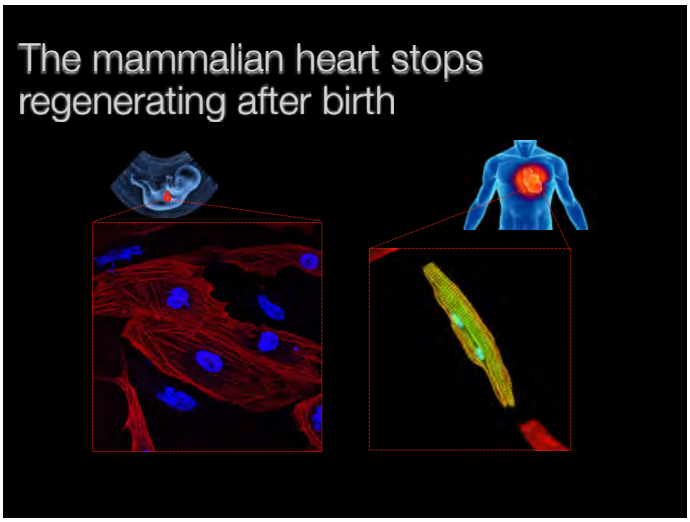
The mammalian heart stops regenerating after birth



The mammalian heart stops regenerating after birth

Mechanism?

- hyperoxic shock?
- mechanical stretch?
- oxidative metabolism?
- lack of exposure to maternal factors (Tregs)?
- loss of angiogenic potential?

Zebrafish/Fetal mammals
- cardiomyocyte proliferation
- cardiac regeneration

- hypoxic environnement
- glycolytic metabolism
- low blood pressure

Adult mammals
- cardiomyocyte cycle arrest
- no cardiac regeneration

- oxygen rich environnement
- oxidative metabolism
- high blood pressure

Zacchigna et al., Cardiovas Res 2014
Puente et al, Cell 2015
Zacchigna et al., Nat Comm, 2018
Gabisonia et al., Nature 2019

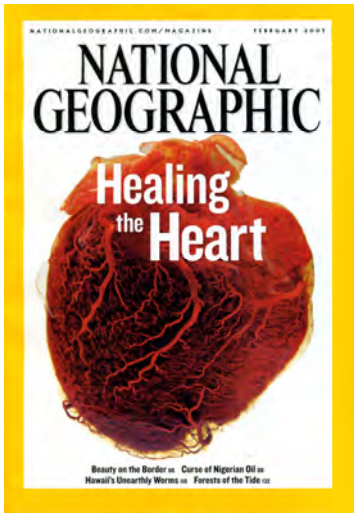
How to mend a broken heart (Bee Gees 1971)

Adult stem cells
- Bone marrow (?)
- Cardiac stem cells

ES cells
- From the embryo
- By cloning
- iPSCs

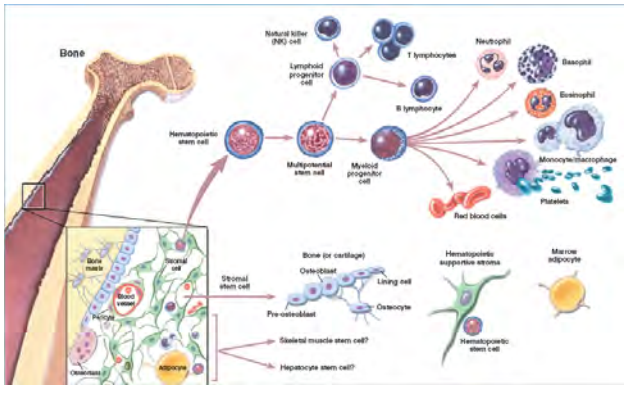
Transdifferentiation

Direct regeneration



"Stem cells" from the bone marrow

BM is a major source of adult stem cells



Neovascularization of ischemic myocardium by human bone-marrow–derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function

A.A. Kocher[1], M.D. Schuster[1], M.J. Szabolcs[2], S. Takuma[1], D. Burkhoff[2], J. Wang[1], S. Homma[2], N.M. Edwards[2] & S. Itescu[1,2]

Kocher AA., Nature Medicine, Apr. 2001

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‖, Ronald McKay‖, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

NATURE | VOL 410 | 5 APRIL 2001

Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells

Kathyjo A. Jackson,[1] Susan M. Majka,[1,2,3] Hongyu Wang,[1] Jennifer Pocius,[4] Craig J. Hartley,[4] Mark W. Majesky,[3,5] Mark L. Entman,[4] Lloyd H. Michael,[4] Karen K. Hirschi,[1,2,3] and Margaret A. Goodell[1]

The Journal of Clinical Investigation | June 2001 | Volume 107 | Number 11

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

** Department of Medicine, New York Medical College, Valhalla, New York 10595, USA*
† Hematopoiesis Section, Genetics and Molecular Biology Branch, NHGRI, and ‡ Laboratory of Molecular Biology, NINDS, NIH, Bethesda, Maryland 20892, USA

Myocardial infarction leads to loss of tissue and impairment of cardiac performance. The remaining myocytes are unable to reconstitute the necrotic tissue, and the post-infarcted heart deteriorates with time[1]. Injury to a target organ is sensed by distant stem cells, which migrate to the site of damage and undergo alternate stem cell differentiation[2–5]; these events promote structural and functional repair[6–8]. This high degree of stem cell plasticity prompted us to test whether dead myocardium could be restored by transplanting bone marrow cells in infarcted mice. We sorted lineage-negative (Lin^-) bone marrow cells from transgenic mice expressing enhanced green fluorescent protein[9] by fluorescence-activated cell sorting on the basis of *c-kit* expression[10]. Shortly after coronary ligation, Lin^- $c\text{-}kit^{POS}$ cells were injected in the contracting wall bordering the infarct. Here we report that newly formed myocardium occupied 68% of the infarcted portion of the ventricle 9 days after transplanting the bone marrow cells. The developing tissue comprised proliferating myocytes and vascular structures. Our studies indicate that locally delivered bone marrow cells can generate *de novo* myocardium, ameliorating the outcome of coronary artery disease.

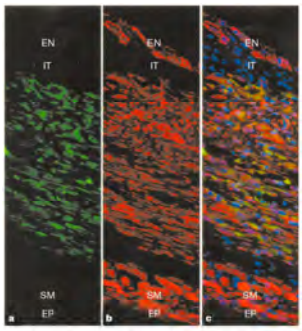
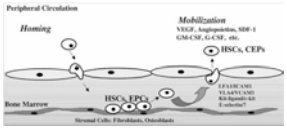


Figure 2 Myocardial infarct injected with Lin^- $c\text{-}kit^{POS}$ cells: myocardium is regenerating from endocardium (EN) to epicardium (EP). **a**, EGFP (green). **b**, cardiac myosin (red). **c**, combination of EGFP and myosin (red–green), and propidium-iodide-stained nuclei (blue). Infarcted tissue (IT) can be seen in the subendocardium, spared myocytes (SM) can be seen in the subepicardium. Original magnification, ×250 (**a**–**c**).

Role of EPCs in adult revascularization



Mobilization

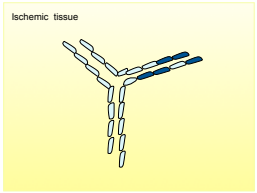
Vascular injury
Organ regeneration
Tumor growth

Endothelial progenitor cells (EPCs)
Origin: Bone Marrow

Circulating endothelial progenitors
Origin: Peripheral Blood

Contribute to neoangiogenesis in:

- Wound healing
- Limb ischemia
- Postmyocardial infarction
- Endothelization of vascular grafts
- Atherosclerosis
- Retinal neovascularization
- Vascularization during tumor growth

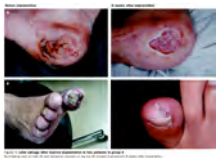


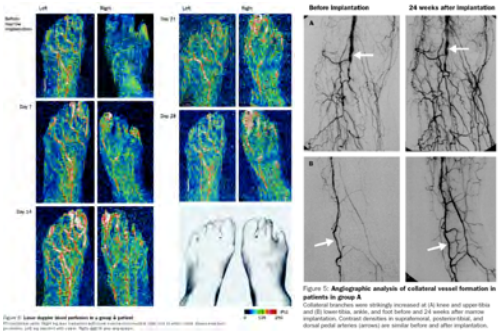
Articles

Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial

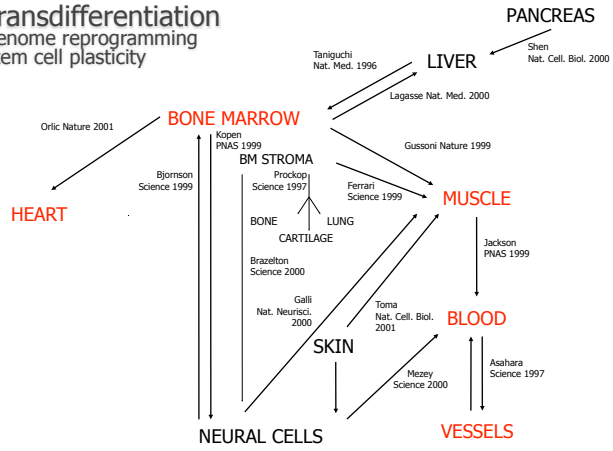
Eriko Tateishi-Yuyama, Hiroaki Matsubara, Toyoaki Murohara, Uichi Ikeda, Satoshi Shintani, Hiroya Masaki, Katsuya Amano, Yuji Kishimoto, Kohji Yoshimoto, Hidetoshi Akashi, Kazuyuki Shimada, Toshiji Iwasaka, Tsutomu Imaizumi, for the Therapeutic Angiogenesis using Cell Transplantation (TACT) Study Investigators*

THE LANCET • Vol 360 • August 10, 2002





Transdifferentiation
Genome reprogramming
Stem cell plasticity



The evolving concept of a stem cell: entity or function?

"…rather then referring to a discrete cellular entity, a stem cell most accurately refers to a biological function that can be induced in many distinct types of cells, even differentiated cells."

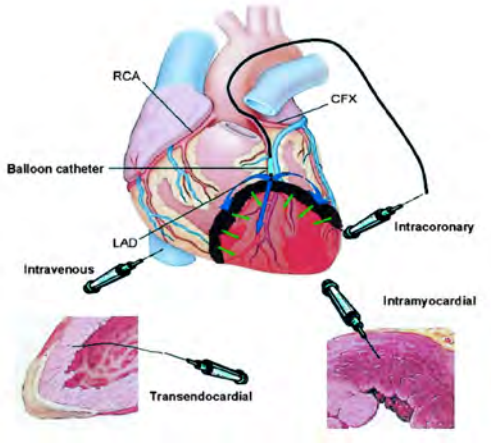
H. Blau. Cell, 2001

Selected Cell-Therapy Trials

Coordinating center	Condition	Subjects	Status
University of Düsseldorf	heart attack	60	completed
University of Frankfurt	heart failure	200	ongoing
University Clinic, Hannover	heart attack	60	ongoing
Hôpital Européen Georges Pompidou	heart attack	300	ongoing
Seoul National University Hospital	heart attack	11	suspended
St. Elizabeth's Medical Center, Boston	blocked arteries	24	ongoing
BioHeart Inc., Weston, Florida	heart failure	15	ongoing
Texas Heart Institute, Houston	blocked arteries/ heart failure	30	ongoing

9 APRIL 2004 VOL 304 SCIENCE

Different ways for BMCs transplantation into the heart



The NOGA system for transmyocardial injection

An injection catheter is 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)

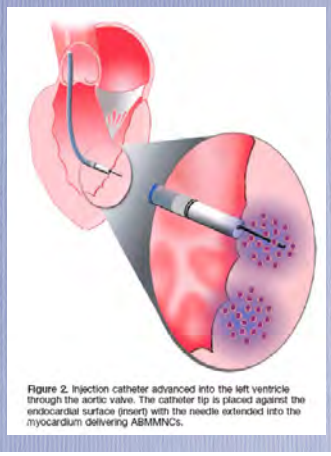
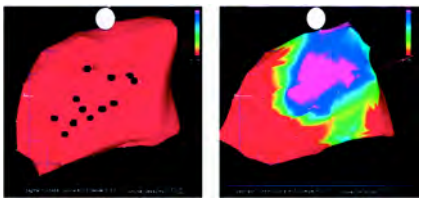


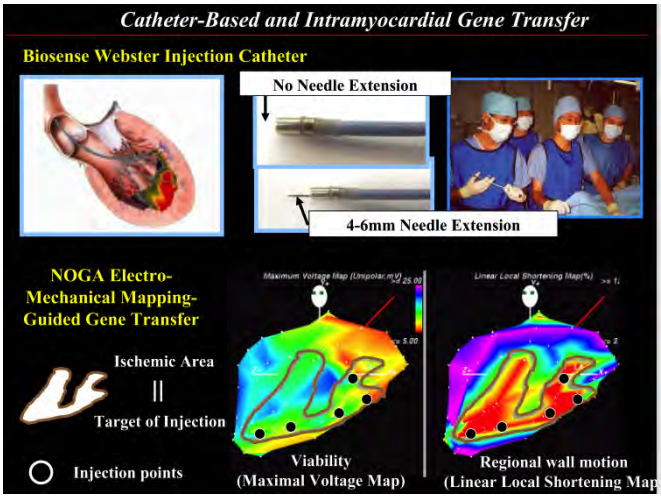
Figure 2. Injection catheter advanced into the left ventricle through the aortic valve. The catheter tip is placed against the endocardial surface (insert) with the needle extended into the myocardium delivering ABMMNCs.

The NOGA system for transmyocardial injection

An injection catheter is 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)

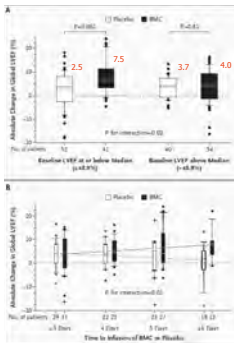


Left, electromechanical linear local shortening map from a stem cell injection procedure. The red color represents low contractility (severe cardiomyopathy). The black dots are injection sites. Right, similar map at 4 month follow-up, showing dramatic improvement in contractility at the site of prior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



REPAIR-AMI

Intracoronary Bone Marrow–Derived Progenitor Cells in Acute Myocardial Infarction



Randomized, placebo controlled, multicentric trial of intracoronary infusion of BMC after successful PCI for acute myocardial infarction

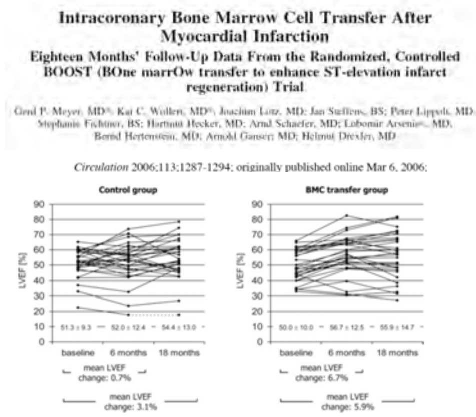
At 4 months, the absolute improvement in the global left ventricular ejection fraction (LVEF) was significantly greater in the BMC group than in the placebo group
mean increase: 5.5±7.3% vs. 3.0±6.5%; P = 0.01

Significant inverse relation between the baseline LVEF and the absolute change in LVEF at 4 months in the BMC group

TOPCARE-CHD

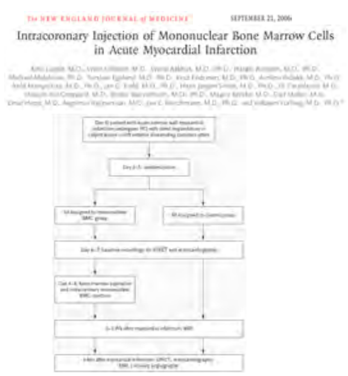
Transcoronary Transplantation of Progenitor Cells after Myocardial Infarction

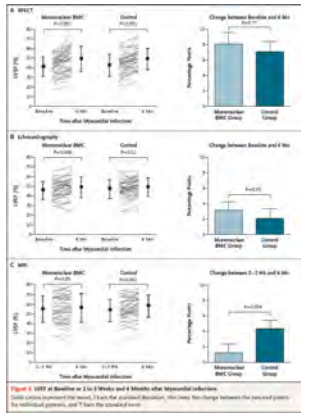
BOOST



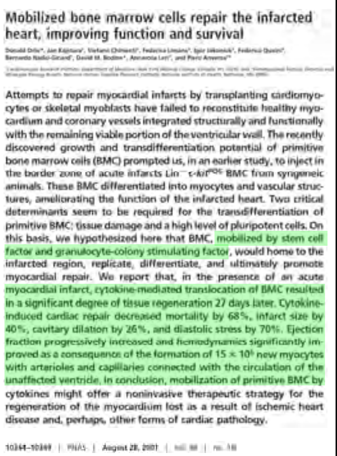
The relative improvement in LVEF after infusion of BMC at 6 months was no longer significant at 18 months
The main effect was an acceleration of recovery

ASTAMI

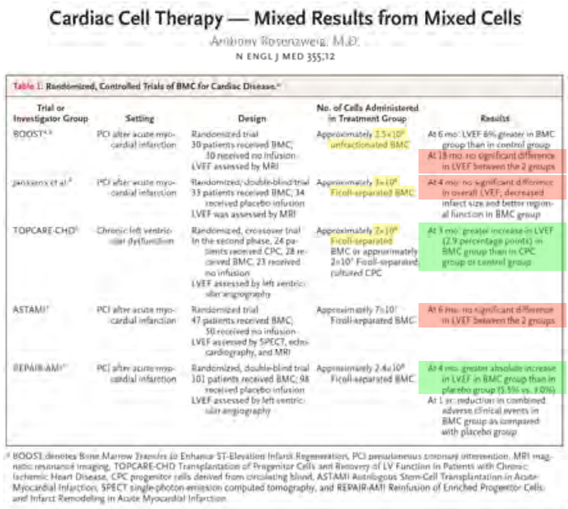




No improved LVEF, no reduction of left ventricular end-diastolic volume or infarct size at 6 months;
the study was powered to have an 80% chance of detecting a change of 5% in LVEF (smaller effects might have been missed)



STEMMI



Cardiac Cell Therapy — Mixed Results from Mixed Cells

Anthony Rosenzweig, M.D.

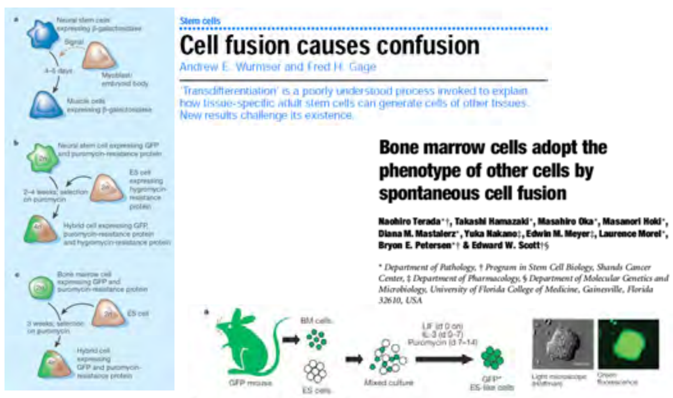
N ENGL J MED 355;12

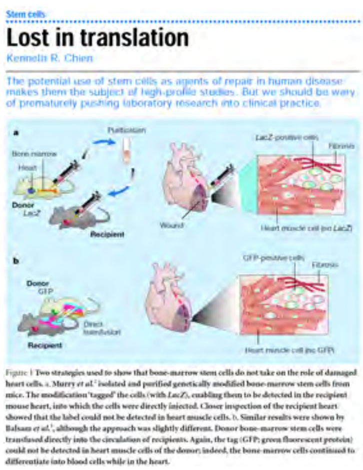
Table 1. Randomized, Controlled Trials of BMC for Cardiac Disease.*

Trial or Investigator Group	Setting	Design	No. of Cells Administered in Treatment Group	Results
BOOST[4,5]	PCI after acute myocardial infarction	Randomized trial; 30 patients received BMC, 30 received no infusion; LVEF assessed by MRI	Approximately 2.5×10^9 unfractionated BMC	At 6 mo: LVEF 6% greater in BMC group than in control group. At 18 mo: no significant difference in LVEF between the 2 groups
Janssens et al.[6]	PCI after acute myocardial infarction	Randomized, double-blind trial; 33 patients received BMC, 34 received placebo infusion; LVEF was assessed by MRI	Approximately 3×10^8 Ficoll-separated BMC	At 4 mo: no significant difference in overall LVEF, decreased infarct size and better regional function in BMC group
TOPCARE-CHD[1]	Chronic left ventricular dysfunction	Randomized, crossover trial; In the second phase, 24 patients received CPC, 28 received BMC, 23 received no infusion; LVEF assessed by left ventricular angiography	Approximately 2×10^8 Ficoll-separated BMC or approximately 2×10^7 Ficoll-separated cultured CPC	At 3 mo: greater increase in LVEF (2.9 percentage points) in BMC group than in CPC group or control group
ASTAMI[7]	PCI after acute myocardial infarction	Randomized trial; 47 patients received BMC, 50 received no infusion; LVEF assessed by SPECT, echocardiography, and MRI	Approximately 7×10^7 Ficoll-separated BMC	At 6 mo: no significant difference in LVEF between the 2 groups
REPAIR-AMI[2]	PCI after acute myocardial infarction	Randomized, double-blind trial; 101 patients received BMC, 98 received placebo infusion; LVEF assessed by left ventricular angiography	Approximately 2.4×10^8 Ficoll-separated BMC	At 4 mo: greater absolute increase in LVEF in BMC group than in placebo group (5.5% vs. 3.0%). At 1 yr: reduction in combined adverse clinical events in BMC group as compared with placebo group

* BOOST denotes Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration, PCI percutaneous coronary intervention, MRI magnetic resonance imaging, TOPCARE-CHD Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Ischemic Heart Disease, CPC progenitor cells derived from circulating blood, ASTAMI Autologous Stem-Cell Transplantation in Acute Myocardial Infarction, SPECT single-photon-emission computed tomography, and REPAIR-AMI Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction.

Is it true plasticity? It might be, but there are other possibilities…



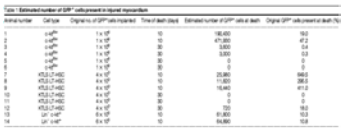


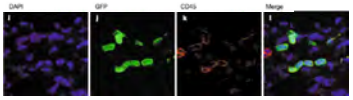
NATURE | doi:10.1038/nature02460 | www.nature.com/nature

Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium

Leora B. Balsam[1], Amy J. Wagers[2,3], Julie L. Christensen[2,3], Theo Kofidis[1], Irving L. Weissman[2,3] & Robert C. Robbins[1]

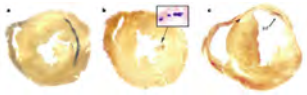
NATURE | VOL 428 | 8 APRIL 2004 | www.nature.com/nature



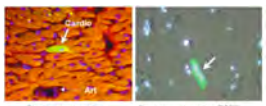


Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts

Charles E. Murry[1], Mark H. Soonpaa[2], Hans Reinecke[1], Hidehiro Nakajima[2], Hisako O. Nakajima[2], Michael Rubart[2], Kishore B. S. Pasumarthi[2+], Jitka Ismail Virag[1], Stephen H. Bartelmez[3], Veronica Poppa[1], Gillian Bradford[2], Joshua D. Dowell[2], David A. Williams[2+] & Loren J. Field[2]



Transgenic mice in which the cardiac-specific MHC promoter drives the expression of a nuclear beta-gal

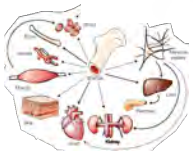


A rare GFP cardiomyocyte in the peri-infarct region, after BMT (MHC staining) and a single rod-shaped enzymatically dispersed cardiomyocyte

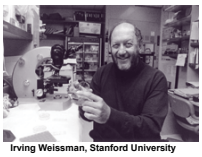
Long-standing biological dogma—that a cell, once committed, can't alter its fate—has been challenged by recent research. But now scientists are taking a more critical look

Plasticity: Time for A Reappraisal?

21 JUNE 2002 VOL 296 SCIENCE



Voice of caution



Irving Weissman, Stanford University

Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells

Amy J. Wagers,* Richard I. Sherwood, Julie L. Christensen, Irving L. Weissman

27 SEPTEMBER 2002 VOL 297 SCIENCE

Frequency of reconstitution (reconstituted mice/total)	
5 weeks	14 weeks
7/22 (32%) L + M 5/22 (23%) L only	4/22 (18%) BTM 1/22 (5%) BT 2/22 (9%) B only
Average reconstitution (% GFP+ PB leukocytes)	
17.6% (range: 0.12–77.6%)	20.2% (range: 0.03–71.6%)

Tissue	No. of sections examined	~No. of cells examined	No. of GFP+ nonhematopoietic cells
Brain	60	13,200,000	1
Liver	18	470,000	7
Kidney	24	990,000	0
Gut	24	360,000	0
Skeletal muscle	23	2,355	0
Cardiac muscle	14	4,346	0
Lung	12	23,000	0

Adult Bone Marrow–Derived Cells for Cardiac Repair

A Systematic Review and Meta-analysis

ARCH INTERN MED/ VOL 167, MAY 28, 2007

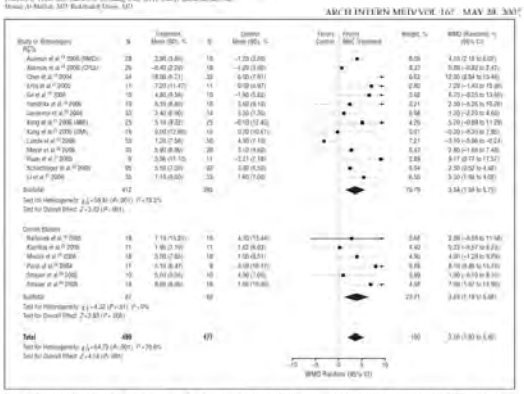


Figure 2. Forest plot of unadjusted difference in mean (with 95% confidence intervals [CIs]) improvement in left ventricular ejection fraction (LVEF) in patients treated with bone marrow–derived cells (BMCs) compared with controls. The figure shows the summary of cohort studies and randomized controlled trials (RCTs). Transplantation with BMCs resulted in a 2.66% (95% CI, 1.93% to 3.40%) increase in mean LVEF. The overall effect was statistically significant in favor of BMC therapy. AMI indicates acute myocardial infarction; CPCs, circulating progenitor cells; CMI, old myocardial infarction; and WMD, weighted mean difference.

No proof of transdifferentation or myocardial regeneration in human trials !

Only 1.3-2.6% of infused bone marrow cells are retained in the heart

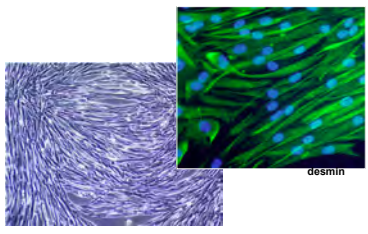
Paracrine effect?

Stem cell therapy

Muscle stem cells

Myoblasts

Skeletal mononucleated unipotent progenitor cells that can be expanded in vitro



desmin

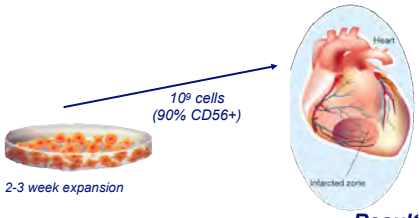
Animal myoblasts form myotubes in the myocardium and mature to become well formed myofibers with a contractile apparatus, with a significant functional improvement in damaged hearts

Long-Term Efficacy of Myoblast Transplantation on Regional Structure and Function After Myocardial Infarction

Said Ghostine, MD; Claire Carrion, MSc; Luiz Cesar Guarita Souza, MD; Pascal Richard, MD; Patrick Bruneval, MD; Jean-Thomas Vilquin, PhD; Bruno Pouzet, MD; Kerry Schwartz, PhD; Philippe Menasche, MD, PhD; Albert Alain Hagege, MD, PhD

(*Circulation*. 2002;106[suppl I]:I-131-I-136.)

Early results of autologous skeletal myoblasts transplantation in patients with severe ischemic heart failure



10^9 cells (90% CD56+)

Multiple sites injections within and at the borders of the scar

2-3 week expansion

Muscle biopsy

5 patients (39-72 years)
– severe impairment of LV function (LVEF < 34%)
– akinetic and metabolically nonviable (PET)
– indication for CABG in remote, viable and ischemic areas

Results:

- *1 early post-operative death*
- *4 patients with uneventful post-operative course*
- *follow-up 2-10 months:*

symptomatic improvement
13% increase in EF
new systolic thickening of the grafted scar
new-onset metabolic viability (PET e NMR)

Menasche' P. (Paris, France)

BIOHEART

Howard Leonhardt founded Bioheart in 1999 around a process, Myocell, which involves biopsying a patient's thigh muscle to obtain skeletal myoblasts, culturing them and expanding them over a course of about four weeks, then injecting them back into the heart using a percutaneous injection catheter

Why myoblasts?

- They differentiate into muscle cells capable of active contraction
- They can survive in ischemic scar tissue better than other types of cells
- Contact inhibition prevents them from over-proliferation

Preliminary results:
1st patient implanted in 2001
15 patients enrolled in Phase I/II study in 2002, with 6 month completed follow-up
20% average improvement by injecting 150 million cells

BIOHEART PRODUCT PIPELINE

Product	Indication	Status/Phase: Research	Pre-Clinical	Clinical: I	II	III	Comments
MyoCell	Myocardial Infarction & congestive heart failure						Phase I/II. Human clinical trials in Europe. In phase I Clinical Trials in U.S.
SR-200	Endoventricular cell & drug delivery device						In Phase I/II Clinical Trials in Europe
MyoCell VT	Ventricular Tachycardia						Modified myoblasts to treat VT conditions. Pre-clinical development. Dr. Charles Murray, University of Washington.
BioPace	Atrial Arrythmia						Pre-clinical development. Dr. Randall Lee, UCSF

genzyme

A surgical approach (500,000 CABG per year represent a significant market)

" Don't tell us you have improved ejection fraction and wall motion. Show us a reduction in major adverse coronary events"

" I do not want to get to the end of the trial with uninterpretable data that do not tell me whether or not I am producing a clinical benefit that anybody would find valuable to pay for"

Duke Collier, executive vice president

A phase II multinational trial started, recruiting 300 randomized patients that have conformed scar following a myocardial infarction (the largest trial to date).
3 arms :

- placebo
- 600 million cells
- 800 million cells

Table 1

Reference	Adjunct	Source	Patients (n)	Cell count	Purity	Sites injected	Results	Complications
Menasche et al.	CABG	Autologous	1	800×10^6	63% CD56+	33	Stabilized in NYHA class II LVEF improved to 30% Improved segmental contractility and perfusion	None
Menasche et al.	CABG	Autologous	10	871×10^6	86±13% (range 67–97%)	37±3 (range 27–37)	LVEF increase (from 23.8±4.9 to 32.1±7.9%) New-onset echocardiographic systolic shortening Improvement in NYHA class (2.7±0.2 to 1.6±0.1)	4 patients with VT
Chachques et al.	CABG	Autologous	5 extended to 18	$300\pm20\times10^6$	62±5%	6±2	Improved regional fractional shortening (9±3 to 20±3%) reduced scar size Improvement in NYHA class	None
Siminiak et al.	CABG	Autologous	1	1×10^8	—	8	Increase in segmental contractility seen on echocardiography	1 episode of sustained VT
Siminiak et al.	CABG	Autologous	10	2×10^7	—	—	No peri operative complications. Improved segmental contractility	Sustained VT in 2 patients, 1 death unrelated to cell transplantation
Nabil et al.	CABG	Autologous	11	$10–300\times10^6$	61–96% CD56+	3–10	Improved LVEF from 21 to 29% MRI and PET scan showed evidence of viability	None
Sim et al.	CABG on beating heart	Autologous	1	3.7×10^8	>98% desmin positive	20	Improved cardiac function on echocardiography Reduction of perfusion defect from 30 to 22% Improved LVEF from 30 to 37% at 6 months	None
Pagani et al.	LVAD		5	300×10^6	81 to 97%	3 to 36	Myofibet staining for myosin heavy chain parallel to host myocardial fibers Increased blood vessel count (72±17 cells vs 229±24 cells, P<0.0001)	Atrial fibrillation (n=2), VT (n=2)
Law et al.	CABG	Allogeneic	2	1.1×10^8 and 1.2×10^8	>98%	18 and 19	Echocardiography showed 14.6 and 10.5% increases in LVEF with no focal hypokinetic regions ^{99m}Tc-tetrofosmine SPECT showed positive dynamics, reduced perfusion defects during exercise and rest	None
Zhang et al.	CABG	Autologous	9	—	—	30–40	Improved LVEF and left ventricular wall thickness on 2-D echocardiography Significant improvement on perfusion scan	Occasional arrhythmia during intensive care unit stay but not observed during the follow-up

CABG, coronary artery bypass grafting; LVAD, left ventricle assist device; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; PET, positron emission tomography; VT, ventricular tachycardia.

Long-Term Engraftment (16 Years) of Myoblasts in a Human Infarcted Heart

MARIE CRAHÈS,[a,b] MARIE-CÉCILE BORIES,[c] JEAN-THOMAS VILQUIN,[d] JEAN-PIERRE MAROLLEAU,[e] MICHEL DESNOS,[b,f] JÉRÔME LARGHERO,[g,h,i] GILLES SOULAT,[b,i] PATRICK BRUNEVAL,[a,b,f] ALBERT A. HAGÈGE,[b,f,k] PHILIPPE MENASCHÉ[b,c,f]

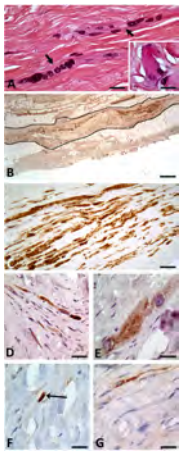
Key Words. Myoblasts • Heart failure • Clinical cell transplantation • Long-term follow-up

ABSTRACT

We report the case of a patient who had undergone injections of myoblasts in an infarct area 16 years before being referred for heart transplantation. The pathological examination of the explanted heart found persisting myotubes embedded in fibrosis. This finding supports the ability of myoblasts to survive in harsh environments, which can make them appealing candidates for transplantation in diseases requiring supply of new myogenic cells. STEM CELLS TRANSLATIONAL MEDICINE *2018;7:705–708*

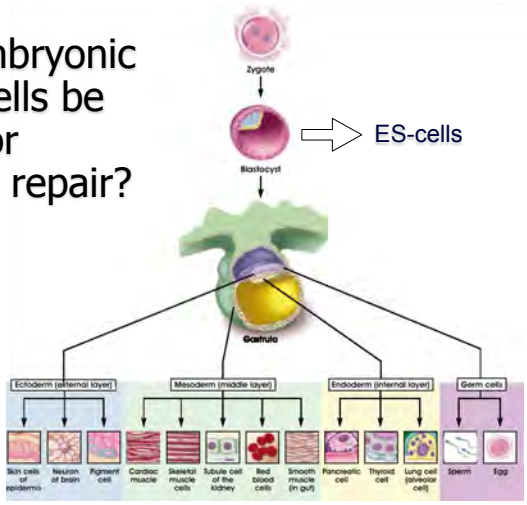
Received January 20, 2018; revised April 12, 2018; accepted for publication April 23, 2018; first published September 13, 2018.

http://dx.doi.org/10.1002/sctm.18-0017

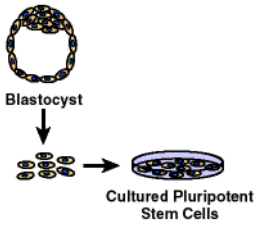


ES cells

Can embryonic stem cells be used for cardiac repair?



Establishment in culture of pluripotent cells from mouse embryos

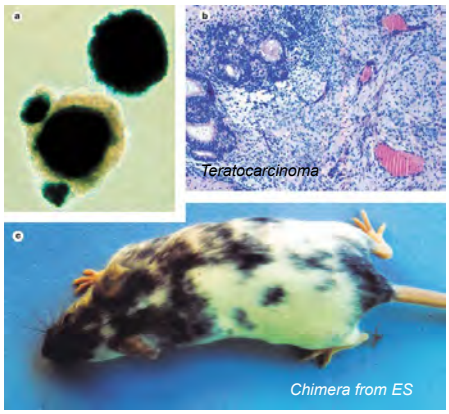


Normal caryotype!
Pluripotency!

Evans MJ and Kaufman MH (1981), Nature 292, 154-156

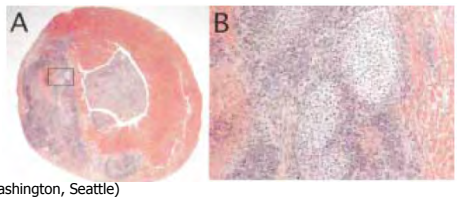
Pluripotency of mouse embryonic stem cells (ES)

Embryoid bodies



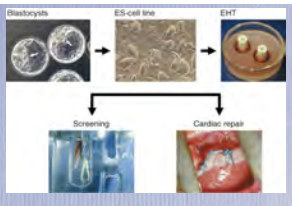
ES cells transplanted into the heart develop into a teratoma:

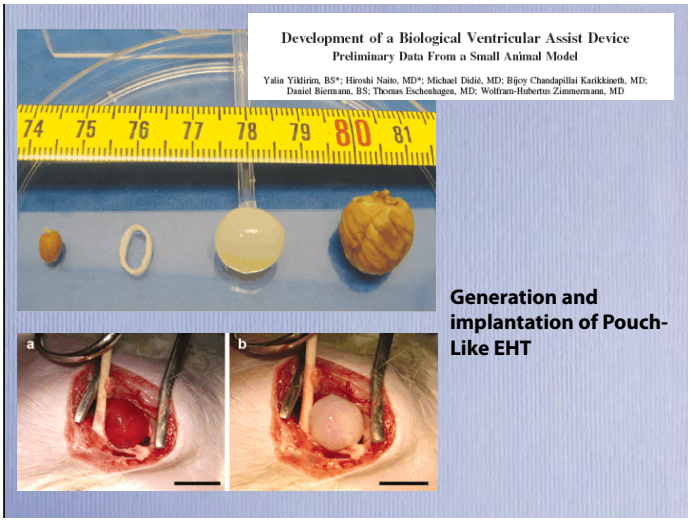
- extensive replacement of the ventricular wall and cavity with tumor, with central necrosis
- Multiple nodules of cartilage (mesodermal) at the interface with host myocardium
- Several poorly differentiated epithelial cells
- Gut epithelium and ciliated respiratory epithelium (endodermal)
- Stratified squamous epithelium (ectodermal)



C. Murry (University of Washington, Seattle)

Stem cell based tissue engineering for myocardial repair





Generation and implantation of Pouch-Like EHT

In Italia la materia è regolamentata dalla legge 40 del 2004

- Max 3 embrioni alla volta, tutti da impiantare
  - ridotta efficienza di gravidanza per ciclo ormonale, necessità di ricorrere a più cicli
  - impossibilità di ricavare nuove linee ES
- Divieto di utilizzo degli embrioni sovrannumerari prodotti in passato (circa 30,000 embrioni intoccabili in Italia)
  - però è possibile usare cellule ES ottenute in altri paesi

Giugno 2005: 4 referendum per abrogare parte della L40

90% dei votanti a favore dell'abrogazione ma solo 26% di affluenza ai seggi

Il problema principale legato alle cellule staminali embrionali non e' pero' di natura scientifica ma
di natura metafisica ed e' legato al concetto di inizio della vita umana

Are we destroying life?

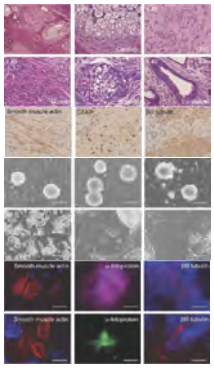
- The potential to treat so many diseases far outweighs the 'cost' of having to destroy these embryos.
- An embryo is not life itself, it just has the potential to become one.
- Blastocysts are just a collection of cells, which have not differentiated into specific types. Making them about as 'human' as skin cells.
- It is estimated that about 18% of zygotes do not implant after conception, making this number far more than the number of embryos used for stem cell research.
- It must be noted that that only unused embryos are used for stem cell research.

Can ES cells be obtained without egg fertilization?

Figure 2 **Cloned frogs. These 19 identical male albino frogs were prepared by nuclear transplantation into unfertilized eggs of the dark green female frog[33]. (Male frogs are about half the size of females.)**

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, **Oct3/4, Sox2, c-Myc**, and **Klf4** in the FBX15 locus, under ES cell culture conditions.



Various tissues present in teratomas derived from iPS

Neural tissues and muscles in teratomas

In vitro embryoid body formation and differentiation

In vitro differentiation into all three germ layers.

These cells, which were designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes.

1- Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers.

2- Following injection into blastocysts, iPS cells contributed to mouse embryonic development, but embryos failed to develop beyond mid-gestation stage.

Derivation and cardiomyocyte differentiation of induced pluripotent stem cells from heart failure patients

Limor Zwi-Dantsis[1,2], Irit Huber[1], Marihal Habib[1], Aaron Winterstern[1], Amira Gepstein[1], Gil Arbel[1] and Lior Gepstein[1,2,*]

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Received April 4, 2011.
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Accepted March 22, 2012.

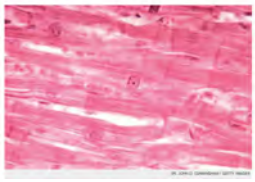
Abstract

Aims Myocardial cell replacement therapies are hampered by a paucity of sources for human cardiomyocytes and by the expected immune rejection of allogeneic cell grafts. The ability to derive patient-specific human-induced pluripotent stem cells (hiPSCs) may provide a solution to these challenges. We aimed to derive hiPSCs from heart failure (HF) patients, to induce their cardiomyocyte differentiation, to characterize the generated hiPSC-derived cardiomyocytes (hiPSC-CMs), and to evaluate their ability to integrate with pre-existing cardiac tissue.

Methods and results Dermal fibroblasts from two HF patients were reprogrammed by retroviral delivery of *Oct4, Sox2*, and *Klf4* or by using an excisable polycistronic lentiviral vector. The resulting HF-hiPSCs displayed adequate reprogramming properties and could be induced to differentiate into cardiomyocytes with the same efficiency as control hiPSCs (derived from human foreskin fibroblasts). Gene expression and immunostaining studies confirmed the cardiomyocyte phenotype of the differentiating HF-hiPSC-CMs. Multi-electrode array recordings revealed the development of a functional cardiac syncytium and adequate chronotropic responses to adrenergic and cholinergic stimulation. Next, functional integration and synchronized electrical activities were demonstrated between hiPSC-CMs and neonatal rat cardiomyocytes in co-culture studies. Finally, *in vivo* transplantation studies in the rat heart revealed the ability of the HF-hiPSC-CMs to engraft, survive, and structurally integrate with host cardiomyocytes.

HEART DISEASE

Scientists Turn Human Skin Cells Into Healthy Heart Cells



theguardian

News | Sport | Comment | Culture | Business | Money | London 201

News › Science › Stem cells

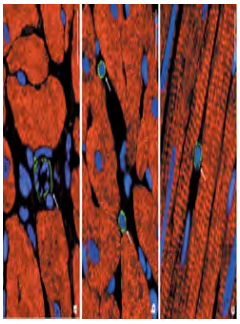
Skin from heart attack patients transformed into beating heart cells

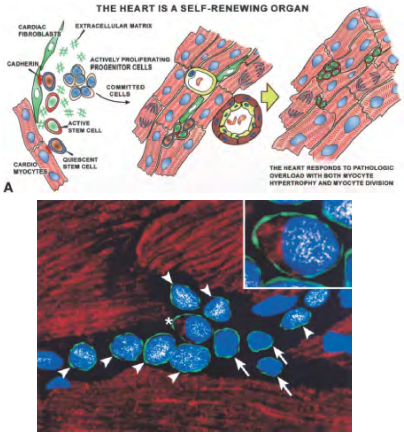
The heart cells created from patients' skin were at the same stage of development as those of a newborn baby

Ian Sample, science correspondent
guardian.co.uk, Wednesday 23 May 2012 00.06 BST
Comments (36)

Does the heart contain resident stem cells?

Cardiac stem cells (CSCs): do they exist?

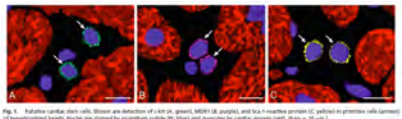


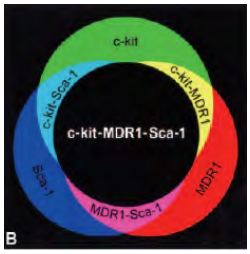


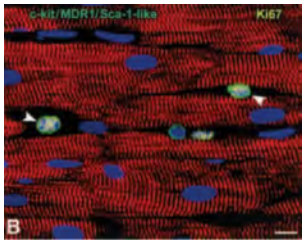
Adult cardiac stem cells are multipotent and support myocardial regeneration.
Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P.

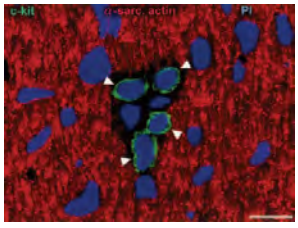
Life and Death of Cardiac Stem Cells
A Paradigm Shift in Cardiac Biology

Piero Anversa, MD; Jan Kajstura, PhD; Annarosa Leri, MD; Roberto Bolli, MD
Circulation March 21, 2006

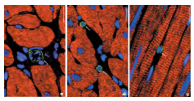








Cardiac stem cells: do they exist?

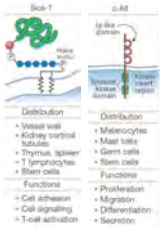


Supplementary Table 2. Comparison of isl1⁺ cardioblasts, cardiac sca-1⁺ cells and cardiac side population (SP) cells.

	isl1⁺ cardioblasts	cardiac sca-1⁺ cells[1]	cardiac SP cells[2]
1. Hoechst 33342 dye efflux	Hoechst dye excluding cells: 4.5%	Hoechst dye excluding cells: 3.6%	Hoechst dye excluding cells: 100%
2. Marker expression	sca1 negative CD31 negative c-kit negative Nkx2.5 positive GATA4 positive myocytic marker negative	sca1 positive CD31 positive c-kit negative Nkx2.5 negative GATA4 positive myocytic marker negative	sca1 positive CD31 negative c-kit positive (low) Nkx2.5 negative GATA4 negative myocytic marker negative
3. *in vivo* localization	• outflow tract • free wall of atria • intra-atrial septum • conus muscle • right ventricle	• adjacent to basal lamina • no preferred heart region	• not determined
4. Progenitor identity determined by lineage tracing	• Isl1 identifies cardiac progenitor cells • established embryonic lineage marker for the heart	• sca-1 surface marker used for cell purification • no cardiac lineage marker	• Abcg2 activity used for Hoechst dye efflux • no cardiac lineage marke r
5. Myocytic differentiation *in vitro*	α-actinin expression with sarcomeric structure : 22% cardiac troponin T : 25%	α-actin expression without sarcomeric structure : 4.6% cardiac troponin I : 2.8%	α-actinin expression without sarcomeric structure : % not determined
6. Myocytic differentiation *in vivo* after cell transplantation	not determined	ischemia/reperfusion injury: ~1.5% differentiation ~1.5% cell fusion	not determined
7. Functional evaluation of *in vitro* differentiated cells	• Ca^{2+} transients • EC coupling • β-adrenergic response • action potentials	not determined	not determined

[1]Oh et al. Cardiac progenitor cells from adult myocardium : Homing, differentiation, and fusion after infarction. *Proc. Natl. Acad. Sci. (USA)*: **100**, 12313-12318 (2003)
[2]Martin et al. Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev. Biol.*: **265**, 262-275 (2004)

Laugwitz, Nature 2005



Resident cardiac stem cells

c-Kit+ cells (Anversa)
Sca-1 cells (Schneider)
Side population cells (Liao)
Islet-1 cells (Chien)
Cardiosphere-forming cells (Messina/Marban)
SSea-4+ cells (Taylor)

One of the least regenerative organ in the body has multiple non-overlapping populations of cardiomyocyte progenitors??

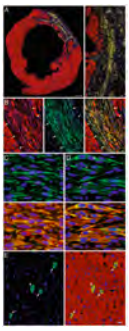
Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function

Buddhadeb Dawn*, Adam B. Stein*, Konrad Urbanek†, Marcello Rota†, Brian Whang†, Raffaella Rastaldo†, Daniele Torella†, Xian-Liang Tang*, Arash Rezazadeh*, Jan Kajstura†, Annarosa Leri†, Greg Hunt*, Jai Varma*, Sumanth D. Prabhu*, Piero Anversa†, and Roberto Bolli*‡

GFP-labeled CSCs delivered to the coronary arteries 4 hr after ischemia-reperfusion

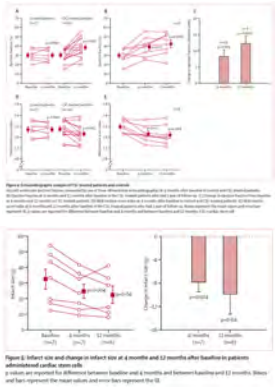
Ventricular function monitored by echocardiography

Myocardial regeneration by histology



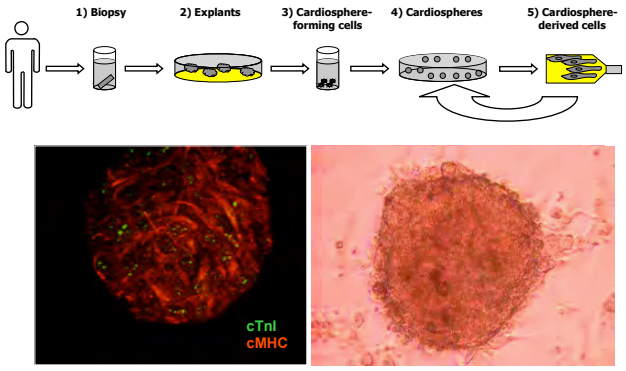
3766–3771 | PNAS | March 8, 2005 | vol. 102 | no. 10

Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial



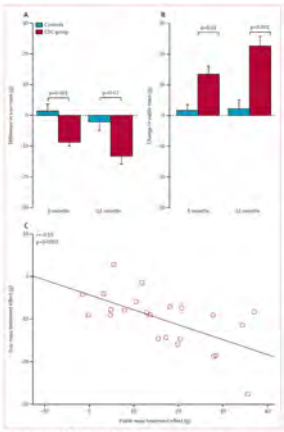
www.thelancet.com Vol 378 November 26, 2011

Cardiospheres



L. Barile

Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial



www.thelancet.com Published online February 14, 2012 DOI:10.1016/S0140-6736(12)60195-0

Harvard and the Brigham call for more than 30 retractions of cardiac stem cell research

STATNEWS, OCTOBER 14, 2018

Harvard Medical School and Brigham and Women's Hospital have recommended that **31 papers** from a former lab director be **retracted from medical journals.**

The papers from the lab of Dr. **Piero Anversa**, who studied cardiac stem cells, "included **falsified and/or fabricated data**"

Anversa has previously corrected 8 of his papers, many for failures to disclose conflicts of interest. He "practically invented the field of cardiac stem cell therapy when he first reported that cardiac cells were capable of regeneration," Cardiobrief and MedPage Today wrote about him last year.

Anversa's work was based on the idea that the heart contains stem cells that could regenerate cardiac muscle. He and his colleagues claimed that they had identified such cells, known as c-kit cells. When various research teams tried to reproduce the results, however, they failed. Scientists have tried to inject c-kit cells into damaged hearts, with mixed results at best.

"For 10 years, he ran everything," said Jeffery Molkentin, a researcher at Cincinnati Children's whose lab was among the first to question the basis of Anversa's results in a 2014 paper in Nature. "It really is a relief that this has been corrected. I think this is good for everybody."

"There are no stem cells in the heart. Quit trying to publish those results."

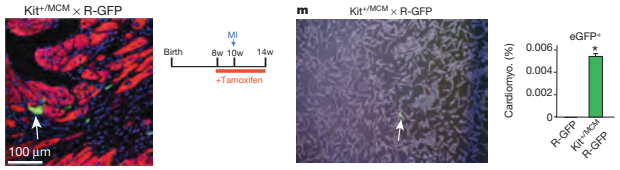
JEFFERY MOLKENTIN, CINCINNATI CHILDREN'S

Still, he said, a small number of researchers continue to publish findings that agree with Anversa's. "Maybe these 31 retractions will keep pushing the pendulum a little further to the right and these people will slowly start to back off even more," he said. "It's just discouraging when you see these papers keep popping up," Molkentin said. "There are no stem cells in the heart. Quit trying to publish those results."

c-kit$^+$ cells minimally contribute cardiomyocytes to the heart

Jop H. van Berlo[1,2]*, Onur Kanisicak[1]*, Marjorie Maillet[1], Ronald J. Vagnozzi[1], Jason Karch[1], Suh-Chin J. Lin[1], Ryan C. Middleton[3], Eduardo Marbán[3] & Jeffery D. Molkentin[1,4]

If and how the heart regenerates after an injury event is highly debated. c-kit–expressing cardiac progenitor cells have been reported as the primary source for generation of new myocardium after injury. Here we generated two genetic approaches in mice to examine whether endogenous c-kit$^+$ cells contribute differentiated cardiomyocytes to the heart during development, with ageing or after injury in adulthood. A complementary DNA encoding either Cre recombinase or a tamoxifen-inducible MerCreMer chimaeric protein was targeted to the *Kit* locus in mice and then bred with reporter lines to permanently mark cell lineage. Endogenous c-kit$^+$ cells did produce new cardiomyocytes within the heart, although at a percentage of approximately 0.03 or less, and if a preponderance towards cellular fusion is considered, the percentage falls to below approximately 0.008. By contrast, c-kit$^+$ cells amply generated cardiac endothelial cells. Thus, endogenous c-kit$^+$ cells can generate cardiomyocytes within the heart, although probably at a functionally insignificant level.



Anversa published at least 55 papers that listed Harvard as an affiliation. In 2014, a former **research fellow described an atmosphere of fear and information control in his lab**.
Anversa, who according to publications was most recently affiliated with the Cardiocentro Ticino and University of Zurich, could not be reached for comment. An email to his address at Cardiocentro Ticino bounced back. A number of Anversa's co-authors either did not immediately respond to a request for comment, or declined.

Anversa was born in Parma, Italy, in 1940 and received his medical degree from the University of Parma in 1965. He gained prominence as a stem-cell researcher at New York Medical College in Valhalla, N.Y., where he worked before moving to Harvard Medical School and the Brigham in 2007. Anversa became a full professor in 2010.

Throughout his career, Anversa has received several commendations, including a research achievement award from the American Heart Association, which in 2004 also named him a "distinguished scientist."
Although journals often act on retraction recommendations by universities, they do not always do so, and it sometimes takes a while. Journals retract roughly 1,400 scholarly papers each year, out of some 3 million total publications. Anversa's total would put him in the top 20 list of scientists with the most retractions in the world. The 10 scientists worldwide with the most retracted papers have at least 39, and in one case — Japanese anesthesiologist Yoshitaka Fujii — 183 such articles.
So what do the calls for retraction mean for cardiology?

"What seems obvious to me is a need for transparency," Yale cardiologist Dr. Harlan Krumholz told STAT and Retraction Watch. "The scientific community needs to know what was found, why papers were retracted, and what is recommended with regard to his work going forward. Also, what has happened to work that was based on his work. Without this knowledge it is hard to know what it means."

Braggadacio, information control, and fear: Life inside a Brigham stem cell lab under investigation

Regular readers of Retraction Watch will note the recent news regarding the work conducted in the laboratory of Piero Anversa at Brigham and Women's Hospital, a Harvard Medical School affiliate. In the early 2000s, his laboratory published a series of papers regarding the regenerative qualities of bone marrow-derived and cardiac-resident "stem cells."
Those initial findings, as well as the research conducted since those early studies, have always been surrounded by controversy, as many have been unsuccessful in efforts to replicate their results. Controversy among competitors is not uncommon in our profession, but this particular one has blossomed into a formal investigation of their findings, and has, to date, led to the retraction of one paper and an expression of concern about another.
The "Science"
I think that most scientists, perhaps with the exception of the most lucky or most dishonest, have personal experience with failure in science—experiments that are unreproducible, hypotheses that are fundamentally incorrect. Generally, we sigh, we alter hypotheses, we develop new methods, we move on. It is the data that should guide the science.
In the Anversa group, a model with much less intellectual flexibility was applied. The "Hypothesis" was that c-kit (cd117) positive cells in the heart (or bone marrow if you read their earlier studies) were cardiac progenitors that could: 1) repair a scarred heart post-myocardial infarction, and: 2) supply the cells necessary for cardiomyocyte turnover in the normal heart.
This central theme was that which supplied the lab with upwards of $50 million worth of public funding over a decade, a number which would be much higher if one considers collaborating labs that worked on related subjects.
In theory, this hypothesis would be elegant in its simplicity and amenable to testing in current model systems. In practice, all data that did not point to the "truth" of the hypothesis were considered wrong, and experiments which would definitively show if this hypothesis was incorrect were never performed (lineage tracing e.g.).
Further, controls that suggested that the data might be artifactual were ignored or not conducted. However, I challenge the readers to determine any of this information from the published manuscripts. So how does this slip through the cracks for years? The fault for this can likely be attributed to multiple sources although a conspicuous lack of stringency in the peer review process of the journals in which they were published come to mind.
Beyond the science, ironically, a certain braggadocio also existed surrounding this hypothesis. Anyone who attended the pertinent sessions at the American Heart Association Scientific Sessions could attest to this. In essence, to Dr. Anversa all investigators who questioned the hypothesis were"morons," a word he used frequently at lab meetings. For one within the group to dare question the central hypothesis, or the methods used to support it, was a quick ticket to dismissal from your position.
Information Segregation + Machiavellian Principles = Successful Lab
The day to day operation of the lab was conducted under a severe information embargo. The lab had Piero Anversa at the head with group leaders Annarosa Leri, Jan Kajstura and Marcello Rota immediately supervising experimentation. Below that was a group of around 25 instructors, research fellows, graduate students and technicians. Information flowed one way, which was up, and conversation between working groups was generally discouraged and often forbidden.
Raw data left one's hands, went to the immediate superior (one of the three named above) and the next time it was seen would be in a manuscript or grant. What happened to that data in the intervening period is unclear.
A side effect of this information embargo was the limitation of the average worker to determine what was really going on in a research project. It would also effectively limit the ability of an average worker to make allegations regarding specific data/experiments, a requirement for a formal investigation.
The general game plan of the lab was to use two methods to control the workforce: Reward those who would play along and create a general environment of fear for everyone else. The incentive was upward mobility within the lab should you stick to message. As ridiculous as it sounds to the average academic scientist, I was personally promised money and fame should I continue to perform the type of work they desired there. There was also the draw of financial security/job stability that comes with working in a very well-funded lab.
On the other hand, I am not overstating when I say that there was a pervasive feeling of fear in the laboratory. Although individually-tailored stated and unstated threats were present for lab members, the plight of many of us who were international fellows was especially harrowing. Many were technically and educationally underqualified compared to what might be considered average research fellows in the United States. Many also originated in Italy where Dr. Anversa continues to wield considerable influence over biomedical research.
This combination of being undesirable to many other labs should they leave their position due to lack of experience/training, dependent upon employment for U.S. visa status, and under constant threat of career suicide in your home country should you leave, was enough to make many people play along.
Even so, I witnessed several people question the findings during their time in the lab. These people and working groups were subsequently fired or resigned. I would like to note that this lab is not unique in this type of exploitative practice, but that does not make it ethically sound and certainly does not create an environment for creative, collaborative, or honest science.
Lessons Learned
So what, if anything, did I learn from spending a period of my life in my scientific nightmare? The conditions I have written about are not unique, although the particulars of how the misconduct happened may be. The simplest explanation is that, in spite of the efforts of ethical watchdogs, these are behaviors that science is selecting for with its current funding and publication mechanisms. I was glad to learn of the investigation regarding this lab but without vigilance and alterations to current structures, newer, more careful versions of Piero Anversa will undoubtedly move in to take his place.

BASIC SCIENCE

Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction

Romain Gallet[1,2†], James Dawkins[†], Jackelyn Valle[1], Eli Simsolo[1], Geoffrey de Couto[1], Ryan Middleton[1], Eleni Tseliou[1], Daniel Luthringer[1], Michelle Kreke[1,3], Rachel R. Smith[1], Linda Marbán[1,3], Bijan Ghaleb[2], and Eduardo Marbán[1*]

Direct regeneration

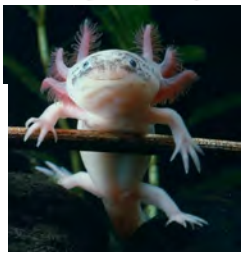
Regenerative responses in urodeles

Regeneration might be a primordial attribute of metazoan that has been lost subsequently for reasons that are not yet understood

An adult newt can regenerate:

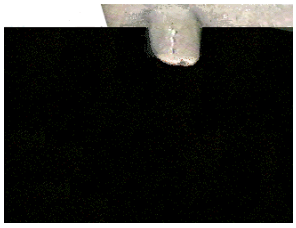
- **Jaws**
- **Lens**
- **Retina**
- **Large sections of the heart**
- **Limbs**
- **Tail**

Regenerative potential of *Ambystoma mexicanum* (Axolotl)



The axolotl does not heal by scarring and is capable of regenerating entire lost appendages in a period of months, and, in certain cases, more vital structures. Some have indeed been found restoring the less vital parts of their brains. They can also readily accept transplants from other individuals, including eyes and parts of the brain —restoring these alien organs to full functionality. In some cases, axolotls have been known to repair a damaged limb as well as regenerating an additional one, ending up with an extra appendage that makes them attractive to pet owners as a novelty.

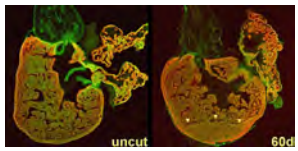
Regenerative potential of *Ambystoma mexicanum* (Axolotl)

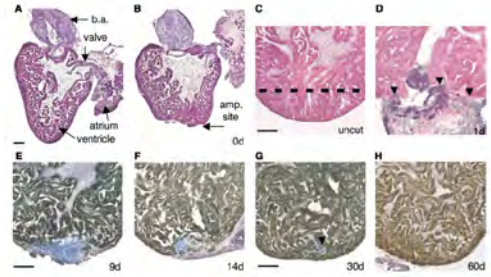


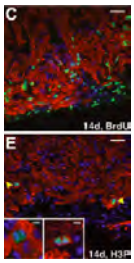
Several species other than mammals regenerate the heart

Heart Regeneration in Zebrafish

Kenneth D. Poss,* Lindsay G. Wilson, Mark T. Keating*



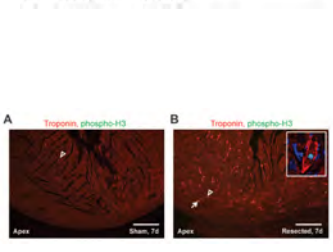


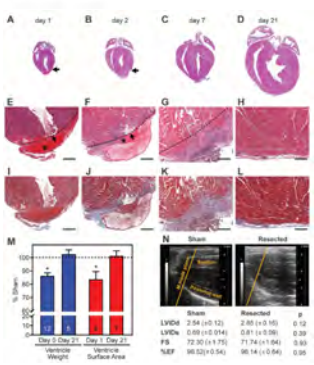


SCIENCE VOL 298 13 DECEMBER 2002

Transient Regenerative Potential of the Neonatal Mouse Heart

Enzo R. Porrello,[1] Ahmed I. Mahmoud,[2] Emma Simpson,[1] Joseph A. Hill,[1,2] James A. Richardson,[1,3] Eric N. Olson,[1]* Hesham A. Sadek[1]*





25 FEBRUARY 2011 VOL 331 SCIENCE www.sciencemag.org

New Hypotheses in Clinical Medicine

Functional Recovery of a Human Neonatal Heart After Severe Myocardial Infarction

Bernhard J. Haubner,* Johanna Schneider,* Ulrich Schweigmann, Thomas Schuetz, Wolfgang Dichtl, Corinna Velik-Salchner, Joerg-I. Stein, Josef M. Penninger

Rationale: **Cardiac remodeling and subsequent heart failure remain critical issues after myocardial infarction despite improved treatment and reperfusion strategies. Recently, cardiac regeneration has been demonstrated in fish and newborn mice after apex resection or cardiac infarctions. Two key issues remain to translate findings in model organisms to future therapies in humans: what is the mechanism and can cardiac regeneration indeed occur in newborn humans?**

Objective: **To assess whether human neonatal hearts can functionally recover after myocardial infarction.**

Methods and Results: **Here, we report the case of a newborn child having a severe myocardial infarction due to coronary artery occlusion. The child developed massive cardiac damage as defined by serum markers for cardiomyocyte cell death, electrocardiograms, echocardiography, and cardiac angiography. Remarkably, within weeks after the initial ischemic insult, we observed functional cardiac recovery, which translated into long-term normal heart function.**

Conclusions: **These data indicate that, similar to neonatal rodents, newborn humans might have the intrinsic capacity to repair myocardial damage and completely recover cardiac function. (*Circ Res.* 2016;118:216-221. DOI: 10.1161/CIRCRESAHA.115.307017.)**

Key Words: angiography ■ cell death ■ heart failure ■ myocardial infarction ■ regeneration

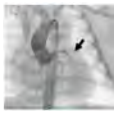
Clinical case

- Boy born at the end of 39th week, uneventful labor, umbilical arterial blood ok
- After birth severe cyanosis, reduced oxygen saturation
- ECG: signs of acute ischemia
- Echocardiography: severe LV dysfunction
- Increased BNP, Troponin T and CK
- Coronary angiography

LAD occlusion

Haubner et al., Circulation Research 2016

- Thrombolysis at 28 hours from first symptoms



LAD re-opening after 3 days

Persisting myocardial damage evident at echocardiography, ECG and blood markers

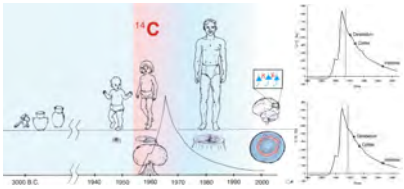
Diagnosis: LAD occlusion for >20 hours, massive MI

MCQ: Outcome of the patient?

1. Complete recovery at 45 days
2. Persisting signs of cardiac dysfunction at repeated follow-up
3. Heart failure at 1 year
4. Death at 2 months

Carbon dating to assess the physiological turnover of human cells

After the Second World War, tests of nuclear bombs spewed carbon-14 pollution into the atmosphere. This isotope was incorporated into plants and the people who consumed them. After above-ground tests were stopped in 1963, levels of the isotope started to fall. The ^{14}C in a cell's DNA corresponds to the amount of the isotope in the atmosphere at the time it was dividing, providing a way to date a cell's birth.

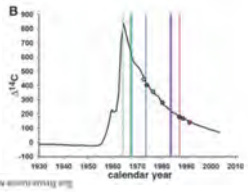


Evidence for Cardiomyocyte Renewal in Humans

Olaf Bergmann,[1,2]* Ratan D. Bhardwaj,[1]* Samuel Bernard,[3] Sofia Zdunek,[1] Fanny Barnabé-Heider,[4] Stuart Walsh,[1] Joel Zupicich,[1] Kanar Alkass,[5] Bruce A. Buchholz,[6] Henrik Druid,[5] Shahram Jovinge,[4]† Jonas Frisén[1]†

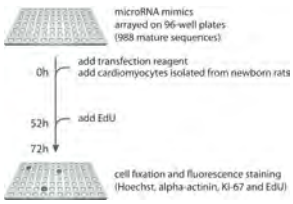
A 25-year-old heart replaces about 1% of all cardiomyocytes over a year; a 75-year-old about half that.

Fewer than 50% of cardiomyocytes are exchanged during a normal life span.

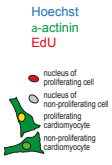


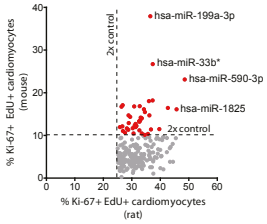
3 APRIL 2009 VOL 324 SCIENCE www.sciencemag.org

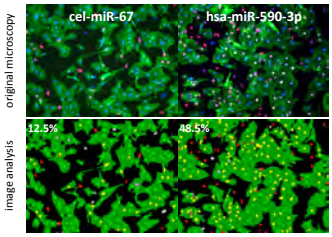
Screening for cardiomyocyte proliferation using a library of microRNA mimics



40 human miRNAs increase both rat and mouse cardiomyocyte proliferation



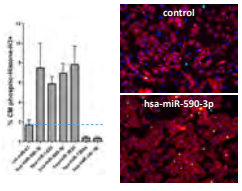




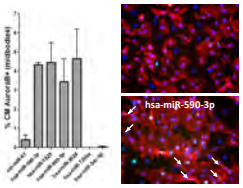
Eulalio et al. 2012. Nature 492, 376

40 human miRNAs increase both rat and mouse cardiomyocyte proliferation

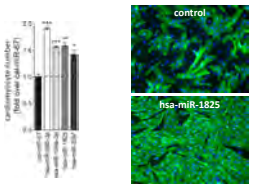
phosphoH3 positivity



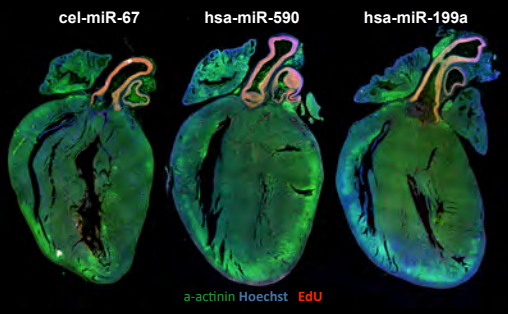
Aurora B midbody localization



Increase in cell number



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



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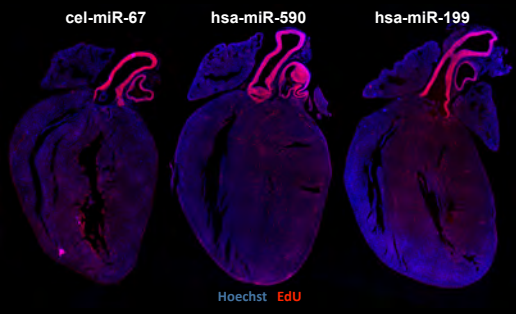
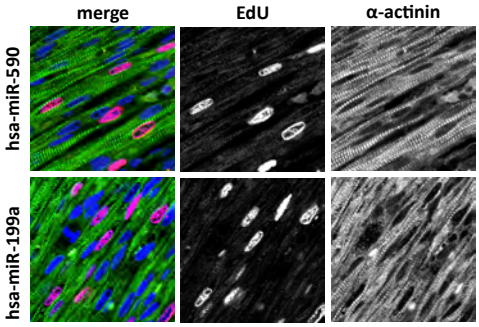
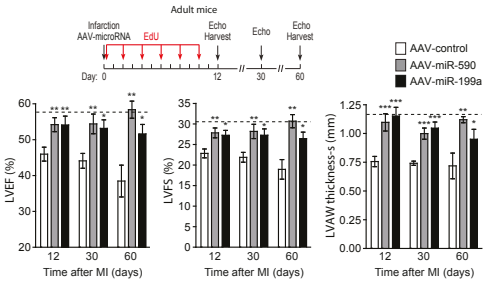


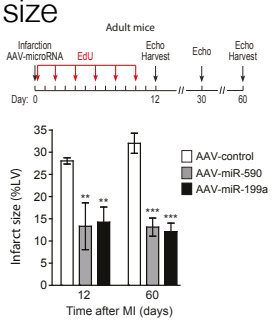
miRNAs increasing CM proliferation *in vivo*

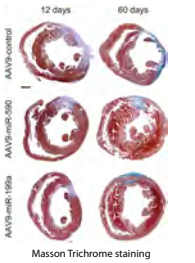


miR-590 and miR-199a preserve myocardial function after MI



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

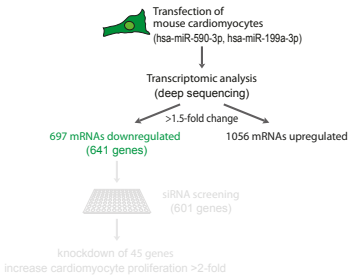




Eulalio et al. 2012. Nature 492, 376

Mechanisms?

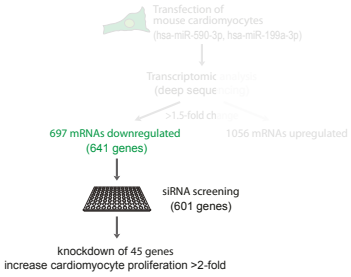
Identification of miR-590-3p and miR-199a-3p target genes



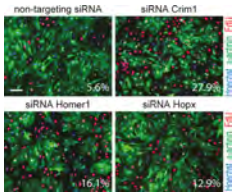
Among the 641 genes downregulated by miR-590-3 and miR-199a-3p there are:

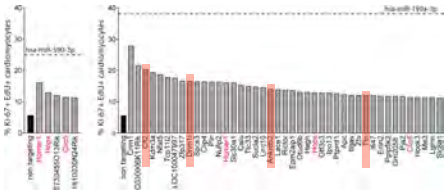
Myomesin 1 (Myom1)
Myomesin 2 (Myom2)
Myosin light polypeptide 4 (Myl4)
Nebulin-related anchoring protein (Nrap)
Myosin IB (Myo1b)
Titin (Ttn)
Troponin T1, skeletal slow (Tnnt1)
Troponin T2 cardiac (Tnnt2)
Cofilin2 (Cofilin2)
Dynamin1-like (Dnm1l)
Ankyrin repeat domain 52 (Ankrd52)
Nebulette (Nbl)

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



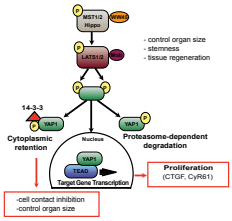
Identification of miR-590 and miR-199a target genes by deep sequencing and HTS siRNA screening

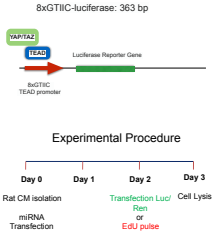




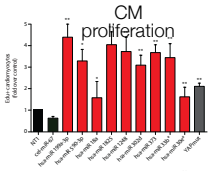
miRNAs promoting cardiomyocyte proliferation activate the YAP transcriptional coactivator

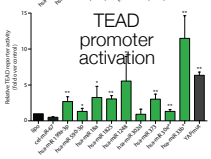
Consuelo Torrini

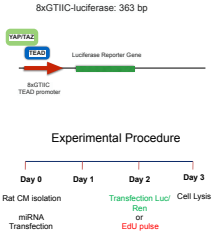




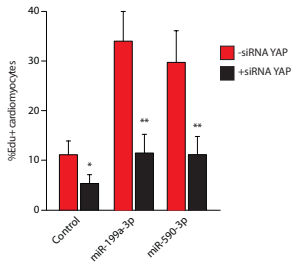
miRNAs promoting cardiomyocyte proliferation activate YAP-induced transcription







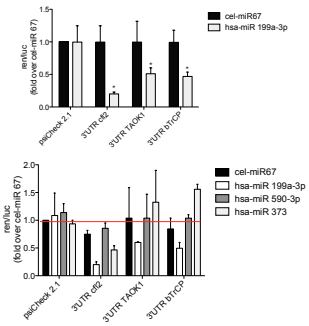
YAP knock down blocks the effects of miR-199a-3p and miR-590-3p



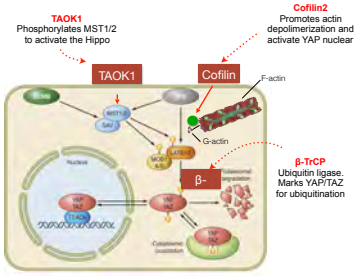
miR-199a-3p direct targets

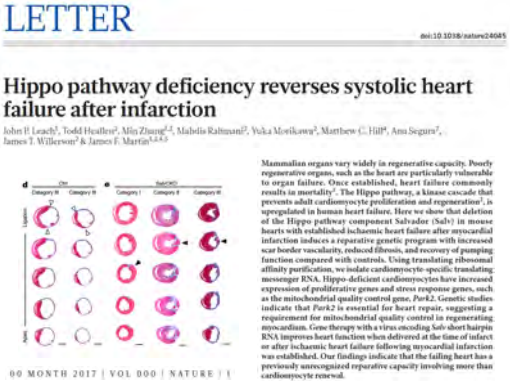
HeLa cells

3' UTR luciferase assays



miR-199a-3p direct targets





LETTER

Hippo pathway deficiency reverses systolic heart failure after infarction

Mammalian organs vary widely in regenerative capacity. Poorly regenerative organs, such as the heart are particularly vulnerable to organ failure. Once established, heart failure commonly results in mortality[1]. The Hippo pathway, a kinase cascade that prevents adult cardiomyocyte proliferation and regeneration[2], is upregulated in human heart failure. Here we show that deletion of the Hippo pathway component Salvador (Salv) in mouse hearts with established ischaemic heart failure after myocardial infarction induces a reparative genetic program with increased scar border vascularity, reduced fibrosis, and recovery of pumping function compared with controls. Using translating ribosomal affinity purification, we isolate cardiomyocyte-specific translating messenger RNA. Hippo-deficient cardiomyocytes have increased expression of proliferative genes and stress response genes, such as the mitochondrial quality control gene, *Park2*. Genetic studies indicate that *Park2* is essential for heart repair, suggesting a requirement for mitochondrial quality control in regenerating myocardium. Gene therapy with a virus encoding Salv short hairpin RNA improves heart function when delivered at the time of infarct or after ischaemic heart failure following myocardial infarction was established. Our findings indicate that the failing heart has a previously unrecognized reparative capacity involving more than cardiomyocyte renewal.

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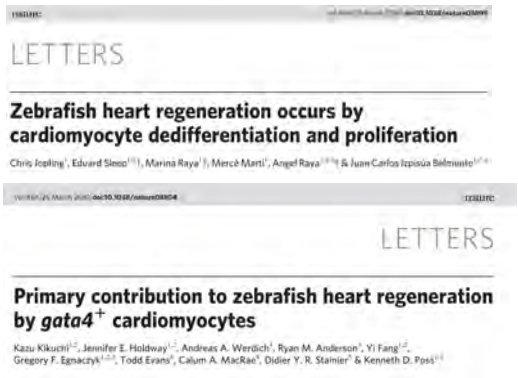


LETTERS

Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation

Chris Jopling[1], Eduard Sleep[1], Marina Raya[1], Mercè Martí[1], Angel Raya[1,2] & Juan Carlos Izpisúa Belmonte[1]

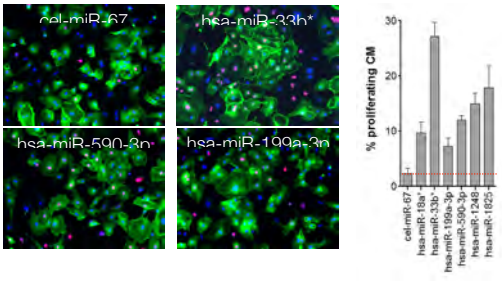
LETTERS

Primary contribution to zebrafish heart regeneration by *gata4*$^{+}$ cardiomyocytes

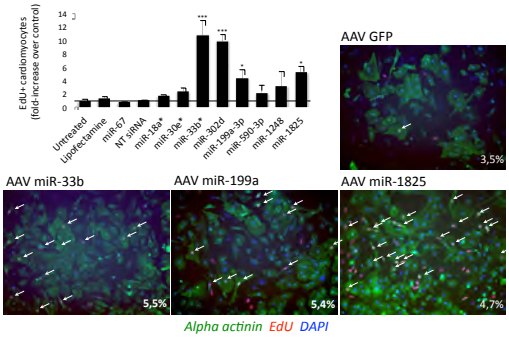
Kazu Kikuchi[1,2], Jennifer E. Holdway[1,2], Andreas A. Werdich[1], Ryan M. Anderson[1], Yi Fang[1,2], Gregory F. Egnaczyk[1,2,3], Todd Evans[3], Calum A. MacRae[3], Didier Y. R. Stainier[2] & Kenneth D. Poss[1,2]

Effect in human cardiomyocytes?

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

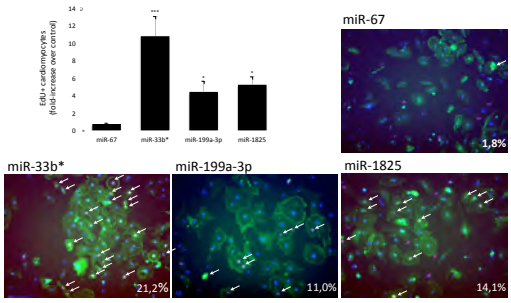


Human fetal cardiomyocyte proliferation (AAV6 pri-miRNA transduction)

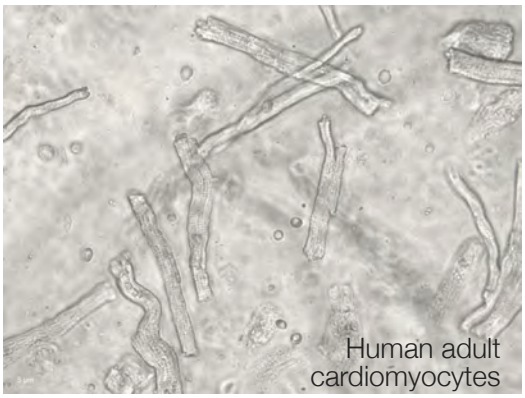


Alpha actinin EdU DAPI

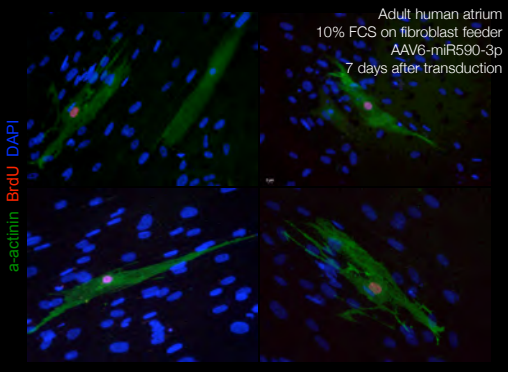
Human fetal cardiomyocyte proliferation (mimic transfection)



Alpha actinin EdU DAPI



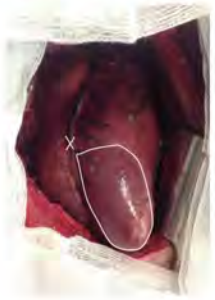
Human adult cardiomyocytes replicating DNA



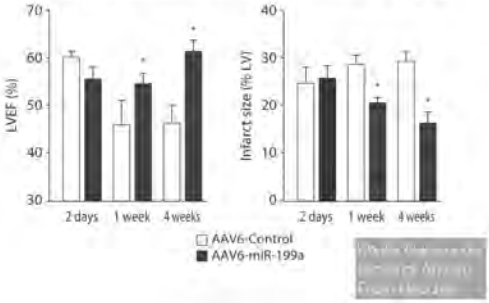
Effect in large animals?

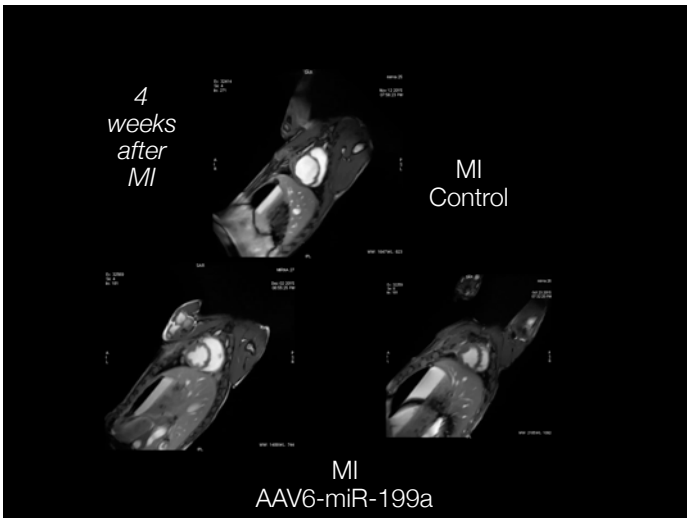
3-6 months old farm pig

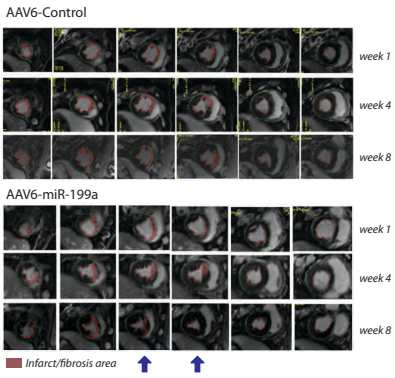
LAD Occlusion after first Diagonal branch for 90 minutes, followed by Reperfusion

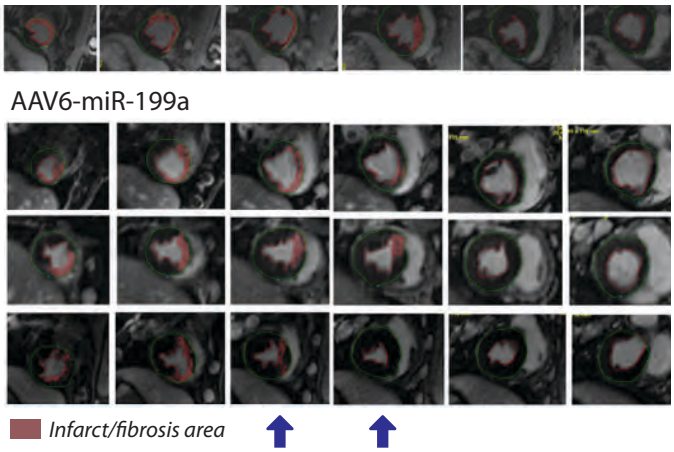


AAV6-miR-199a reduces infarct size and improves cardiac function after MI in pigs

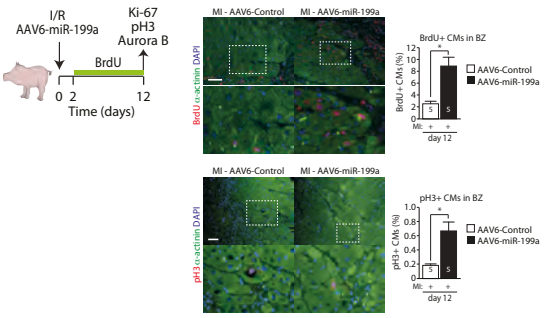


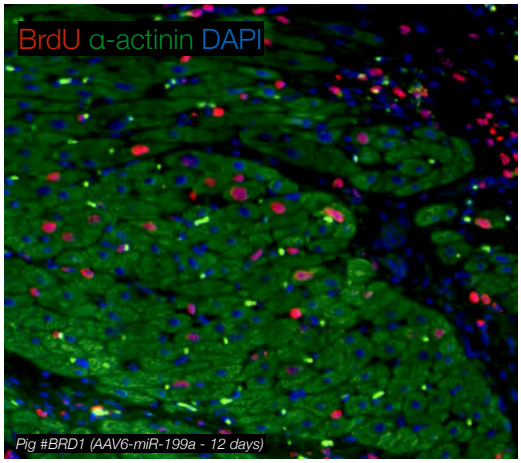




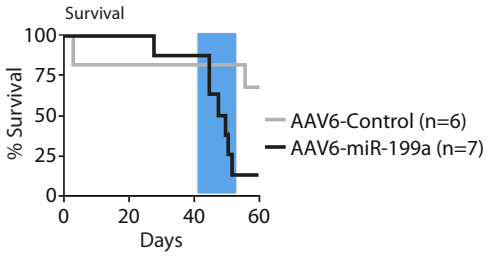


Cardiomyocyte proliferation in the infarct border zone in AAV6-miR-199a-treated pigs

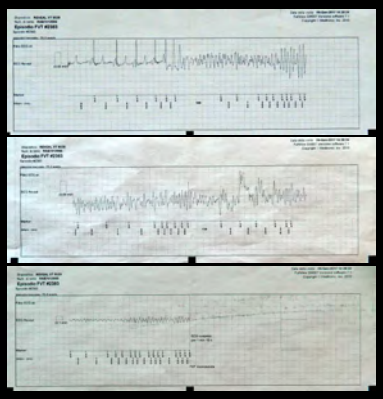




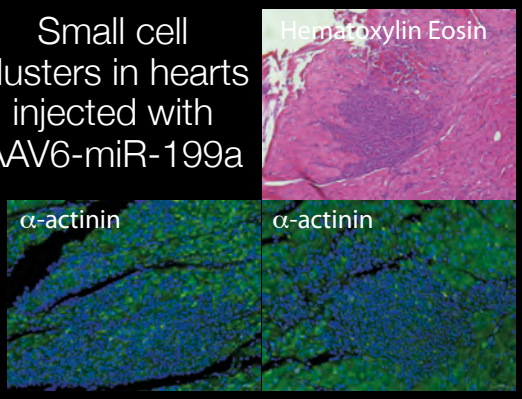
Sudden cardiac death of AAV6-miR-199a-treated pigs



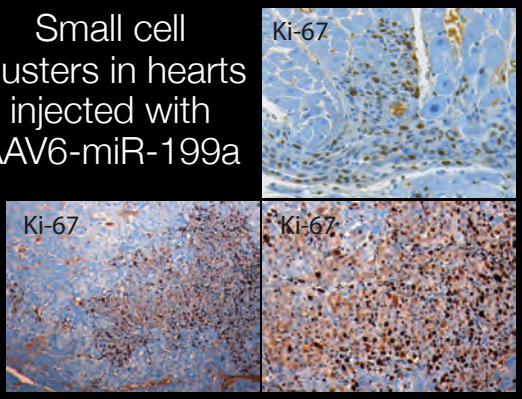
Episodes of fatal arrhythmias after AAV-miR-199a delivery in pigs



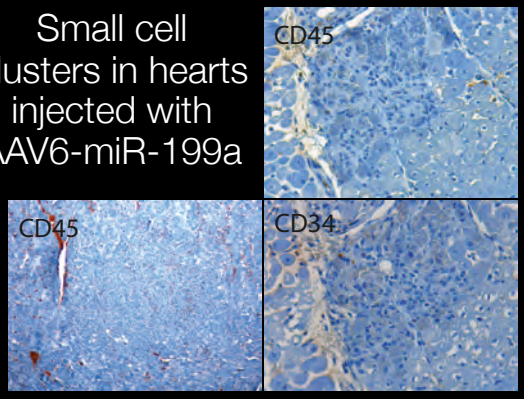
Small cell clusters in hearts injected with AAV6-miR-199a



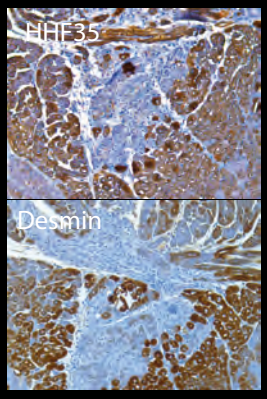
Small cell clusters in hearts injected with AAV6-miR-199a



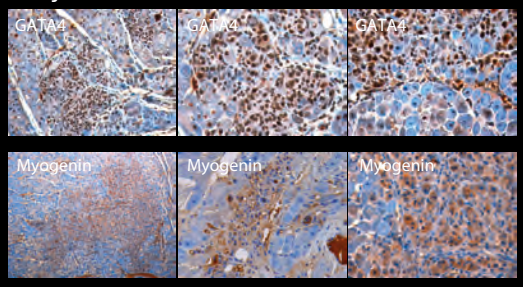
Small cell clusters in hearts injected with AAV6-miR-199a



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

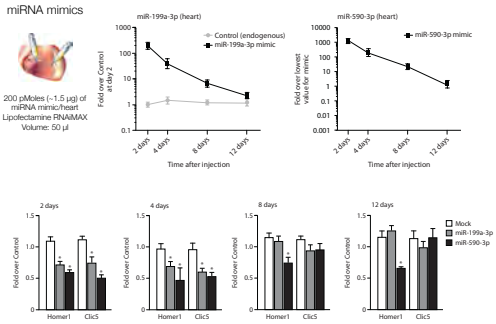


Small cell clusters in hearts injected with AAV6-miR-199a

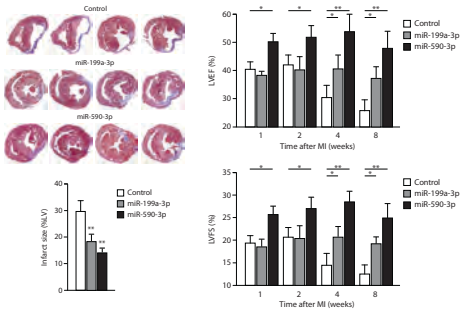


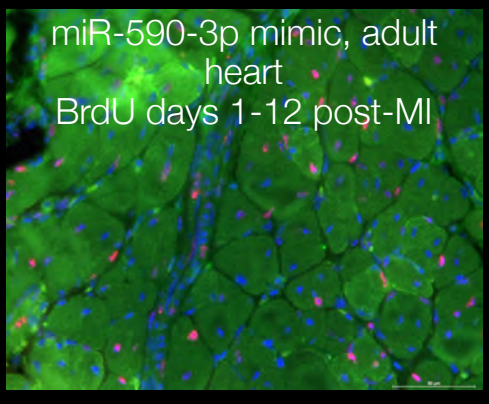
Delivery?

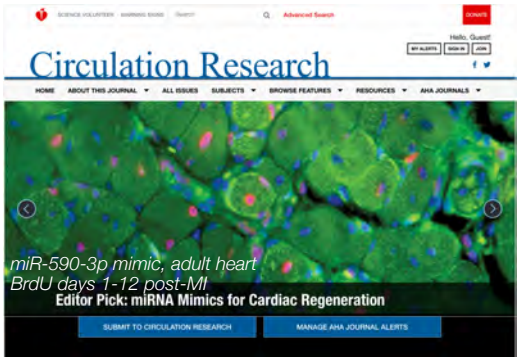
Prolonged effect of miRNA mimics after intracardiac injection



miRNA mimics stimulate myocardial repair after MI







Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors

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In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes

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The reprogramming of adult cells into pluripotent cells or directly into alternative adult cell types holds great promise for regenerative medicine. We reported previously that cardiac fibroblasts, which represent 50% of the cells in the mammalian heart, can be directly reprogrammed to adult cardiomyocyte–like cells *in vitro* by the addition of Gata4, Mef2c and Tbx5 (GMT). Here we use genetic lineage tracing to show that resident non-myocytes in the murine heart can be reprogrammed into cardiomyocyte–like cells *in vivo* by local delivery of GMT after coronary ligation. Induced cardiomyocytes became binucleate, assembled sarcomeres and had cardiomyocyte–like gene expression. Analysis of single cells revealed ventricular cardiomyocyte–like action potentials, beating upon electrical stimulation, and evidence of electrical coupling. *In vivo* delivery of GMT decreased infarct size and modestly attenuated cardiac dysfunction up to 3 months after coronary ligation. Delivery of the pro-angiogenic and fibroblast-activating peptide, thymosin β4, along with GMT, resulted in further improvements in scar area and cardiac function. These findings demonstrate that cardiac fibroblasts can be reprogrammed into cardiomyocyte–like cells in their native environment for potential regenerative purposes.

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How to mend a broken heart (Bee Gees 1971)

- Adult stem cells
  - Bone marrow (?)
  - Cardiac stem cells
- ES cells
  - From the embryo
  - By cloning
  - iPSCs
- Transdifferentiation
- Direct regeneration