Gene therapy of cardiovascular disorders

The problem

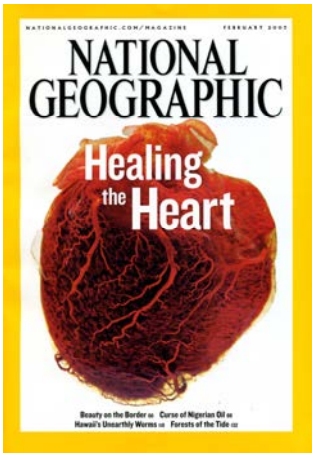
Therapeutic nucleic acids

Gene delivery vehicles

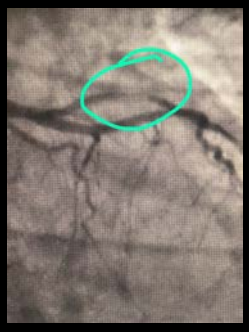
Route of administration

Clinical applications

Expected future developments

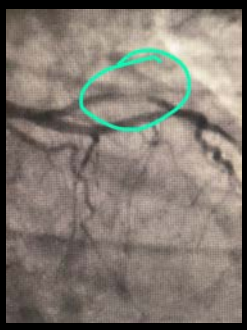


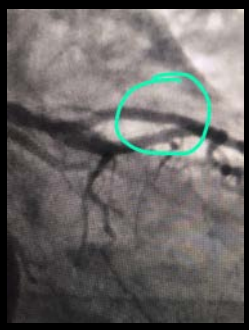
A personal story… man, 75 years



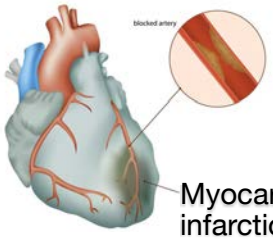
90% stenosis of a major coronary artery

A personal story… man, 75 years

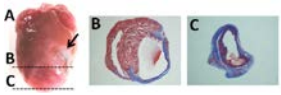




What would have happened if not revascularized?

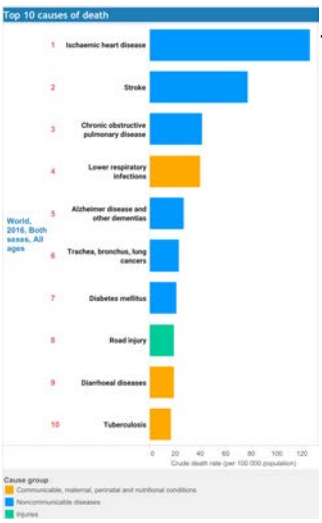


Myocardial infarction



⟶ Loss of contractile tissue

⟶ Heart failure



The tremendous burden of cardiovascular disorders

About **17.6 million people** have died from CVDs in 2016, representing 31% of all global deaths (www.who.int/mediacentre/factsheets/fs317), killing more people than all cancers.

Of these, 85% deaths were due to **heart attack** and **stroke**. It is estimated that, by 2030, almost 23.6 million people will die from CVDs.

Over three quarters of CVD deaths take place in low- and middle-income countries.

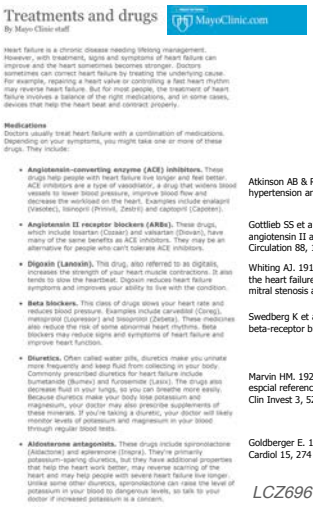
Causes of death
World Health Organization, 2018

Heart failure is prevalent, deadly, and expensive

HEART FAILURE STATISTICS (US MARKET)	
Number of patients with congestive heart failure	4,900,000
Annual number of new heart failure cases	400,000
Percentage of heart attack patients that develop heart failure within 6 years	20%
Five-year mortality rate for heart failure	50%
Percentage of heart failure patients over the age of 65% (Medicare patients)	75%
Number of hospital admission each year for which congestive heart failure is the primary diagnosis	750,000
Total costs associated with heart failure	$40BN

Source: American Heart Association

Medical therapies for heart failure


Treatments and drugs

By Mayo Clinic staff

MayoClinic.com

Heart failure is a chronic disease needing lifelong management. However, with treatment, signs and symptoms of heart failure can improve and the heart sometimes becomes stronger. Doctors sometimes can correct heart failure by treating the underlying cause. For example, repairing a heart valve or controlling a fast heart rhythm may reverse heart failure. But for most people, the treatment of heart failure involves a balance of the right medications, and in some cases, devices that help the heart beat and contract properly.

Medications

Doctors usually treat heart failure with a combination of medications. Depending on your symptoms, you might take one or more of these drugs. They include:

- **Angiotensin-converting enzyme (ACE) inhibitors.** These drugs help people with heart failure live longer and feel better. ACE inhibitors are a type of vasodilator, a drug that widens blood vessels to lower blood pressure, improve blood flow and decrease the workload on the heart. Examples include enalapril (Vasotec), lisinopril (Prinivil, Zestril) and captopril (Capoten).
- **Angiotensin II receptor blockers (ARBs).** These drugs, which include losartan (Cozaar) and valsartan (Diovan), have many of the same benefits as ACE inhibitors. They may be an alternative for people who can't tolerate ACE inhibitors.
- **Digoxin (Lanoxin).** This drug, also referred to as digitalis, increases the strength of your heart muscle contractions. It also tends to slow the heartbeat. Digoxin reduces heart failure symptoms and improves your ability to live with the condition.
- **Beta blockers.** This class of drugs slows your heart rate and reduces blood pressure. Examples include carvedilol (Coreg), metoprolol (Lopressor) and bisoprolol (Zebeta). These medicines also reduce the risk of some abnormal heart rhythms. Beta blockers may reduce signs and symptoms of heart failure and improve heart function.
- **Diuretics.** Often called water pills, diuretics make you urinate more frequently and keep fluid from collecting in your body. Commonly prescribed diuretics for heart failure include bumetanide (Bumex) and furosemide (Lasix). The drugs also decrease fluid in your lungs, so you can breathe more easily. Because diuretics make your body lose potassium and magnesium, your doctor may also prescribe supplements of these minerals. If you're taking a diuretic, your doctor will likely monitor levels of potassium and magnesium in your blood through regular blood tests.
- **Aldosterone antagonists.** These drugs include spironolactone (Aldactone) and eplerenone (Inspra). They're primarily potassium-sparing diuretics, but they have additional properties that help the heart work better, may reverse scarring of the heart and may help people with severe heart failure live longer. Unlike some other diuretics, spironolactone can raise the level of potassium in your blood to dangerous levels, so talk to your doctor if increased potassium is a concern.

Atkinson AB & Robertson JI. 1979. Captopril in the treatment of clinical hypertension and cardiac failure. Lancet 2, 836-9 — '70s

Gottlieb SS et al. 1993. Hemodynamic and neurohormonal effects of tghe angiotensin II antagonist Losartan in patients with congestive heart failure. Circulation 88, 1602-1609 — '90s

Whiting AJ. 1918. On the comparative value of the digitalis series of remedies in the heart failure of auricular fibrillation and the changes in the clinical features of mitral stenosis after fibrillation of the auricle. Proc R Soc Med 11, 1-52 — '10s

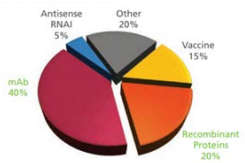
Swedberg K et al. 1979. Prolongation of survival in congestive cardiomyopathy by beta-receptor blockade. Lancet 30, 1374-6 — '70s

Marvin HM. 1927. Digitalis and diuretics in heart failure with regular rhythm, with especial reference to the importance of etiologic classification of heart disease. J Clin Invest 3, 521-39 — '20s

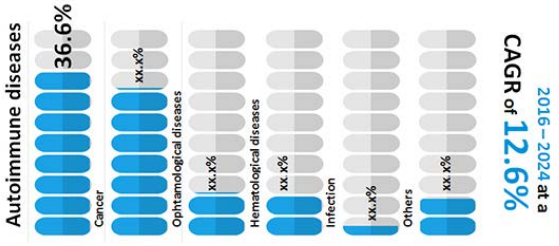
Goldberger E. 1965. Aldosterone and the edema of congestive heart failure. Am J Cardiol 15, 274 — '60s

LCZ696? SGLT2 inhibitors?

Biotherapeutics



Global Monoclonal Antibody Therapeutics Market Share By Application (2016)

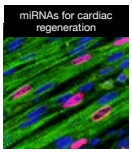


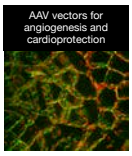
Biotherapeutics for myocardial ischemia and heart failure

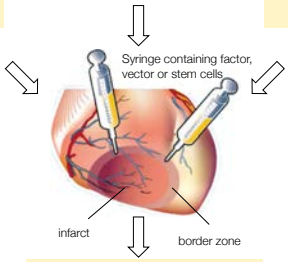
Recombinant proteins

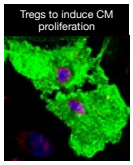
Gene Therapy
AAV vectors, miRNAs

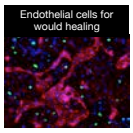
Cells





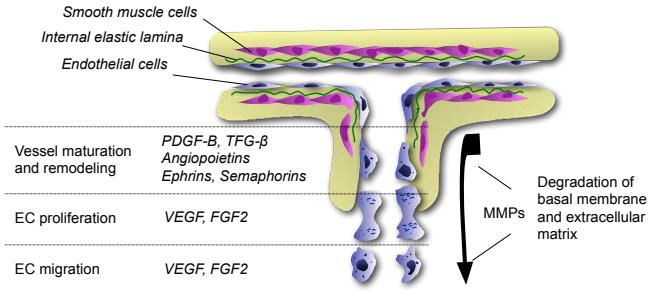




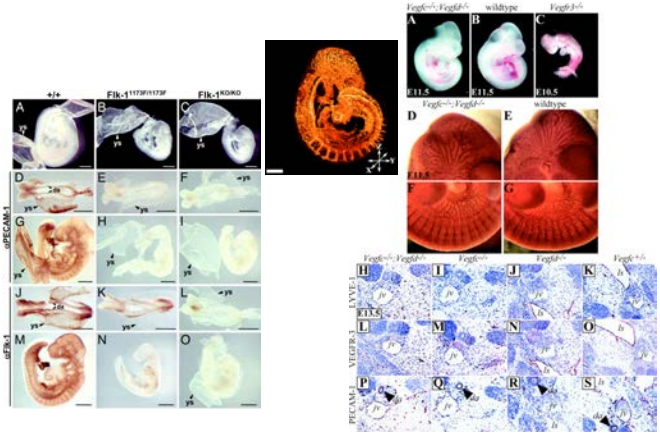


Neoangiogenesis
Cardioprotection
Cardiac regeneration

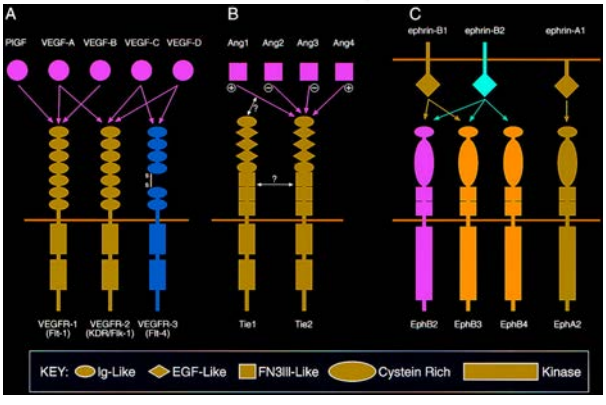
Molecular players in angiogenesis



Lessons from KO animal models



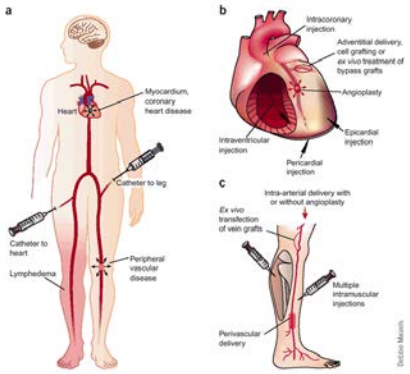
Ligands and RTK families involved in vascular development



From: N. W. Gale & G. D. Yancopulos. 1999. Genes Dev 9, 1055 - 1066

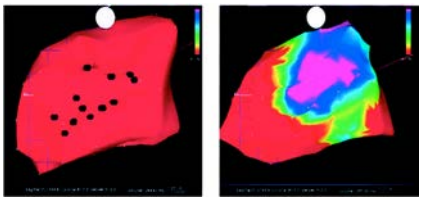
Gene delivery routes to induce therapeutic vascular growth

Intravenous
Intracoronary
Intramyocardial (trans-endocardial)
Intramyocardial (trans-pericardial)
Intrapericardial
Perivascular
Coronary sinus

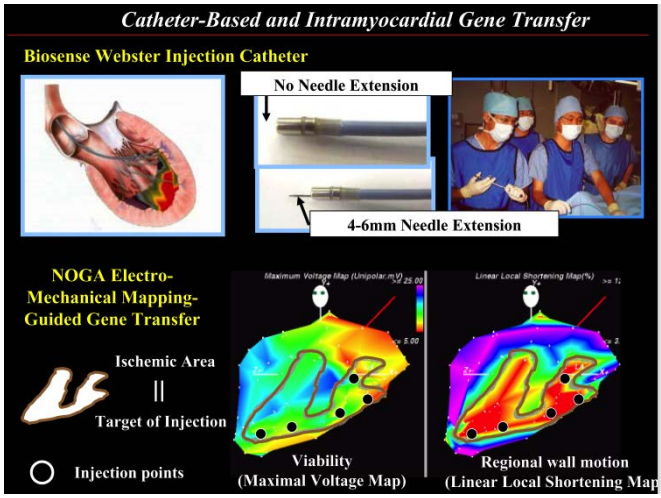


The NOGA system for transmyocardial injection

An injection catheter incorporates the mapping capabilities of the system. This provide a means by which tissues with different degrees of viability and ischemia can be mapped in detail, allowing therapy to be precisely targeted (eg, at the border zone of an infarct)



Left, electromechanical linear local shortening map from an injection procedure. The red color represents low contractility (severe cardiomyopathy). The black dots are injection sites. Right, similar map at 4 month follow-up, showing dramatic improvement in contractility at the site of injection.



Therapeutic angiogenesis: Key observations from animal studies

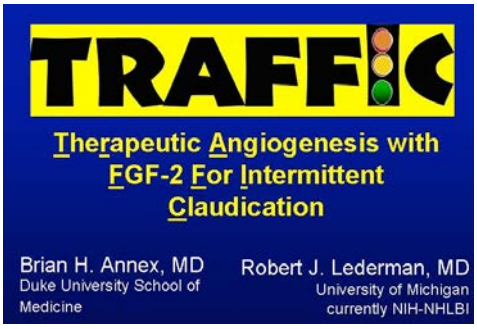
- Single administration of recombinant angiogenic proteins (VEGF, FGF)as effective as 4 week infusion
- Young and healthy animals very responsive to exogenous growth factors in the context of ischemia

Large randomized placebo-controlled trials for therapeutic angiogenesis

Table 2 Phase 2 and 3 angiogenesis trials

Trial	Therapeutic agent	Disease target	n	Endpoint	Results[a]	Reference
VIVA trial	Recombinant VEGF protein	CHD	178	ETT[b] at 60 d	Negative	97
FIRST trial	Recombinant FGF-2 protein	CHD	337	ETT at 90 d	Negative	98
TRAFFIC trial	Recombinant FGF-2 protein	PAOD	190	ETT at 90 d	Positive	99
GM-CSF trial	Recombinant GM-CSF protein	CHD	21	Invasive collateral flow index at 2 weeks	Positive	100
AGENT trial	Adenovirus-FGF-4	CHD	79	ETT at 4 weeks	Positive[c]	101
VEGF peripheral vascular disease trial	Adenovirus-$VEGF_{165}$ Plasmid/liposome $VEGF_{165}$	PAOD	54	Increased vascularity in angiography at 3 months	Positive	102
KAT trial	Adenovirus-$VEGF_{165}$ Plasmid/liposome$VEGF_{165}$	CHD	103	Improved myocardial perfusion at 6 months	Positive (adenovirus group only)	103
REVASC trial (Biobypass-CAD)	Adenovirus-$VEGF_{121}$	CHD	67	Time to 1 mm ST segment depression on ETT at 26 weeks	Positive	104
RAVE trial (Biobypass-PAD)	Adenovirus $VEGF_{121}$	PAOD	105	Peak walking time at 12 weeks	Negative	105
Euroinject One Trial	Plasmid $VEGF_{165}$	CHD	74	Improved myocardial perfusion at 3 months	Negative[d]	106

CHD, coronary heart disease; PAOD, peripheral vascular disease. [a]Efficacy measured as the study protocol–defined primary or secondary endpoint. [b]ETT, exercise tolerance test. [c]Only one dose-group showed positive results. [d]Positive results were obtained after excluding results from two of the six study centers where patient recruitment might have been a confounding issue.



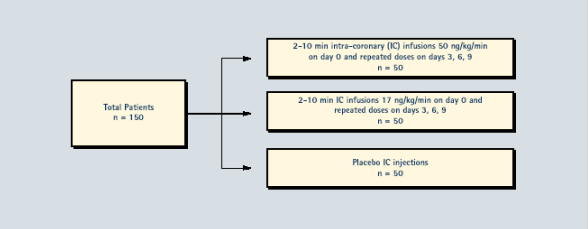
Study design

Phase II: double-blind, placebo-controlled
Groups: Placebo, SINGLE, DOUBLE
Route: Bilateral intra-arterial, days 1 and 30
Primary Endpoint: Change in PWT at day 90

VIVA

PRINCIPAL INVESTIGATOR: Edward R. McCluskey, MD Phd
Genetech, Inc.

Intra-coronary infusion of recombinant human VEGF-A to improve perfusion and function of ischemic myocardium



VEGF In Vascular Angiogenesis

ACC 1999; JACC 1999; 33(suppl A): #874-5.

PRIMARY ENDPOINT

CLINICAL: Change in exercise treadmill time at 60 days
ANGIOGRAPHIC: Collateral circulation assessment (in a subset of patients)
STATUS: Complete

Results

No changes in the clinical parameters in any of the groups

Conclusion

Intracoronary infusion of VEGF failed to improve perfusion and function of ischemic myocardium compared to placebo

Gene or protein therapy?

Protein therapy:

Advantages:
- Controlled dosing at administration
- No transmission of foreign genetic material

Disadvantages:
- Unstable in circulation
- Variable stability in tissues
  - < 2 weeks under best circumstances
  - requires polymers for delivery if >2 weeks

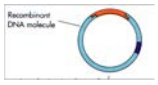
Gene therapy:

Advantages:
- Persistent expression
- Potential for single-dose regimens and cell specific therapy

Disadvantages:
- Difficult to produce and administer
- No present ability to regulate the dose
- Safety issues

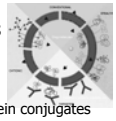
Delivery systems for human gene therapy

Direct uptake of oligonucleotides or plasmid DNA



Viral vectors
- Gammaretroviruses
- Lentiviruses
- Adenoviruses
- AAV
- Herpesviruses

Chemical methods
- Liposomes
- Cationic lipids
- Cationic polymers
- Lipid- or polymer-protein conjugates



Physical methods
- (Microinjection)
- Electroporation
- Hydroporation
- Sonoporation
- Jet injection
- Bombardment with DNA-coated gold beads (gene gun)

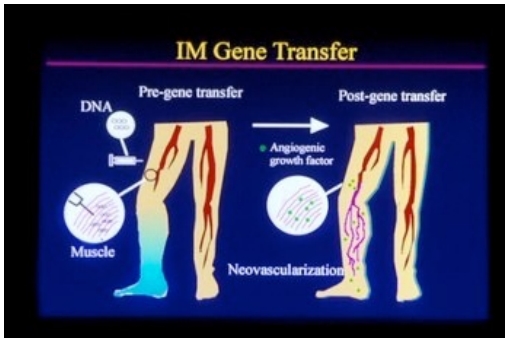
Protein-DNA complexes
- Transferrin/DNA conjugates
- Lactoferrin/DNA complexes
- Poly-L-lysine/DNA conjugates
- Asialoglycoprotein/DNA conjugates
- Adenovirus/DNA conjugates

DNA-complexed nanoparticles

Neo-organs

Therapeutic angiogenesis for peripheral artery disease

J. Isner



Initial clinical results with naked plasmid DNA (J. Isner's group)

Arterial transfer of a plasmid expressing VEGF165 in one patient with severe limb ischemia
Isner J.M. et al. 1996. Lancet 348, 370-374

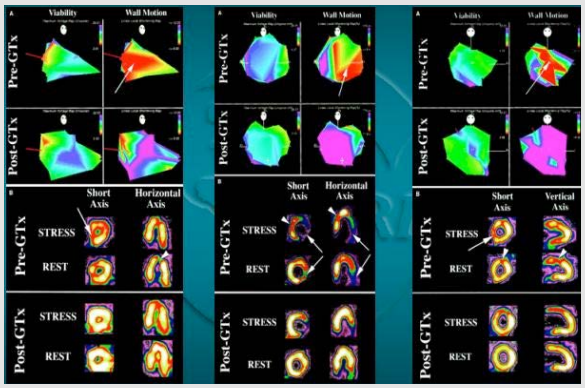
Intramuscolar injection of VEGF165 plasmid in 9 patients with non healing ischemic ulcers and/or rest pain
Baumgartner I. et al. 1998. Circulation 97, 11114-11123

Direct myocardial injection of VEGF165 expression plasmid for myocardial ischemia
Losordo D.W. et al. 1998. Circulation 98, 2800-2804

Treatment of thromboangioitis obliterans (Burger's disease) by intramuscular injection of VEGF165 plasmid
Isner J.M. et al.. 1998. J. Vasc. Surg. 28, 964-975

NB: None of these studies were placebo-controlled and based upon objective end-points!

VEGF Gene Transfer in Humans



Vale P. et al., Circulation 2000

Phase 1/2 Placebo-Controlled, Double-Blind, Dose-Escalating Trial of Myocardial Vascular Endothelial Growth Factor 2 Gene Transfer by Catheter Delivery in Patients With Chronic Myocardial Ischemia

Douglas W. Losordo, MD*; Peter R. Vale, MD*; Robert C. Hendel, MD; Charles E. Milliken, MS; F. David Fortuin, MD; Nancie Cummings, RN; Richard A. Schatz, MD; Takayuki Asahara, MD; Jeffrey M. Isner, MD; Richard E. Kuntz, MD

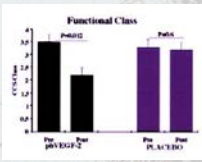
Number of patients: 19
12 randomized to phVEGF2 GTX
7 randomized to placebo injection

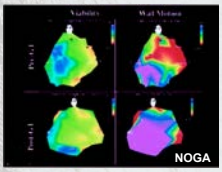
Total 6 injections at one time
Followed-up for 12 weeks

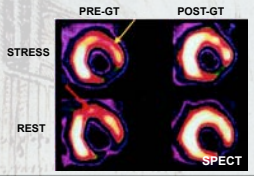
Primary endpoint:
CCS anginal class status

No adverse events

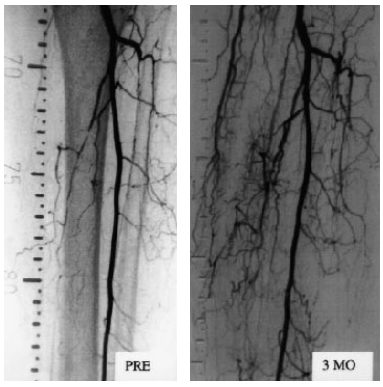
Larger randomized trials have been undertaken





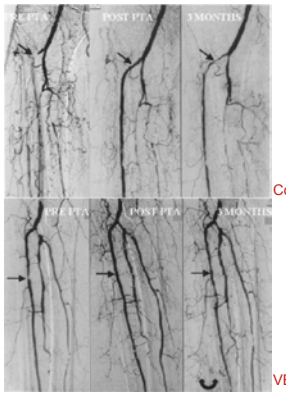


Angiography of the lower extremity of **a patient** with limb ischemia before (PRE) and 3 months after (3 MO) the transfection of a VEGF165 plasmid/ liposome expression vector

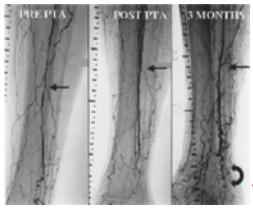


From: N. Ferrara & K. Alitalo. 1999. Nature Medicine 5, 1359 - 1364

Angiography after VEGF gene transfer to human lower limb artery: a randomized, placebo-controlled, double-blinded phase II study



Control



VEGF-P/L

VEGF-Ad

Primary endpoint: DSA at 3 months follow-up as compared with the post-PTA angiograms

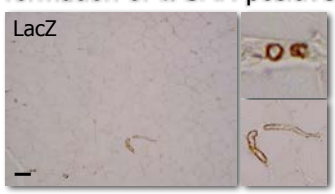
Significant increase in vascularity in the treated groups

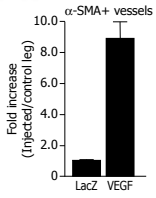
Makinen et al, Mol Ther. 2002

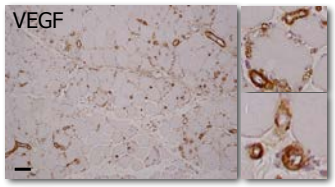
Table 3 Clinical phase II/III randomized controlled gene therapy trials in PAD

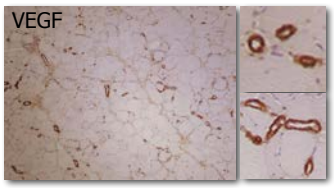
Trial	Therapeutic [target] application	Therapeutic agent	Administration	Control treatment	*n*	Primary endpoint	*Results	Reference
VEGF peripheral vascular disease trial	Therapeutic angiogenesis in PAD (claudication)	AdVEGF$_{165}$ or Plasmid/liposome VEGF$_{165}$	Intraarterial injection at the angioplasty site	Ringer's lactate	54	Increased vascularity in angiography at 3 months	Positive	14
RAVE trial	Therapeutic angiogenesis in PAD (claudication)	AdVEGF$_{121}$	Intramuscular injections	Vehicle (no virus)	105	PWT at 12 weeks	Negative	124
WALK	Therapeutic angiogenesis in PAD (claudication)	AdHIF-1a/VP16	Intramuscular injections	Vehicle	300	PWT at 6 months	Ongoing	133 (Unpublished)
DELTA-1	Therapeutic angiogenesis in PAD (claudication)	Plasmid-expressing Del-1 formulated with poloxamer 188	Intramuscular injections	Vehicle	157	PWT at 3 months	Negative	135 (Unpublished)
Groningen trial	Therapeutic angiogenesis in PAD (CLI)	Naked VEGF$_{165}$ Plasmid	Intramuscular injections	Saline	54	Decrease in amputation rate	Negative (secondary endpoints positive)	17
HGF-STAT	Therapeutic angiogenesis in PAD (CLI)	Naked HGF plasmid	Intramuscular injections	Saline	48 (planned)	Wound healing, amputation rate, rest pain, ABI	Ongoing	134 (Unpublished)
TALISMAN 201	Therapeutic angiogenesis in PAD (CLI)	Naked FGF-1 plasmid	Intramuscular injections	Vehicle	107	Ulcer healing at 6 months	Negative (secondary endpoints positive)	136 (Unpublished)
PM 202	Therapeutic angiogenesis in PAD (CLI)	Naked FGF-1 plasmid	Intramuscular injections	Vehicle	71	Change in transcutaneous pO2	Negative	137 (Unpublished)
Prevent III	Vein graft failure in PAD (CLI)	Edifoligide (an E2F transcription factor decoy)	*Ex vivo* pressure-mediated delivery	Buffered saline	1,404	Time to graft reintervention or major amputation due to graft failure	Negative (secondary endpoint positive)	35

Transduction with AAV-VEGF-A$_{165}$ induces massive formation of α-SMA-positive arterioles





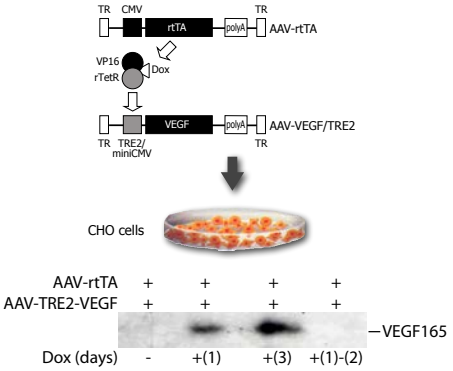




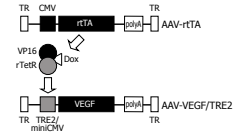
α-SMA

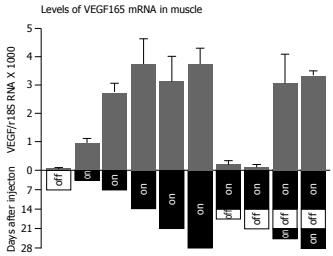
Zacchigna, S, *et al.* 2008. J. Clin. Invest. 118, 2062
Zentilin, L, et al. 2006. Blood 107, 3546.
Zacchigna, S, et al. 2005. Am J Pathol 167, 981.
Arsic N. *et al.* 2003. Mol. Ther. 7. 450

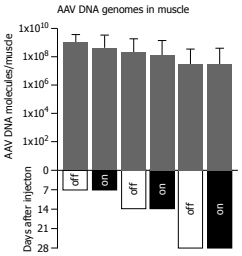
Tet-on/AAV inducible system: controlled in vivo delivery of VEGF



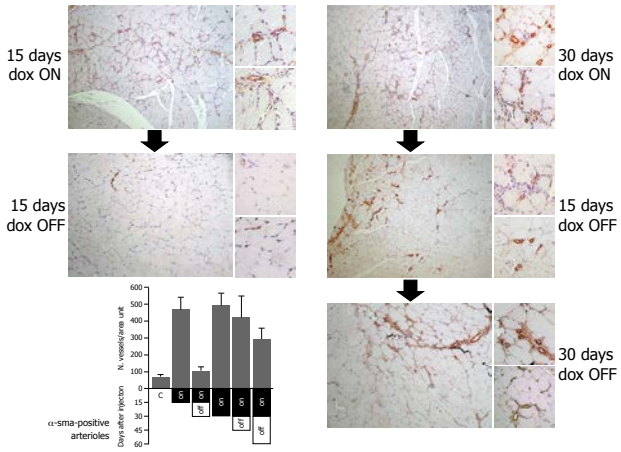
Doxycycline-mediated control of in vivo transgene expression

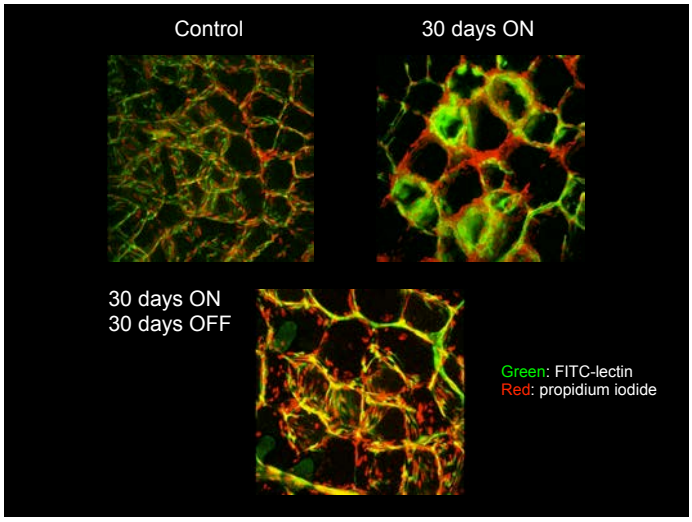


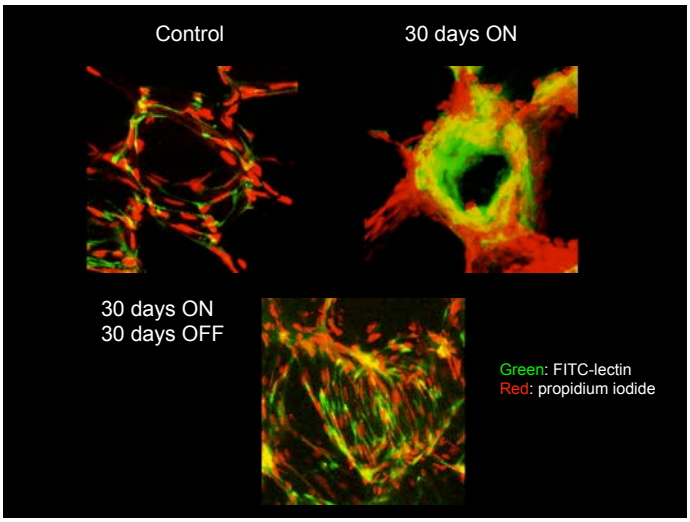




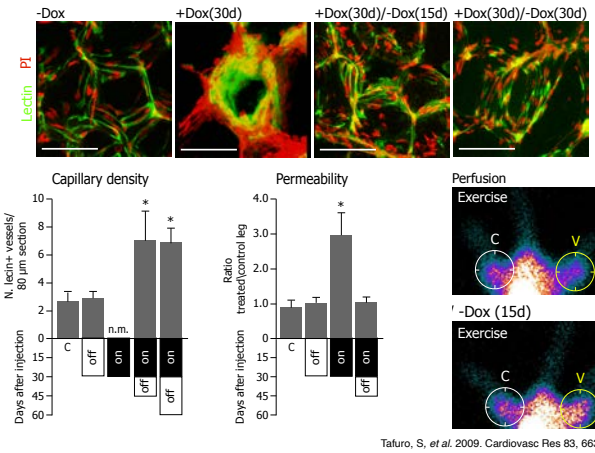
Stable vessel formation requires prolonged VEGF stimulation







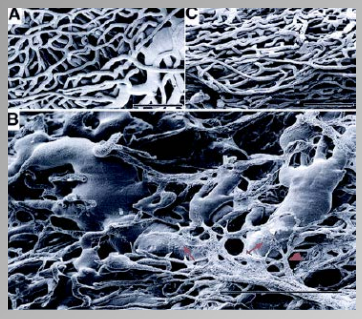
Vessel maturation upon withdrawal of the VEGF stimulus

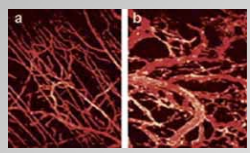


Tafuro, S, *et al.* 2009. Cardiovasc Res 83, 663.

Fate of new blood vessels: inducible promoters

Control 4 wks on, 8 d off

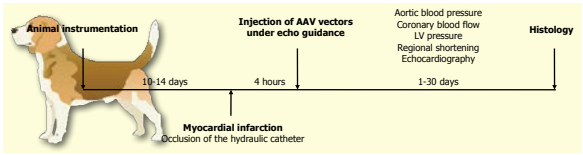


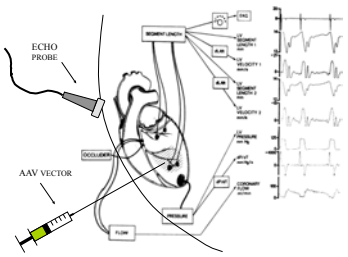


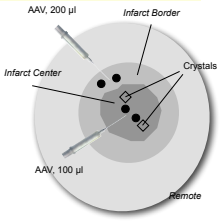
a. normal vasculature
b. tumor vasculature

4 wks of VEGF on

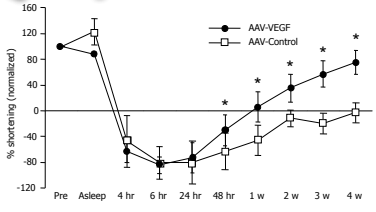
Transduction of AAV-VEGFA$_{165}$ to the infarcted myocardium in chronically instrumented dogs

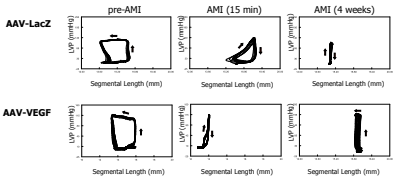




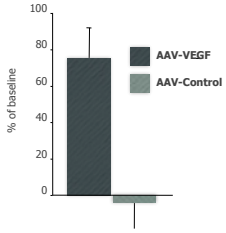


AAV-VEGFA$_{165}$ transduction dramatically improves functional recovery of infarcted myocardium in chronically instrumented dogs



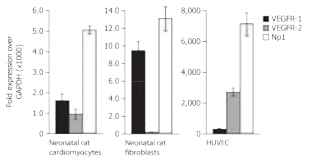


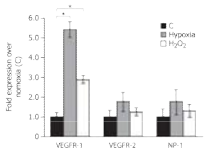
Fractional shortening at 4 weeks

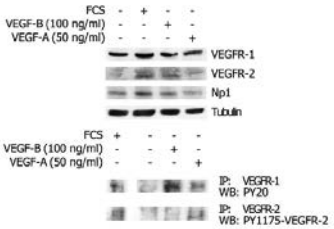


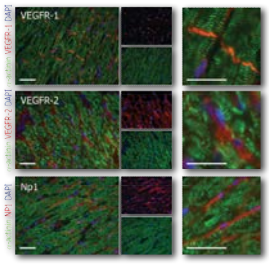
Ferrarini, M, et al. 2006. Circ Res 98, 954.

Cardiomyocytes express functional VEGF receptors - VEGF-R1 expression increases in hypoxia

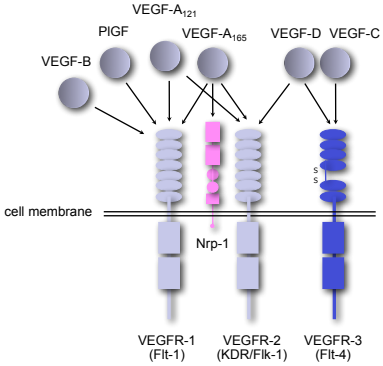




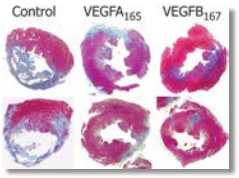


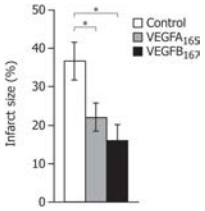


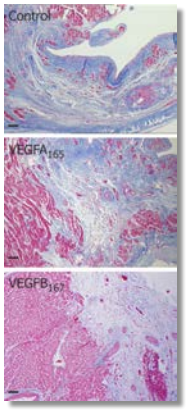
The VEGF family of angiogenic factors and their receptors



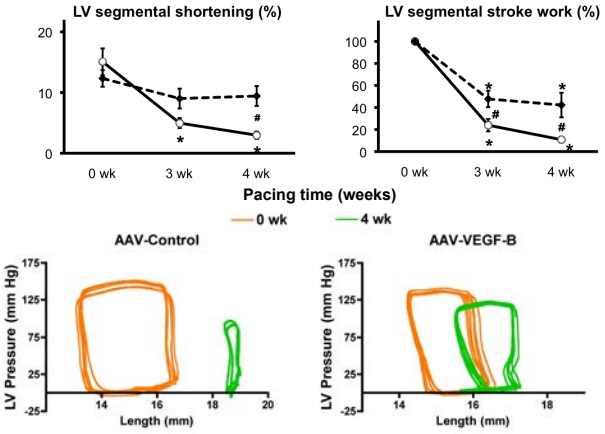
Reduction of infarct size in rats injected with AAV-VEGFA$_{165}$ or AAV-VEGFB$_{167}$



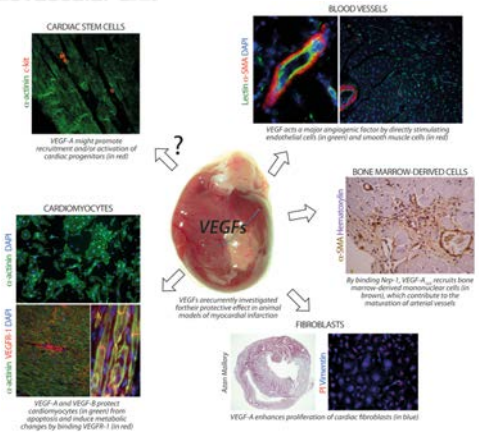




Effects of AAV9-VEGFB transduction in pacing-induced heart failure in dogs



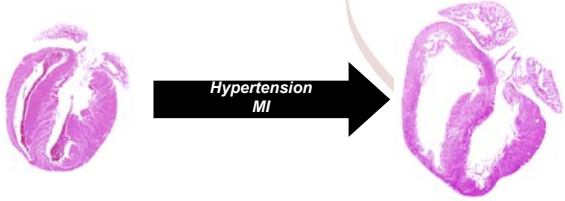
Multiple effects of VEGF family members at the cardiovascular unit



Giacca M. and Zacchigna S., Gene Therapy 2012

Heart Failure

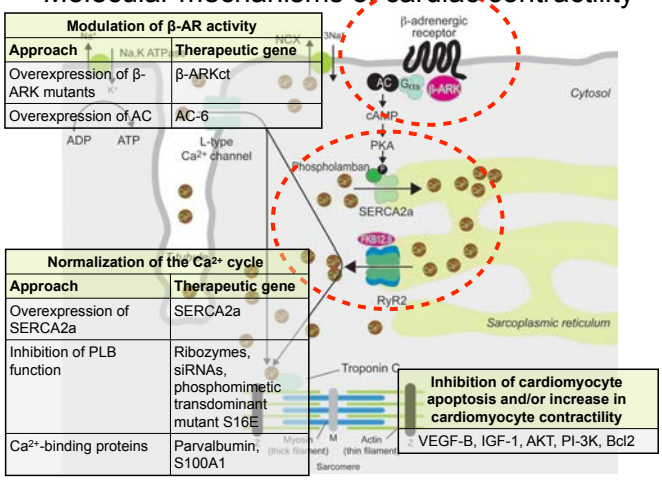
- *>5 million affected with >400,000 new cases per year*
- *50% mortality within 5 years*
- *Over $30 billion/year in health care costs*



Molecular mechanisms of cardiac contractility

Modulation of β-AR activity	
Approach	**Therapeutic gene**
Overexpression of β-ARK mutants	β-ARKct
Overexpression of AC	AC-6

Normalization of the Ca^{2+} cycle	
Approach	**Therapeutic gene**
Overexpression of SERCA2a	SERCA2a
Inhibition of PLB function	Ribozymes, siRNAs, phosphomimetic transdominant mutant S16E
Ca^{2+}-binding proteins	Parvalbumin, S100A1



Journal of Cardiac Failure Vol. 15 No. 3 2009

Clinical Trials

Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID Trial), a First-in-Human Phase 1/2 Clinical Trial

BRIAN E. JASKI, MD, FACC,[1] MARIELL L. JESSUP, MD,[2] DONNA M. MANCINI, MD,[3] THOMAS P. CAPPOLA, MD,[2] DANIEL F. PAULY, MD, PhD,[4] BARRY GREENBERG, MD,[5] KENNETH BOROW, MD,[6] HOWARD DITTRICH, MD,[7] KRISZTINA M. ZSEBO, PhD,[8] AND ROGER J. HAJJAR, MD,[9] ON BEHALF OF THE CALCIUM UP-REGULATION BY PERCUTANEOUS ADMINISTRATION OF GENE THERAPY IN CARDIAC DISEASE (CUPID) TRIAL INVESTIGATORS.

San Diego, California; Philadelphia, Pennsylvania; New York, New York; Gainesville, Florida; Wayne, Pennsylvania; La Jolla, California

ABSTRACT

Background: SERCA2a deficiency is commonly seen in advanced heart failure (HF). This study is designed to investigate safety and biological effects of enzyme replacement using gene transfer in patients with advanced HF.

Methods and Results: A total of 9 patients with advanced HF (New York Heart Association [NYHA] Class III/IV, ejection fraction [EF] ≤30%, maximal oxygen uptake [VO_2 max] <16 mL·kg·min, with maximal pharmacological and device therapy) received a single intracoronary infusion of AAV1/SERCA2a in the open-label portion of this ongoing study. Doses administered ranged from 1.4×10^{11} to 3×10^{12} DNase resistant particles per patient. We present 6- to 12-month follow-up data for these patients. AAV1/SERCA2a demonstrated an acceptable safety profile in this advanced HF population. Of the 9 patients treated, several demonstrated improvements from baseline to month 6 across a number of parameters important in HF, including symptomatic (NYHA and Minnesota Living with Heart Failure Questionnaire, 5 patients), functional (6-minute walk test and VO_2 max, 4 patients), biomarker (NT-ProBNP, 2 patients), and LV function/remodeling (EF and end-systolic volume, 5 patients). Of note, 2 patients who failed to improve had preexisting anti-AAV1 neutralizing antibodies.

Conclusions: Quantitative evidence of biological activity across a number of parameters important for assessing HF status could be detected in several patients without preexisting neutralizing antibodies in this open-label study, although the number of patients in each cohort is too small to conduct statistical analyses. These findings support the initiation of the Phase 2 double-blind, placebo-controlled portion of this study. *J Cardiac Fail 2009;15:171–181*)

Key Words: Gene therapy, heart failure, SERCA2a, cardiovascular disease.

The CUPID Trial with MYDICAR® (AAV1-SERCA2a)

9 patients with advanced HF (NYHA Class III/IV, EF<30%)

Single intracoronary infusion of AAV1-SERCA2a in three doses (Cohort#1: $1.4x10^{11}$; Cohort#2: $6x10^{11}$; Cohort 3: $3x10^{12}$ viral particles)

6-12 month follow up:
- Safe
- Significant clinical improvement

Phase II trial started

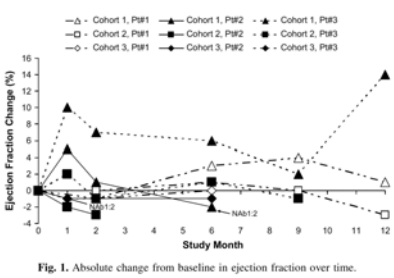
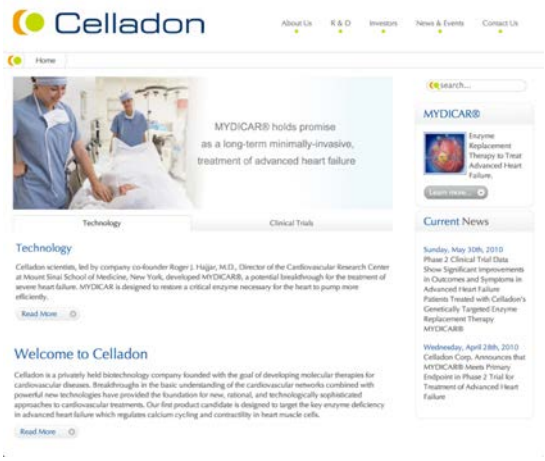
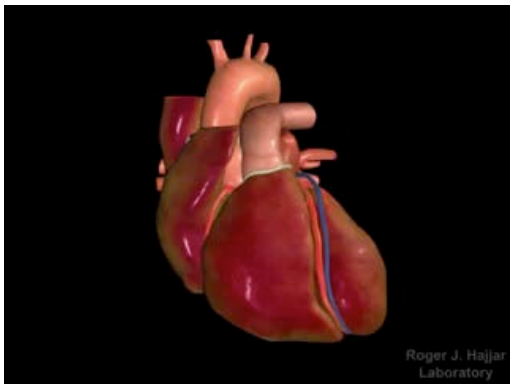
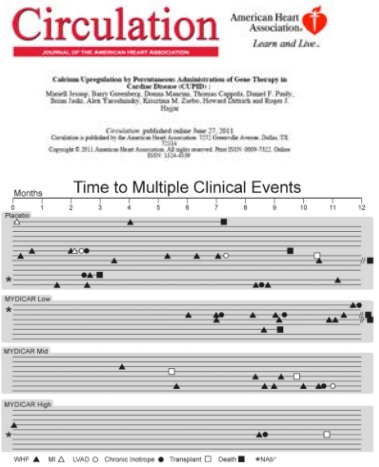


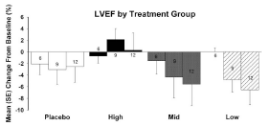
Fig. 1. Absolute change from baseline in ejection fraction over time.







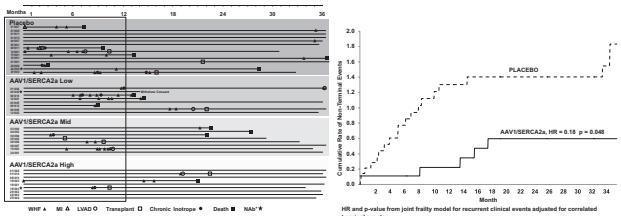
Significant differences in the treated versus control patients are found when multiple-efficacy domain analysis is applied, which simultaneously takes into consideration a series of clinical and instrumental parameters, but not when ejection fraction alone (a precise measurement of cardiac function) is analyzed. This is a possible indication that treatment at this stage slows progression but does not reverse the condition.



A substantially larger number of patients will be required to address this issue directly.

Long-Term Effects of AAV1/SERCA2a Gene Transfer in Patients With Severe Heart Failure: Analysis of Recurrent Cardiovascular Events and Mortality

Krisztina Zsebo, Alex Yaroshinsky, Jeffrey J. Rudy, Kim Wagner, Barry Greenberg, Mariell Jessup and Roger J. Hajjar



Note: Clinical events up to and including a terminal event (death, transplant, LVAD) or Month 12, whichever came first, were adjudicated; events thereafter were not adjudicated.

HR and p-value from joint frailty model for recurrent clinical events adjusted for correlated terminal events.

Evidence of long-term transgene presence was observed in high-dose patients.

No safety concerns were noted during the 3-year follow-up.

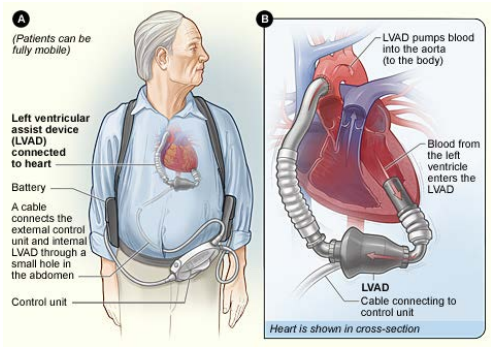
After a single intracoronary infusion of AAV1/SERCA2a in patients with advanced heart failure, positive signals of cardiovascular events persist for years.

Circulation Research January 3, 2014

Celladon

Aug 11, 2014

Celladon Announces Initiation of Clinical Trial to Investigate MYDICAR in Patients With Heart Failure and a Left Ventricular Assist Device (LVAD)



Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial

Barry Greenberg, Javed Butler, G Michael Felker, Piotr Ponikowski, Adriaan A Voors, Akshay S Desai, Denise Barnard, Alain Bouchard, Brian Jaski, Alexander R Lyon, Janice M Pogoda, Jeffrey J Rudy, Krisztina M Zsebo

Lancet 2016; 387: 1178–86
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http://dx.doi.org/10.1016/S0140-6736(16)00062-9
See Comment page 1137

UC San Diego Sulpizio Cardiovascular Center, La Jolla, CA, USA (Prof B Greenberg MD, Prof D Barnard MD); Stony Brook University, Stony Brook, NY, USA (Prof J Butler MD); Duke University School of Medicine, Durham, NC, USA (Prof G M Felker MD); Wroclaw Medical University and Military Hospital, Wroclaw, Poland (Prof P Ponikowski MD); University of Groningen, Groningen, Netherlands (Prof A A Voors MD); Cardiovascular Division, Brigham and Women's Hospital, Boston, MA, USA (A S Desai MD); Cardiology PC, Birmingham, AL, USA (A Bouchard MD); San Diego Cardiac Center, Sharp Memorial Hospital, San Diego, CA, USA (B Jaski MD); Royal Brompton Hospital, London, UK (A R Lyon MD); Imperial College London, London, UK (A R Lyon); Celladon Corporation, San Diego, CA, USA (J M Pogoda PhD, J J Rudy BSc) and Biovect Consulting, Santa Barbara, CA, USA

Summary

Background Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) activity is deficient in the failing heart. Correction of this abnormality by gene transfer might improve cardiac function. We aimed to investigate the clinical benefits and safety of gene therapy through infusion of adeno-associated virus 1 (AAV1)/SERCA2a in patients with heart failure and reduced ejection fraction.

Methods We did this randomised, multinational, double-blind, placebo-controlled, phase 2b trial at 67 clinical centres and hospitals in the USA, Europe, and Israel. High-risk ambulatory patients with New York Heart Association class II–IV symptoms of heart failure and a left ventricular ejection fraction of 0·35 or less due to an ischaemic or non-ischaemic cause were randomly assigned (1:1), via an interactive voice and web-response system, to receive a single intracoronary infusion of 1×10^{13} DNase-resistant particles of AAV1/SERCA2a or placebo. Randomisation was stratified by country and by 6 min walk test distance. All patients, physicians, and outcome assessors were masked to treatment assignment. The primary efficacy endpoint was time to recurrent events, defined as hospital admission because of heart failure or ambulatory treatment for worsening heart failure. Primary efficacy endpoint analyses and safety analyses were done by modified intention to treat. This trial is registered with ClinicalTrials.gov, number NCT01643330.

Findings Between July 9, 2012, and Feb 5, 2014, we randomly assigned 250 patients to receive either AAV1/SERCA2a (n=123) or placebo (n=127); 243 (97%) patients comprised the modified intention-to-treat population. Patients were followed up for at least 12 months; median follow-up was 17·5 months (range 1·8–29·4 months). AAV1/SERCA2a did not improve time to recurrent events compared with placebo (104 *vs* 128 events; hazard ratio 0·93, 95% CI 0·53–1·65; p=0·31). No safety signals were noted. 20 (16%) patients died in the placebo group and 25 (21%) patients died in the AAV1/SERCA2a group; 18 and 22 deaths, respectively, were adjudicated as being due to cardiovascular causes.

Interpretation CUPID 2 is the largest gene transfer study done in patients with heart failure so far. Despite promising results from previous studies, AAV1/SERCA2a at the dose tested did not improve the clinical course of patients with heart failure and reduced ejection fraction. Although we did not find evidence of improved outcomes at the dose of AAV1/SERCA2a studied, our findings should stimulate further research into the use of gene therapy to treat patients with heart failure and help inform the design of future gene therapy trials.

Funding Celladon Corporation.

The microRNA pathway

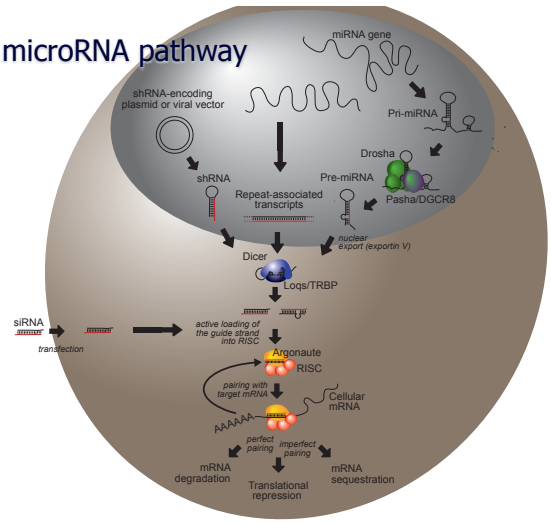
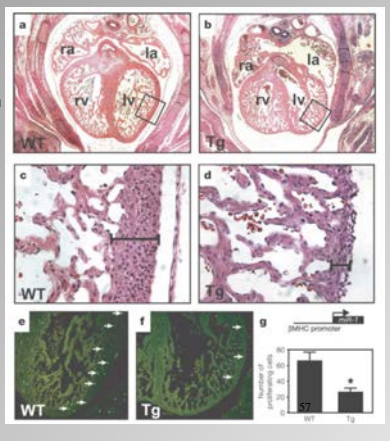


Table 1. Table 1. MiRs Reported Dysregulated in Array Analyses to Date

Downregulated MiRs	Upregulated MiRs	No Change	Species/Model or Disease
1, 7d*, 10a/b, 26a/b, 29a/c, 30a-3p/a-5p/b/ c/d/e/e*, 139, 149, 150, 151, 155, 185, 194, 218, 378	15b, **21**, 23a/b, 24, 27a/b, 31, 103, 107, 125b, 127, 140/*, 195, 199a/a*/b, 214, 221, 222, 351, let-7b/c	133a/b	mouse/TAC[91]
29c, 30c, **93**, 133a/b, **150, 181b**	10b, 19a, **21, 23a**/b, **24**, 25, 27a/b, 125b, 126, 154, **195, 199a**/a*, 210, **214**, 217, 218, 330, 351		mouse/TAC and CnA Tg[88]
29a/b/c, **30c**, 126-5p, 133a/b, 149, **150, 185, 451, 486**	**21, 27a/b**, 146, **214, 341, 424**		mouse/TAC[89]
30b/c, 150	17-5p, 18b, 19b, 20b, **21, 23a, 25, 29a**, 106a, **125b**, 140, 142-3p, 153, 184, 200a, 208, 210, 211, 221, 222		mouse/TAC[90]
187, 292-5p, 373, 466	*18b*, 20b, **21, 23a**, 106a, **125b**, 133a	**25, 29a**	rCM/PHE[90]
	23*, **24, 125b, 195, 199a, 214**	21, 27, 29c, 93, 150, 181b	Human/HF[88]
16, 17-5p, 19b, 22, 23b, 24, 27a, 30a-5p/b/c/e-5p, 107, 126, 130b, 135a, 136, 148a, 150, 182, 186, 192, 199a*, 218, 299-5p, 302b*, 302c*, 325, 339, 342, 452/*, 494, 495, 497, 499, 507, 512-5p, 515-5p, 520d*/h, 520, 523, 526b/b*	*1*, 7a/b/c/d/e/f, 10b, 106b, 17-3p, *21*, 26a, 28, 29a/b/c, 32, 34b, 98, 125a, 126*, *129*-3p, 130a, 132, 196a, 199b, 200c, 204, 205, 208, 210, 211, *212*, 213, 215, 292-3p, 294, 295, 296, 297, 300, 302a, *320*, 322, 330, 331, 333, 340, 341, 343, 365, 367, 372, 373, 377, 381, 382, *423*, 424, 429, 432, 500, 520c, 525*		Human/HF[92]

Bold indicates miRs reported validated by Northern blots; italics, miR reported validated by PCR; CnA Tg, calcineurin A transgenic mice; rCM, neonatal rat cardiomyocytes; PHE, phenylephrine; HF, end-stage heart failure. †Expression found variable.

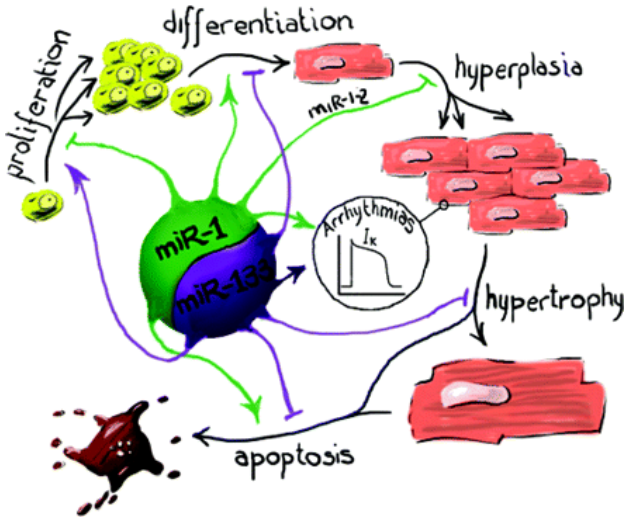
miR-1 regulates pool of proliferating ventricular cardiomyocytes and ventricular expansion.

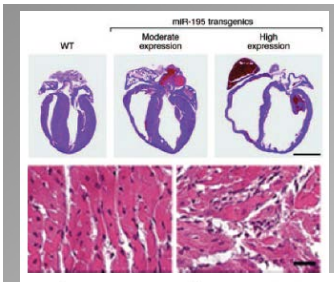


a–d, Transverse sections of wild-type (a) or beta-MHC-miR-1 transgenic (b) hearts at E13.5. Boxed areas are shown below at higher magnification (c, d), with the black bar illustrating the narrowed width of the compact layer in transgenic hearts. e, f, Immunohistochemical staining in wild-type (e) and transgenic (f) hearts using an antibody specific to phosphohistone H3, to mark proliferating cells. Arrows indicate proliferating cells. g, Quantification of cycling cells shows a statistically significant decrease (asterisk) in the number of proliferating cells in miR-1 transgenic hearts (n = 3). Error bars indicate standard deviations. Abbreviations: la, left atrium; lv, left ventricle; ra, right atrium; rv, right ventricle.

Yong Zhao, Eva Samal and Deepak Srivastava

Nature 436, 214-220 (14 July 2005)

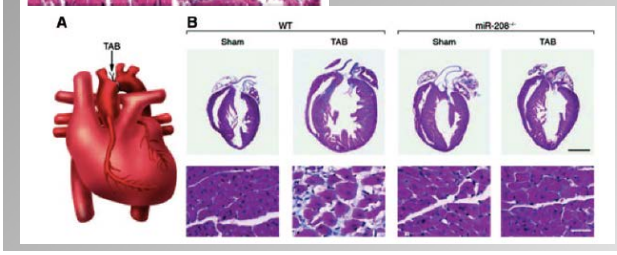




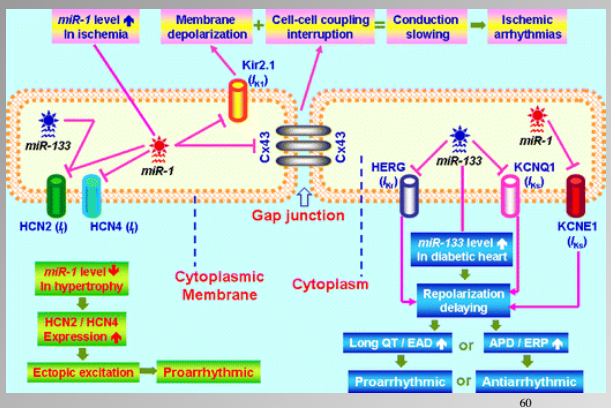
Cardiac-specific overexpression of miR-195 is sufficient to drive cardiomyopathy

Requirement of miR-208 for cardiomyocyte hypertrophy and fibrosis.

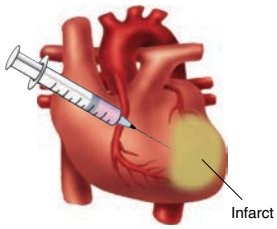
van Rooij E, Olson EN. MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. *J Clin Invest*. 2007;117: 2369-2376.



miRNAs and cardiac arrhythmias



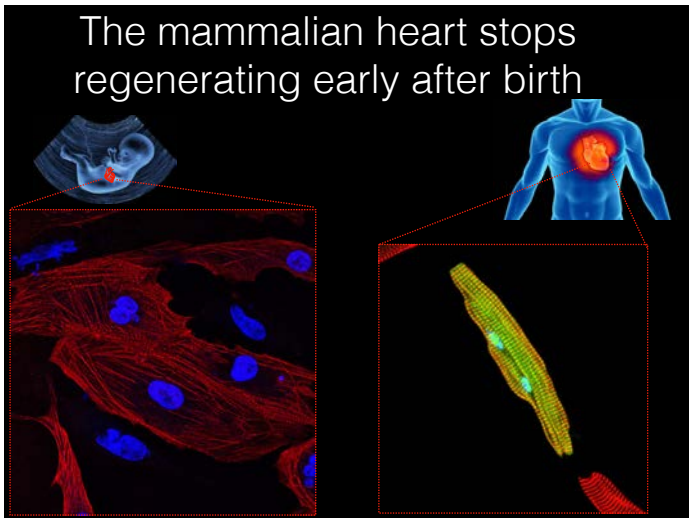
The problem in cardiac regeneration



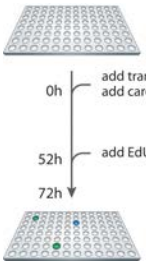
2-4 billion cardiomyocytes are lost from the left ventricle during myocardial infarction

Search for biotherapeutics promoting cardiomyocyte proliferation

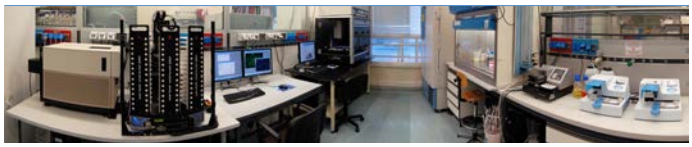
The mammalian heart stops regenerating early after birth



Screening for cardiomyocyte proliferation using a library of microRNA mimics

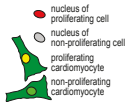


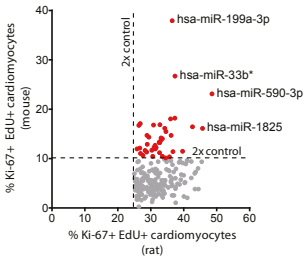
Ana Eulalio

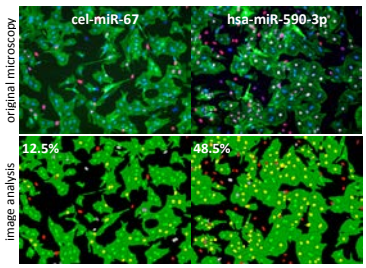


40 human miRNAs increase both rat and mouse cardiomyocyte proliferation

Hoechst
a-actinin
EdU



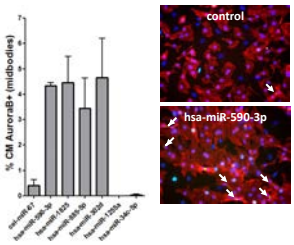




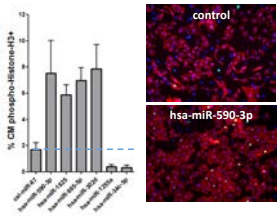
Eulalio et al. 2012. Nature 492, 376

40 human miRNAs increase both rat and mouse cardiomyocyte proliferation

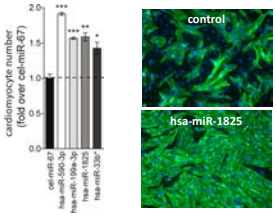
Aurora B midbody localization



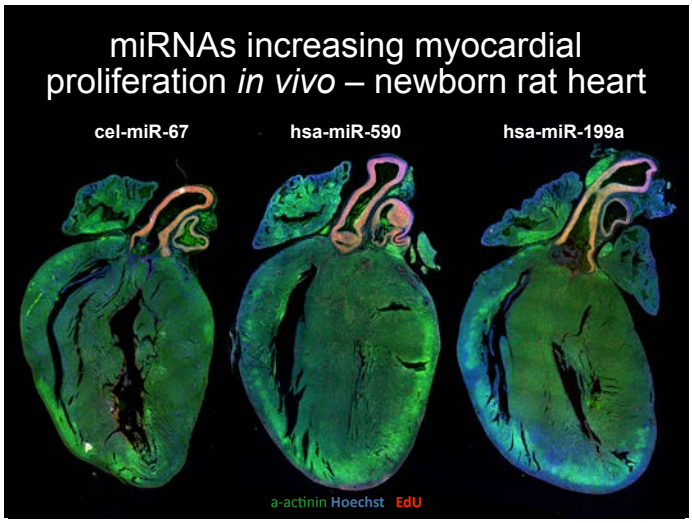
phosphoH3 positivity



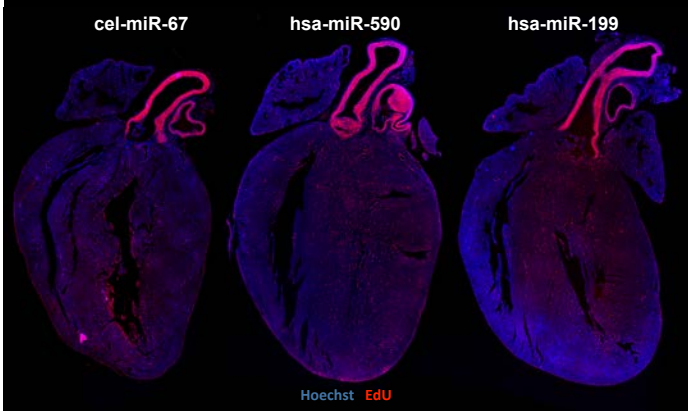
Increase in cell number



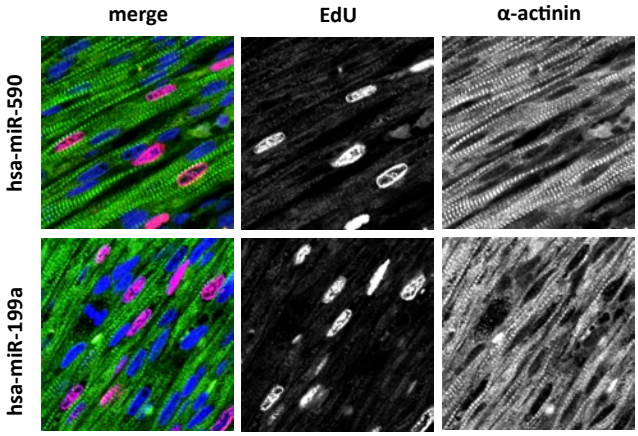
miRNAs increasing myocardial proliferation *in vivo* – newborn rat heart



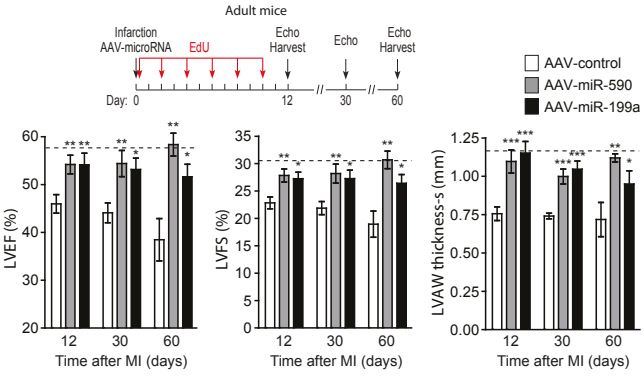
miRNAs increasing myocardial proliferation *in vivo* – newborn rat heart



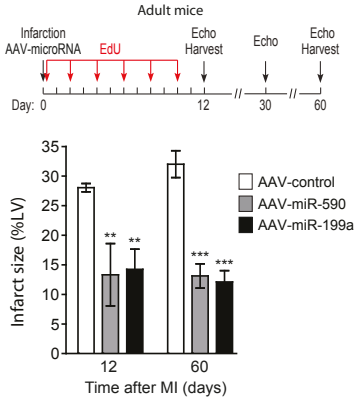
miRNAs increasing CM proliferation *in vivo*

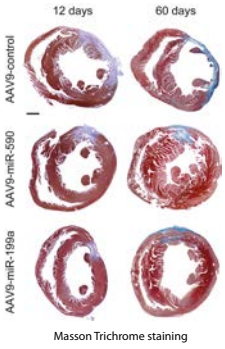


miR-590 and miR-199a preserve myocardial function after MI



miR-590 and miR-199a markedly reduce infarct size

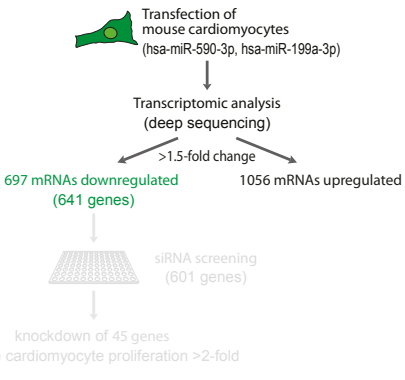




Eulalio et al. 2012. Nature 492, 376

Mechanisms?

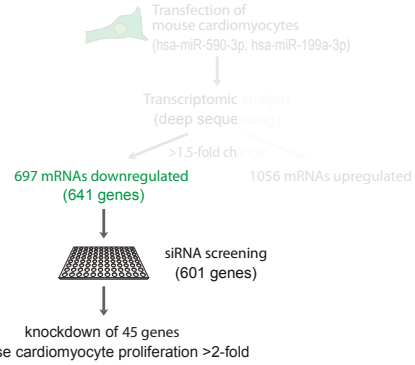
Identification of miR-590-3p and miR-199a-3p target genes



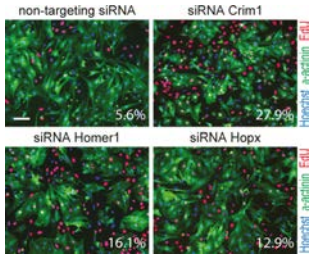
Among the 641 genes downregulated by miR-590-3 and miR-199a-3p there are:

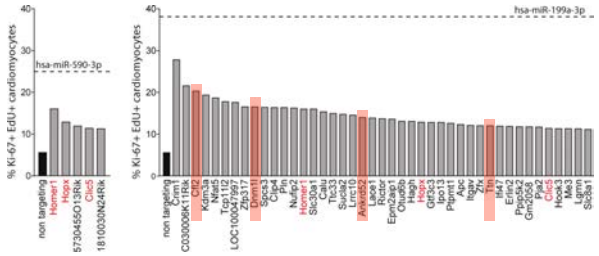
Myomesin 1 (Myom1)
Myomesin 2 (Myom2)
Myosin light polypeptide 4 (Myl4)
Nebulin-related anchoring protein (Nrap)
Myosin IB (Myo1b)
Titin (Ttn)
Troponin T1, skeletal slow (Tnnt1)
Troponin T2 cardiac (Tnnt2)
Cofilin2 (Cofilin2)
Dynamin1-like (Dnm1l)
Ankyrin repeat domain 52 (Ankrd52)
Nebulette (Nbl)

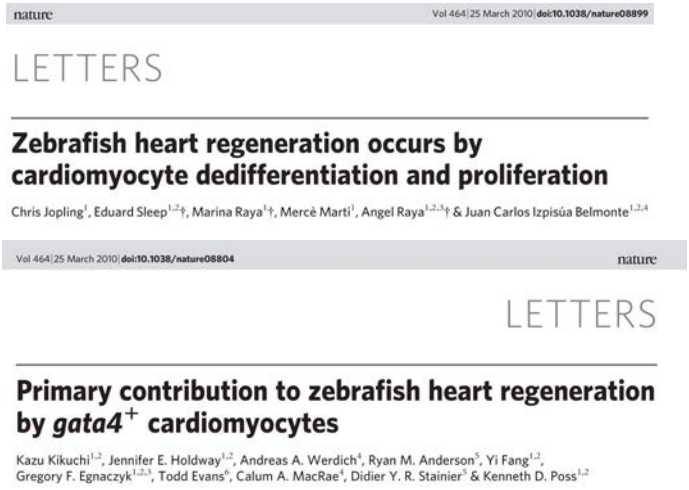
Identification of miR-590-3p and miR-199a-3p target genes



Identification of miR-590 and miR-199a target genes by deep sequencing and HTS siRNA screening

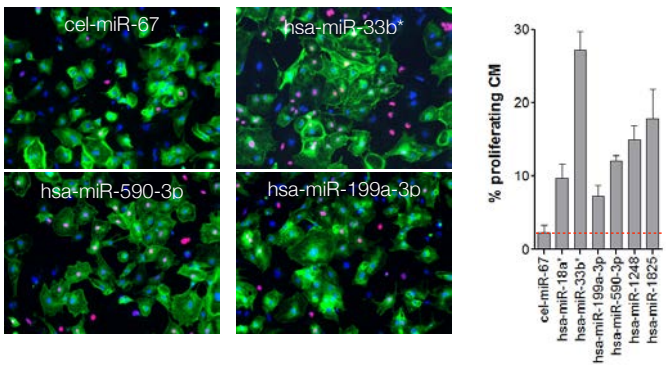




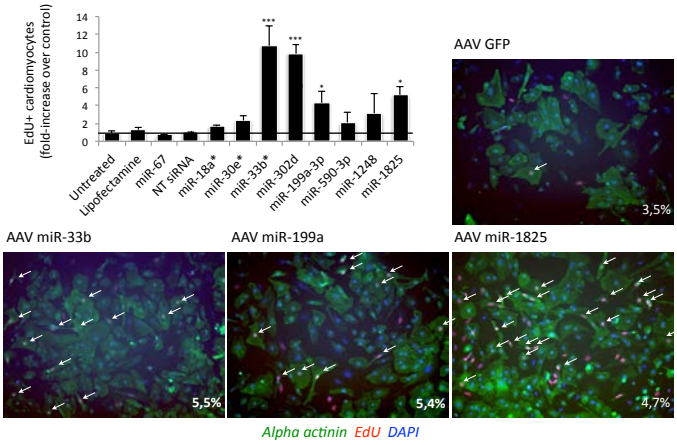


Effect in human cardiomyocytes?

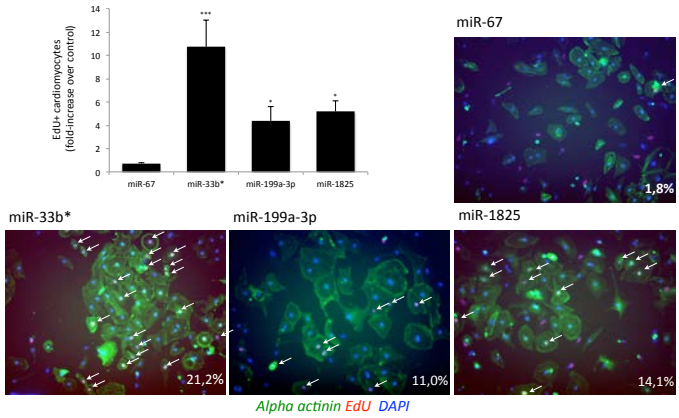
Effect of selected miRNAs on the proliferation of human ES cell-derived cardiomyocytes

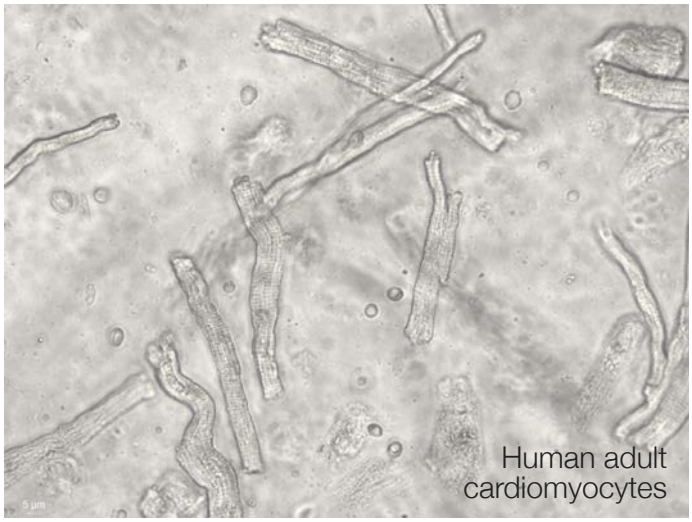


Human fetal cardiomyocyte proliferation (AAV6 pri-miRNA transduction)

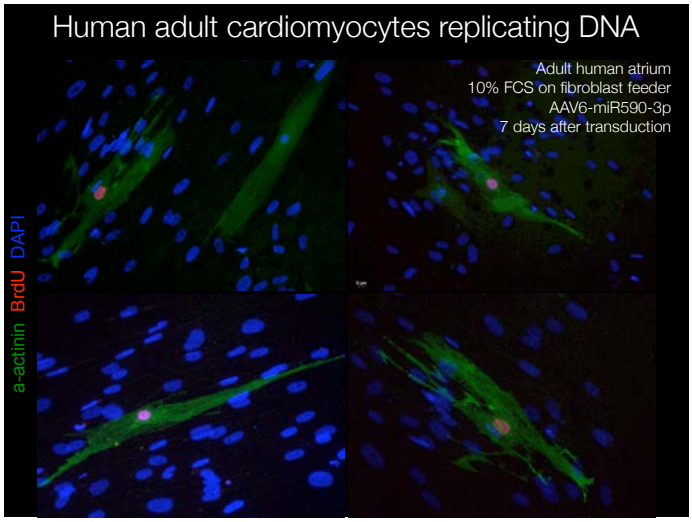


Human fetal cardiomyocyte proliferation (mimic transfection)





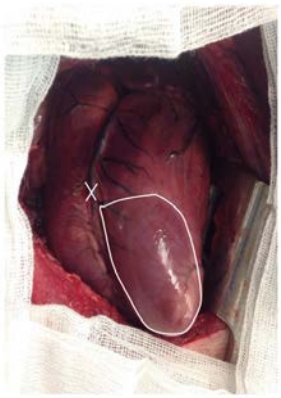
Human adult cardiomyocytes replicating DNA



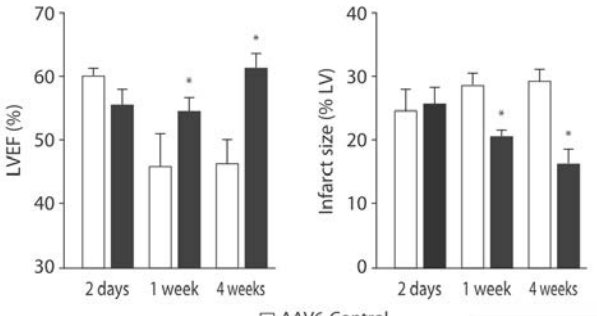
Effect in large animals?

3-6 months old farm pig

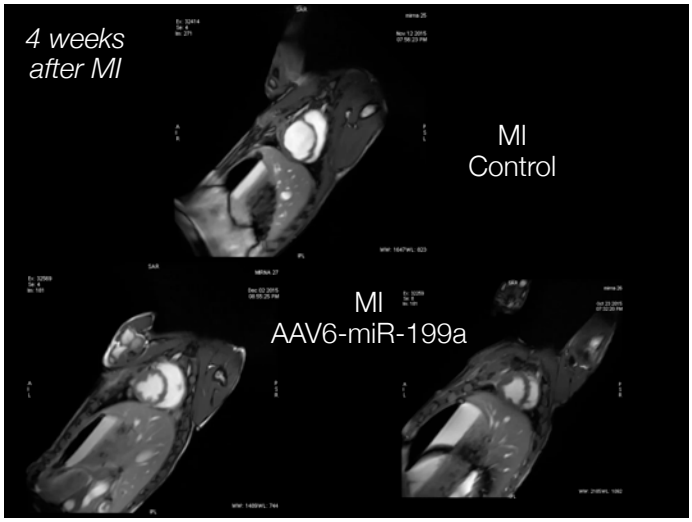
LAD Occlusion after first Diagonal branch for 90 minutes, followed by Reperfusion

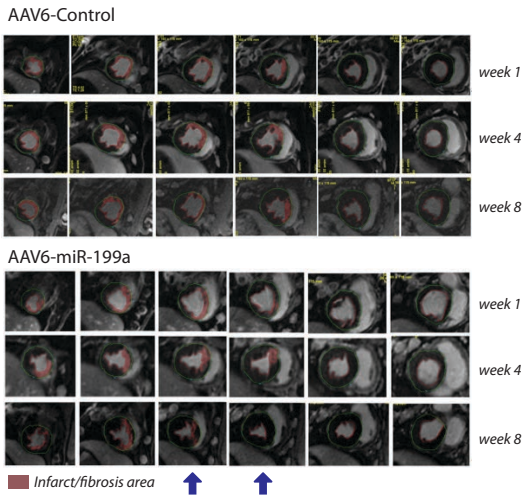


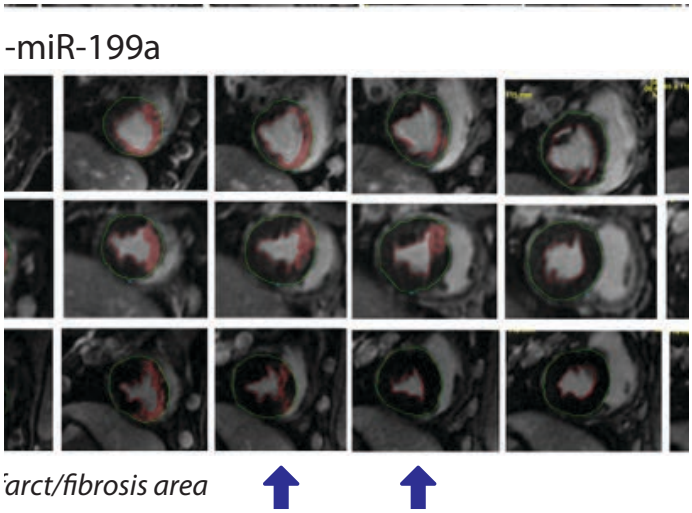
AAV6-miR-199a reduces infarct size and improves cardiac function after MI in pigs



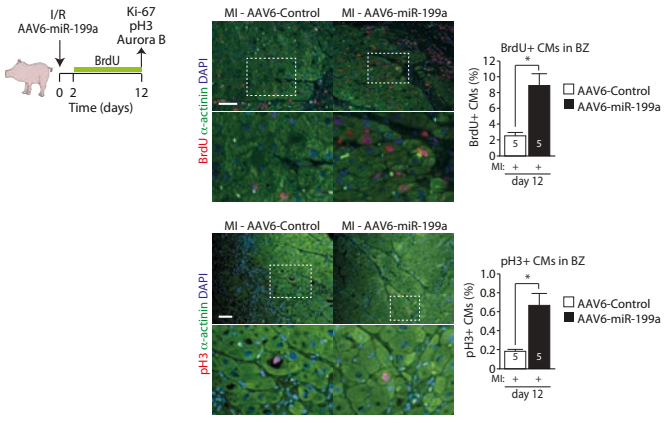
Khatia Gabisonda
Giovanni Aquaro
Fabio Recchia

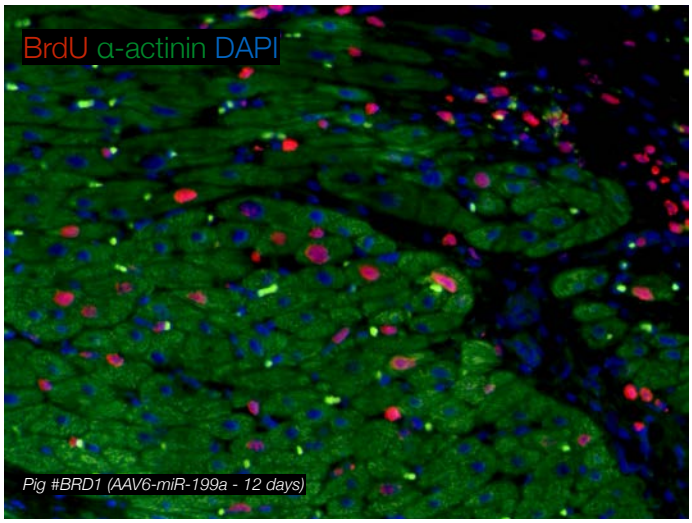




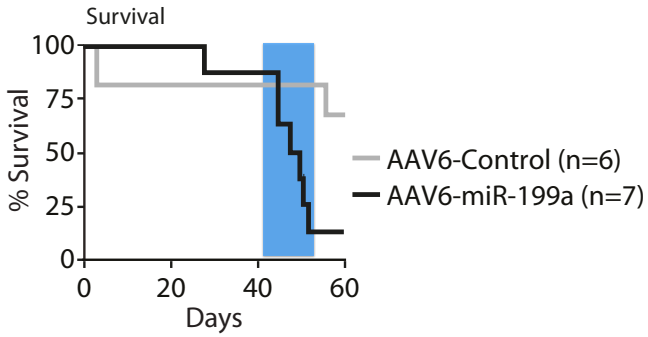


Cardiomyocyte proliferation in the infarct border zone in AAV6-miR-199a-treated pigs

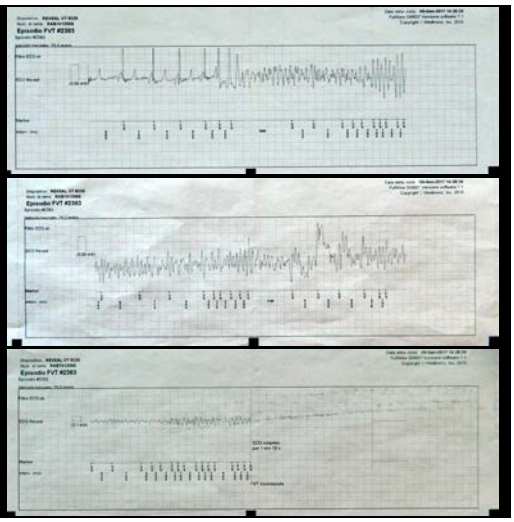




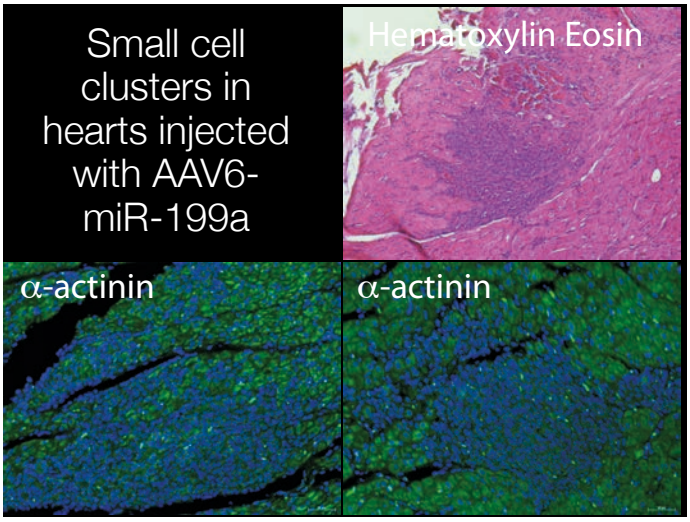
Sudden cardiac death of AAV6-miR-199a-treated pigs



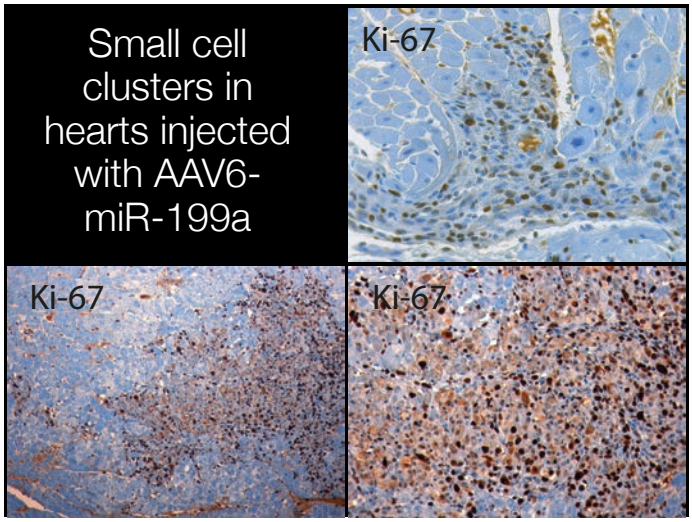
Death of pig#66 at 8 weeks after MI and injection of AAV6-miR-199a



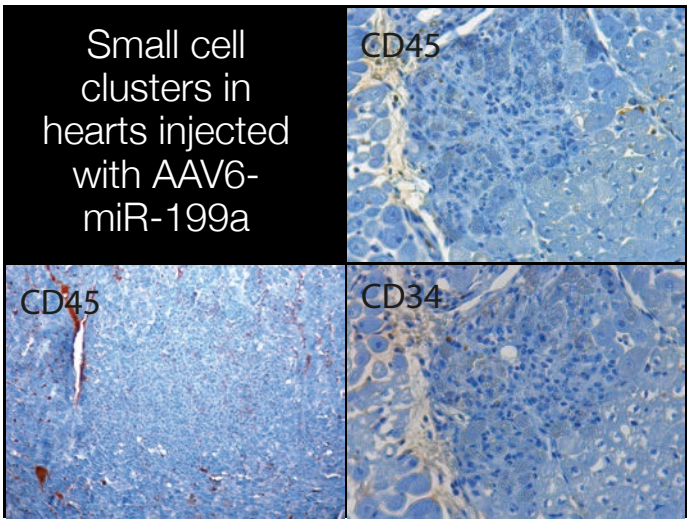
Small cell clusters in hearts injected with AAV6-miR-199a



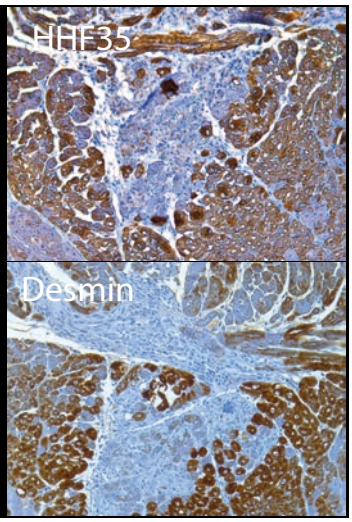
Small cell clusters in hearts injected with AAV6-miR-199a

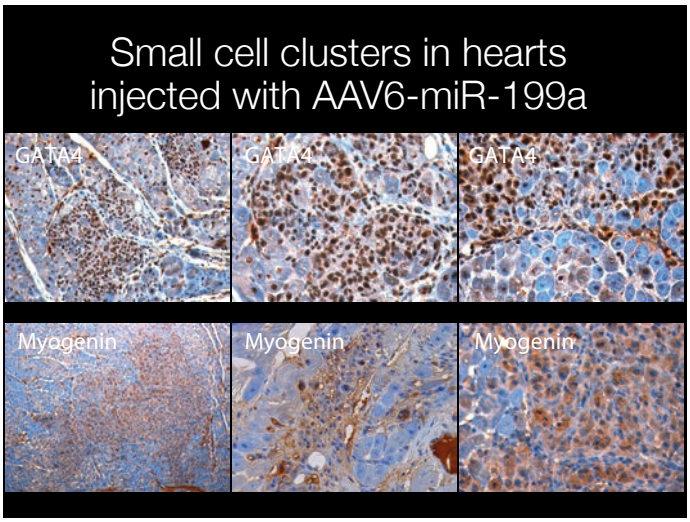


Small cell clusters in hearts injected with AAV6-miR-199a



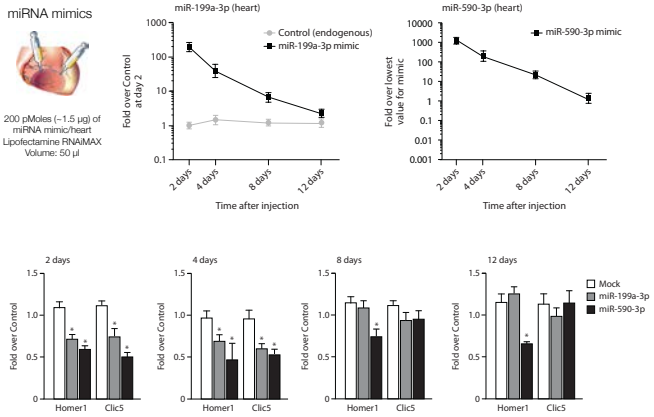
Small cell clusters in hearts injected with AAV6-miR-199a





Delivery?

Prolonged effect of miRNA mimics after intracardiac injection



miRNA mimics stimulate myocardial repair after MI