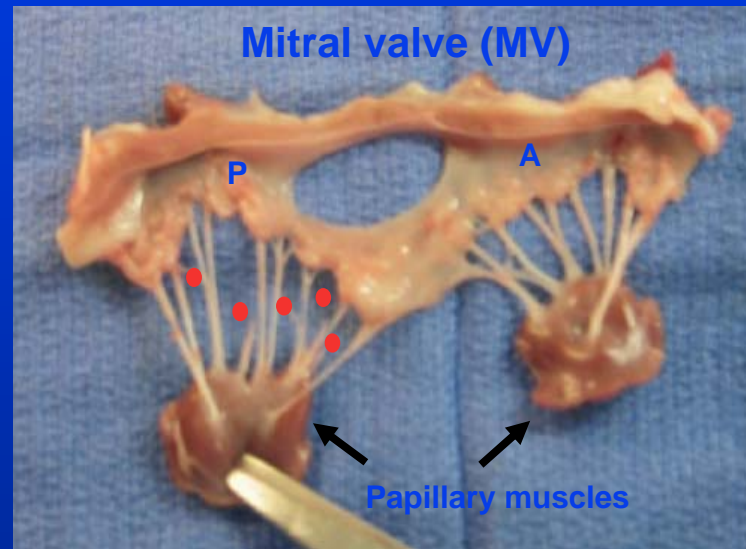
SERCA2a Gene Transfer Improves Survival in Pressure-Overload Rats



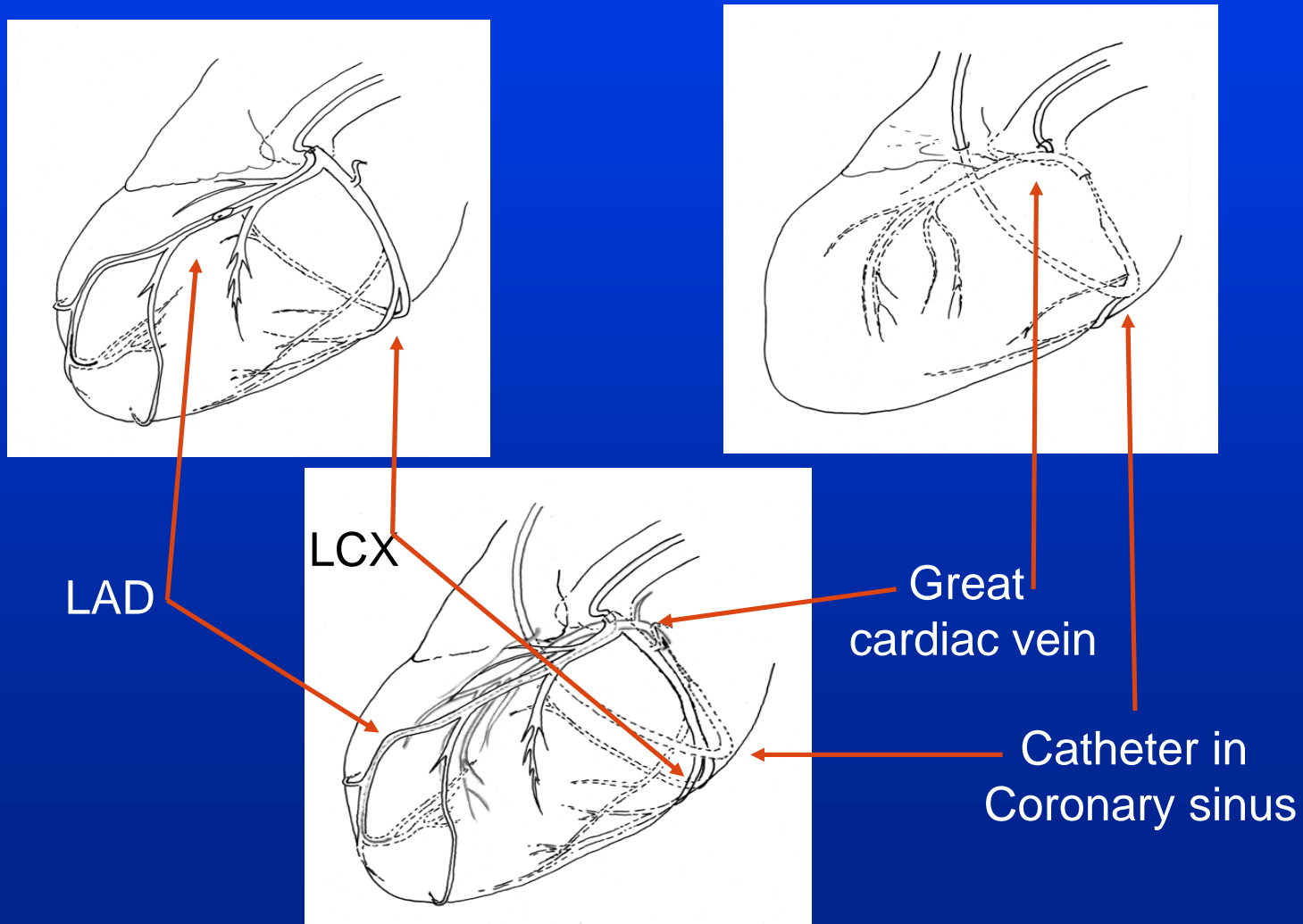
AAV6-CMV-SERCA2a Gene Transfer: Preclinical Data

- Intracoronary delivery of AAV6-CMV-SERCA2a in a swine model of mitral regurgitation with heart failure
 - Mitral regurgitation created using percutaneous technique
 - 30 days: Gene transfer using AAV6-CMV-SERCA2a or AAV6-CMV- β gal
 - 60 to 120 days: Invasive hemodynamics, functional studies, molecular studies

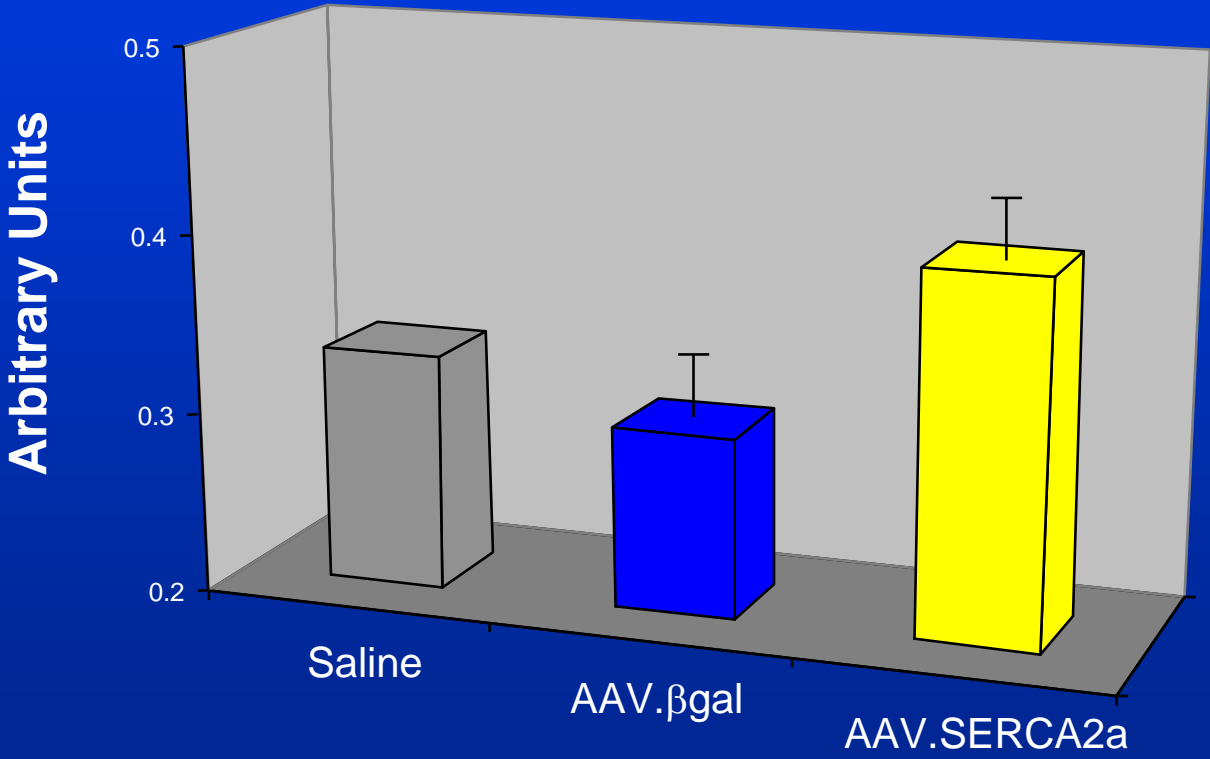
Mitral valve cordes rupture to create MR using a percutaneous biotome



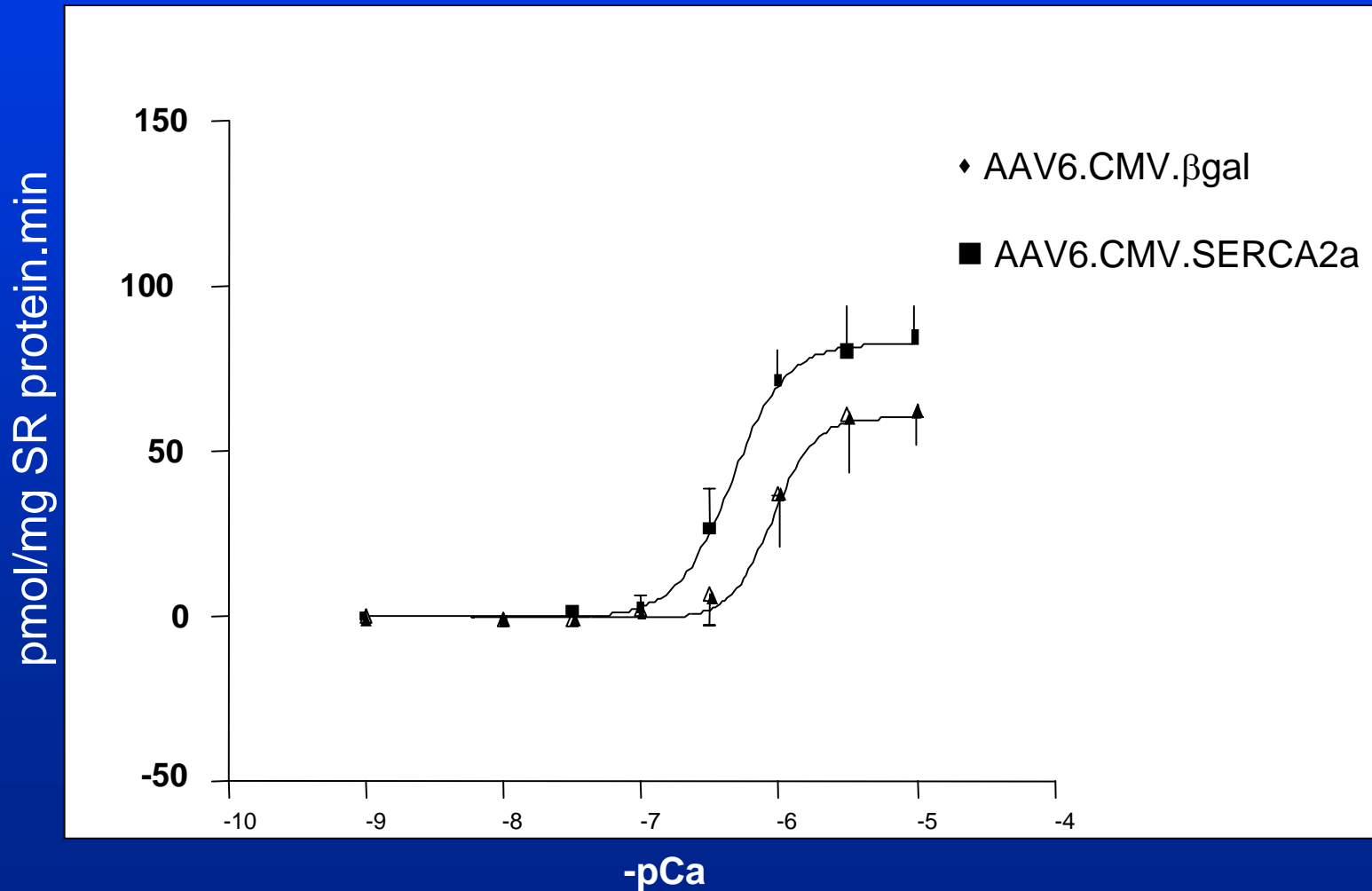
Intracoronary Delivery of $5 \times 10^{13}/\text{ml}$ AAV6-CMV-SERCA2a



SERCA2a Protein Expression in LV Sections 6 Weeks Post-Delivery

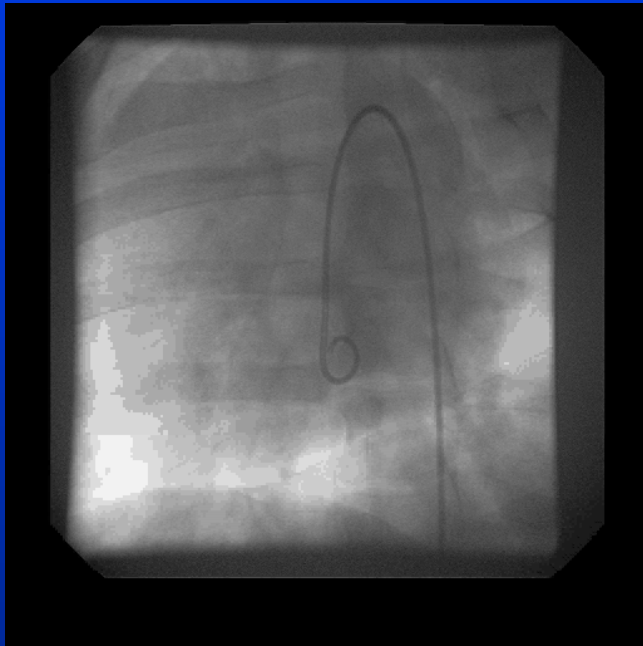


SR ATPase Activity in LV Sections 12 weeks Post-Delivery

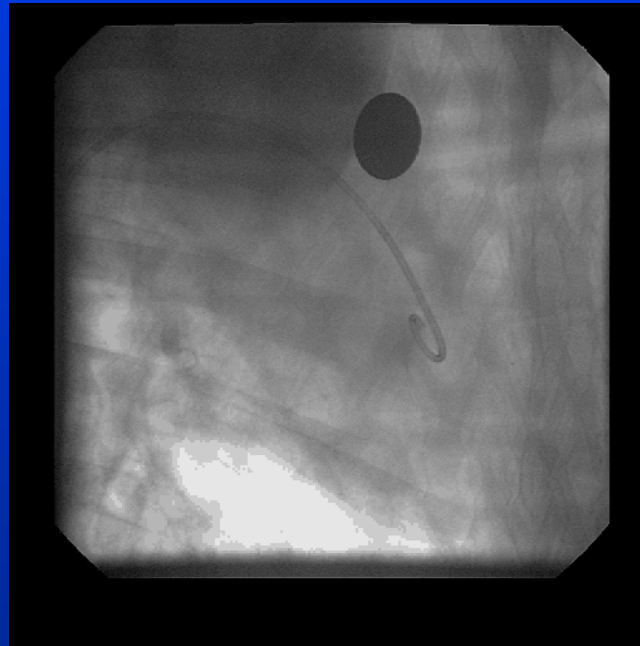


Porcine Model of Heart Failure

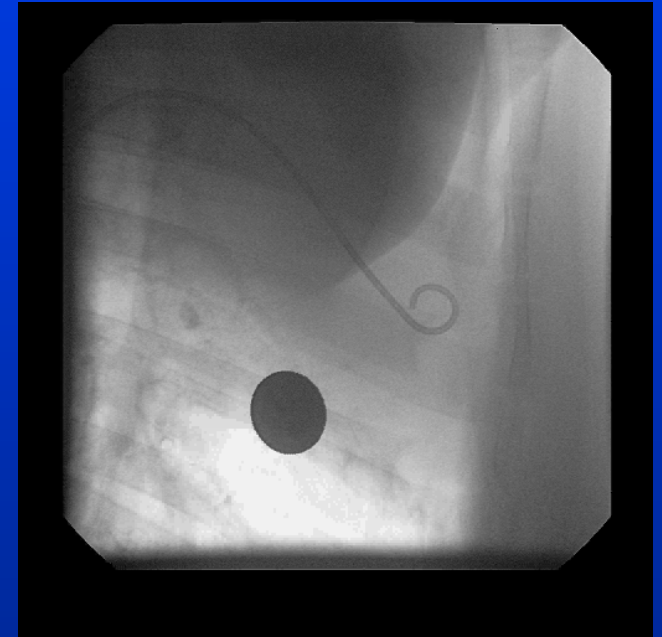
Chronic mitral regurgitation



Mitral Regurgitation



3-month post
Mitral Regurgitation



+AAV-SERCA

Hemodynamic Parameters: AAV6-CMV-SERCA2a Gene Transfer

	Control	MR	MR + AAV β gal	MR + AAVSERCA2a
Heart Rate (bpm)	102 \pm 8	156 \pm 6	162 \pm 18	126 \pm 6
Fractional Shortening (%)	42.3 \pm 4	33.0 \pm 5	18.5 \pm 6	37.3 \pm 4
Stroke volume (ml)	34.2 \pm 1.3	24.7 \pm 2.1	19.2 \pm 1.0	31.7 \pm 2
+dP/dt (mmHg/sec)	1865 \pm 324	1240 \pm 245	1156 \pm 412	1398 \pm 344
-dP/dt (mmHg/sec)	-1562 \pm 388	-1114 \pm 191	-1033 \pm 422	-1514 \pm 101
LV Systolic Pressure (mmHg)	92 \pm 5	89 \pm 5	82 \pm 4	98 \pm 4
LV End-diastolic Pressure (mmHg)	7 \pm 2	14 \pm 1	19 \pm 4	10 \pm 3
# of animals	6	5	8	9

AAV6-CMV-SERCA2a Gene Transfer: Canine Pacing Study

- Direct injection of AAV6-CMV-SERCA2a during cardiopulmonary bypass in a canine pacing model of heart failure
 - Transvenous Pacemaker, Pacing at 230 bpm
 - 30 days: Gene transfer using AAV6-CMV-SERCA2a (n=8) or AAV6-CMV- β gal (n=4) by direct injection
 - Pacing at 190 bpm, serial blood testing, EKG, echocardiographic and hemodynamic studies
 - 75 days: Cellular, molecular, histological, immunological, tissue distribution studies

AAV6-CMV-SERCA2a Gene Transfer: Enrollment

- Subjects with nonischemic cardiomyopathy, age 18-65, will be enrolled at the time of LVAD placement at the University of Pittsburgh or Massachusetts General Hospital
- Decompensated heart failure as a bridge to transplant
- Randomize 8 patients to AAV6-CMV-SERCA2a and 8 subjects to saline injection

AAV6-CMV-SERCA2a Gene Transfer: Exclusion Criteria

- Acute myocarditis (signs, symptoms, biopsy)
- Coronary artery disease (>50% lesion)
- Postpartum (6 months)
- Fever, leukocytosis, positive blood cultures
- Unable to provide consent

AAV6-CMV-SERCA2a Gene Transfer: Gene Delivery

- Two 3x3 grids: LV anterior and lateral walls
 - 5×10^{11} /ml in low dose grid
 - 5×10^{12} /ml in high dose grid
 - 1 cm between each injection site
 - 5 mm deep
 - 0.1 mls per injection

AAV6-CMV-SERCA2a Gene Transfer: Follow-up Testing on LVAD

- Serum (weekly x 4, then biweekly)
 - Myocardial toxicity (CPK, Troponin)
 - Kidney, liver function tests
 - Inflammation (WBC, CRP, ESR)
 - Antibodies to SERCA2a, AAV6
 - Viral DNA

AAV6-CMV-SERCA2a Gene Transfer: Weaning Protocol

- 4-6 weeks after implantation, good clinical status
- Metabolic stress testing
 - $MVO_2 > 17$ ml O_2 /kg/min on VAD
 - $MVO_2 > 15$ ml O_2 /kg/min off VAD
- Tissue-Doppler Echocardiography (automated border detection, regional wall motion)
 - On 90% support, EF > 40%; Off support
- Right Heart Catheterization
 - PCW < 20 mmHg, CI > 2.2 l/min, HR stable

AAV6-CMV-SERCA2a Gene Transfer: Analysis at Time of Transplant

- Tissue (high dose, low dose, uninjected areas)
 - SERCA2a expression (RNA, protein, activity)
 - Inflammation, fibrosis (Histology, immunohistochem)
 - Myocytes size, apoptosis
 - Expression of muscle genes (Microarray: NCX, ANF, phospholamban, myosin heavy chain, collagen)
- Isolated Myocytes
 - Shortening velocity, systolic and diastolic calcium

AAV6-CMV-SERCA2a Gene Transfer: Endpoints

- Safety
 - Inflammation, kidney, liver damage
 - Worsened heart function (increased RVADs)
 - Arrhythmias
- Local Efficacy
 - Tissue, myocytes (vs. controls, function of dose)
 - Regional wall motion by echocardiography
- Global Efficacy
 - Ability to wean off LVAD

Acknowledgements

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