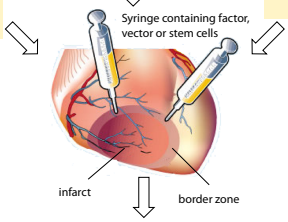
Novel biotherapeutics for myocardial ischemia and heart failure

Synthetic peptides or recombinant proteins

Gene Therapy
Protein-coding cDNA? siRNA? miRNA? Which vector?

Cell Therapy
Stem cell? Source?



Neoangiogenesis
Myocardial protection
Myocardial regeneration

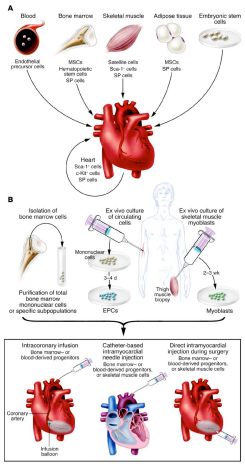
Source of stem cells for potential heart injection

BM mononuclear cells
EPCs (CD133+ CD34+ VEGR2+)
Culture-expanded myelomonocytic EPCs (CD14+ CD34-)
Mesenchymal Stem Cells (CD34- CD133-)
Skeletal myoblasts
Resident Cardiac Stem Cells
Embryonic Stem Cells

Modes of cell delivery

Transvascular
- Intracoronary (stop-flow balloon cathether)
- Intravenous
- After progenitor cell mobilization

Direct injection in the ventricular wall
- Transendocardial injection
- Transepicardial injection (during CABG)
- Transcoronary vein injection



The Journal of Clinical Investigation
Volume 115 Number 3 March 2005

"Stem cells" from the bone marrow

Sources of adult stem cells

Bone marrow:	*HSC and MSC*
Peripheral blood:	*HSC, hemangioblast?*
Brain and spinal cord:	*NSC*
Skin:	*bulge zone cells, SKP in the dermis*
Liver:	*oval cells*
Pancreas:	*ductal stem cells*
Eye:	*corneal and retinal stem cells*
Skeletal muscle:	*satellite cells and SP*

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[2], D. Burkhoff[2], J. Wang[1], S. Homma[2], N.M. Edwards[1] & 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

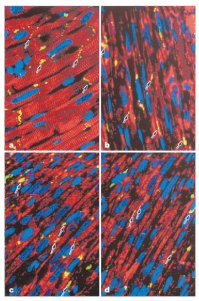


Figure 4 Myocardial repair and connexin 43. **a**, Border zone; **b**–**d**, regenerating myocardium. Shown are connexin 43 (yellow–green; arrows indicate contacts between myocytes) and α-sarcomeric actin (red), and PI-stained nuclei (blue). Original magnification, ×500 (**a**), ×800 (**b**–**d**).

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*
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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-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.

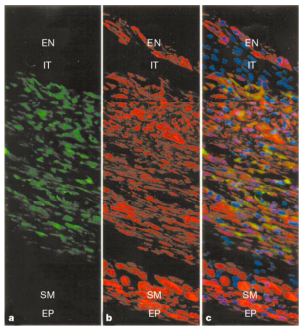
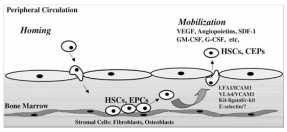


Figure 2 Myocardial infarct injected with Lin^-c-kit^{POS} cells; myocardium is regenerating from endocardium (EN) to epicardium (EP). **a**, EGFP (green); **b**, cardiac myosin (red); **c**, combination of EGFP and myosin (red–green), and propidium-iodide-stained nuclei (blue). Infarcted tissue (IT) 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

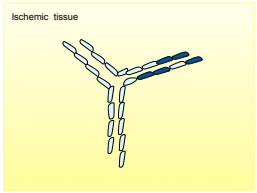
Vascular injury
Organ regeneration
Tumor growth

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



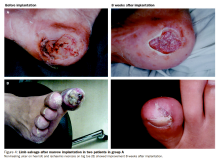
ARTICLES

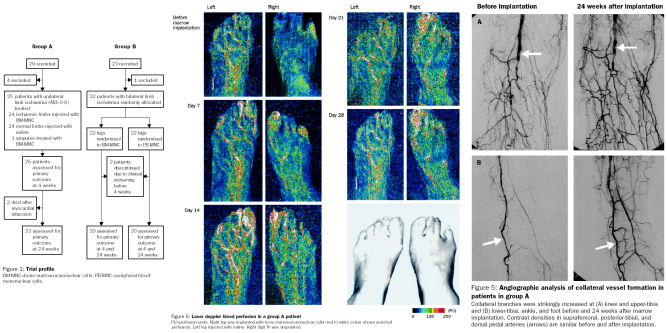
Articles

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

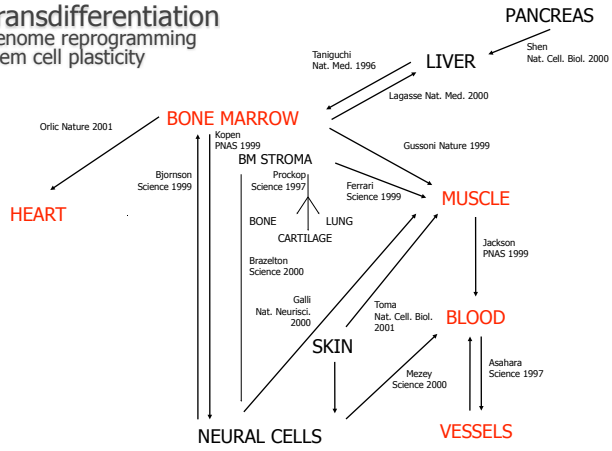
*Eriko Tateishi-Yuyama, Hiroaki Matsubara, Toyoaki Murohara, Ukichi Ikeda, Satoshi Shintani, Hiraya Masaki, Katsuya Amano, Yuji Kishimoto, Kenji Yoshimoto, Hidetoshi Akaishi, Kazuyuki Shimada, Toshiji Hamaoka, Tsutomu Imaizumi, for the Therapeutic Angiogenesis using Cell Transplantation (TACT) Study Investigators**

THE LANCET • Vol 360 • August 10, 2002





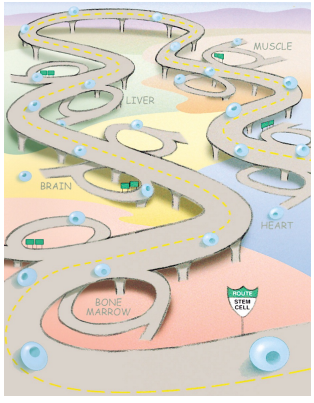
Transdifferentiation
Genome reprogramming
Stem cell plasticity



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

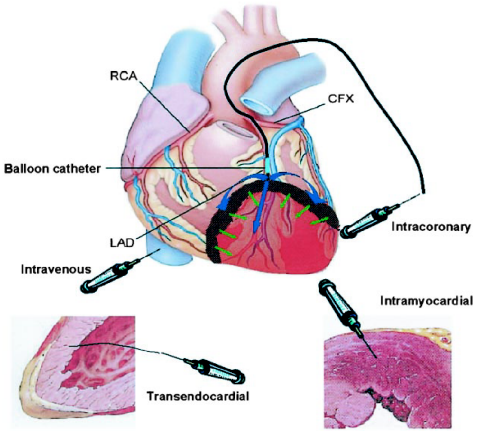


Selected Cell-Therapy Trials

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

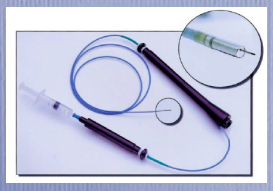
9 APRIL 2004 VOL 304 SCIENCE

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)



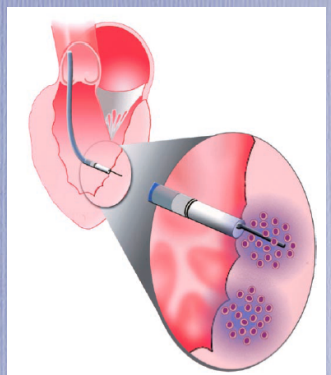
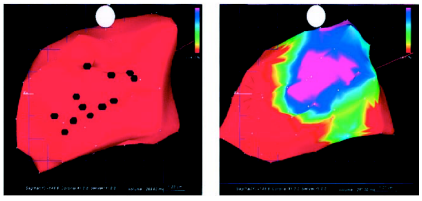


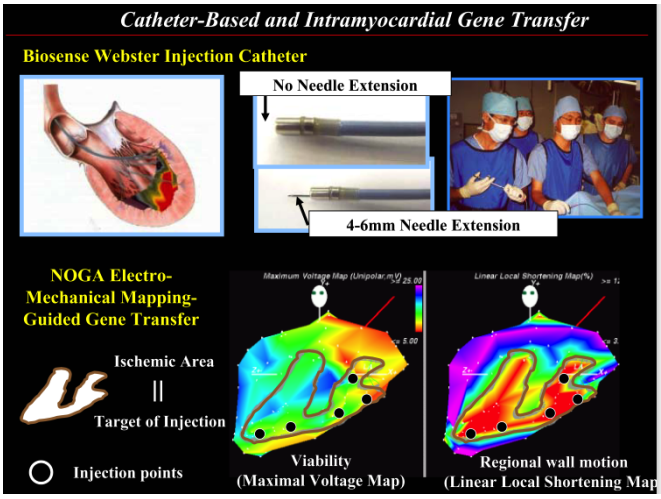
Figure 2. Injection catheter advanced into the left ventricle through the aortic valve. The catheter tip is placed against the endocardial surface (insert) with the needle extended into the myocardium delivering ABMMNCs.

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

Volker Schächinger, M.D., Sandra Erbs, M.D., Albrecht Elsässer, M.D., Werner Haberbosch, M.D., Rainer Hambrecht, M.D., Hans Hölschermann, M.D., Jiangtao Yu, M.D., Roberto Corti, M.D., Detlef G. Mathey, M.D., Christian W. Hamm, M.D., Tim Süselbeck, M.D., Birgit Assmus, M.D., Torsten Tonn, M.D., Stefanie Dimmeler, Ph.D., and Andreas M. Zeiher, M.D., for the REPAIR-AMI Investigators*

ABSTRACT

BACKGROUND

Pilot trials suggest that the intracoronary administration of autologous progenitor cells may improve left ventricular function after acute myocardial infarction.

METHODS

In a multicenter trial, we randomly assigned 204 patients with acute myocardial infarction to receive an intracoronary infusion of progenitor cells derived from bone marrow (BMC) or placebo medium into the infarct artery 3 to 7 days after successful reperfusion therapy.

RESULTS

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 [SD] increase, 5.5±7.3% vs. 3.0±6.5%; P=0.01). Patients with a baseline LVEF at or below the median value of 48.9% derived the most benefit (absolute improvement in LVEF, 5.0%; 95% confidence interval, 2.0 to 8.1). At 1 year, intracoronary infusion of BMC was associated with a reduction in the prespecified combined clinical end point of death, recurrence of myocardial infarction, and any revascularization procedure (P=0.01).

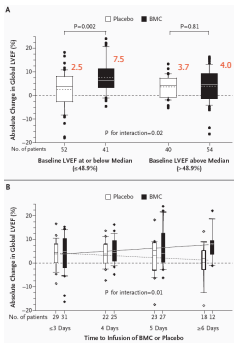
CONCLUSIONS

Intracoronary administration of BMC is associated with improved recovery of left ventricular contractile function in patients with acute myocardial infarction. Large-scale studies are warranted to examine the potential effects of progenitor-cell administration on morbidity and mortality. (ClinicalTrials.gov number, NCT00279175.)

From the Department of Internal Medicine III, Johann Wolfgang Goethe University, Frankfurt (V.S., B.A., S.D., A.M.Z.); the Department of Cardiology, Herzzentrum Leipzig, Leipzig (S.E., R.H.); the Department of Cardiology, Kerckhoff Klinik, Bad Nauheim (A.E., C.W.H.); the Department of Internal Medicine, Dreifaltigkeitskum Sahl, Sahl (W.H.); the Department of Cardiology, Universitätsklinikum Giessen, Giessen (H.H.); the Department of Cardiology, Zentralklinikum, Bad Berka (J.Y.); the Hamburg University Cardiovascular Center, Hamburg (D.G.M.); the Department of Cardiology, Universitäts-klinikum, Mannheim (T.S.); and the Institute for Transfusion Medicine and Immunohematology, Red Cross Blood Donor Service Baden–Württemberg–Hessen, Frankfurt (T.T.) — all in Germany; and the Department of Cardiology, Universitätsspital, Zurich, Switzerland (R.C.). Address reprint requests to Dr. Zeiher at the Department of Internal Medicine III, J.W. Goethe University, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany, or at zeiher@em.uni-frankfurt.de.

*Members of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) study group are listed in the Appendix.

N Engl J Med 2006;355:1210-21.



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

Birgit Assmus, M.D., Jörg Honold, M.D., Volker Schächinger, M.D., Martina B. Britten, M.D., Ulrich Fischer-Rasokat, M.D., Ralf Lehmann, M.D., Claudius Teupe, M.D., Katrin Pistorius, M.D., Hans Martin, M.D., Nasreddin D. Abolmaali, M.D., Torsten Tonn, M.D., Stefanie Dimmeler, Ph.D., and Andreas M. Zeiher, M.D.

ABSTRACT

BACKGROUND

Pilot studies suggest that intracoronary transplantation of progenitor cells derived from bone marrow (BMC) or circulating blood (CPC) may improve left ventricular function after acute myocardial infarction. The effects of cell transplantation in patients with healed myocardial infarction are unknown.

METHODS

After an initial pilot trial involving 17 patients, we randomly assigned, in a controlled crossover study, 75 patients with stable ischemic heart disease who had had a myocardial infarction at least 3 months previously to receive either no cell infusion (23 patients) or infusion of CPC (24 patients) or BMC (28 patients) into the patent coronary artery supplying the most dyskinetic left ventricular area. The patients in the control group were subsequently randomly assigned to receive CPC or BMC, and the patients who initially received BMC or CPC crossed over to receive CPC or BMC, respectively, at 3 months' follow-up.

RESULTS

The absolute change in left ventricular ejection fraction was significantly greater among patients receiving BMC (+2.9 percentage points) than among those receiving CPC (−0.4 percentage point, P=0.003) or no infusion (−1.2 percentage points, P<0.001). The increase in global cardiac function was related to significantly enhanced regional contractility in the area targeted by intracoronary infusion of BMC. The crossover phase of the study revealed that intracoronary infusion of BMC was associated with a significant increase in global and regional left ventricular function, regardless of whether patients crossed over from control to BMC or from CPC to BMC.

CONCLUSIONS

Intracoronary infusion of progenitor cells is safe and feasible in patients with healed myocardial infarction. Transplantation of BMC is associated with moderate but significant improvement in the left ventricular ejection fraction after 3 months. (ClinicalTrials.gov number, NCT00289822.)

Table 2. Quantitative Variables Pertaining to Left Ventricular Function, as Assessed by Left Ventricular Angiography.*

Variable	Baseline	3 Months' Follow-up	Absolute Change	P Value
Global LVEF (%)				
Control group	43±13	42±13	−1.2±3.0	0.12
CPC group	39±10	38±10	−0.4±2.2	0.60
BMC group	41±11	43±10	+2.9±3.6	0.001
P value for all 3 groups	0.68	0.31	0.001	
Regional contractility in central target area (SD from normal/chord)				
Control group	−1.55±0.40	−1.50 ±0.47	−0.06±0.33	0.62
CPC group	−1.72±0.36	−1.75±0.41	−0.03±0.30	0.70
BMC group	−1.63±0.40	−1.38±0.42	+0.26±0.43	0.006
P value for all 3 groups	0.44	0.03	0.09	
Extent of regional left ventricular dysfunction (% circumference)				
Control group	45±24	45±22	0±5	0.41
CPC group	52±18	50±19	−3±6	0.15
BMC group	45±18	42±19	−3±10	0.31
P value for all 3 groups	0.51	0.50	0.37	
End-diastolic volume (ml/m² of BSA)				
Control group	90±38	87±33	−3±17	0.45
CPC group	96±34	93±30	−3±18	0.47
BMC group	79±29	79±29	0±10	0.95
P value for all 3 groups	0.14	0.26	0.62	
End-systolic volume (ml/m² of BSA)				
Control group	55±36	55±32	−1±12	0.91
CPC group	62±31	60±26	−2±13	0.57
BMC group	49±26	47±26	−2±5	0.09
P value for all 3 groups	0.21	0.26	0.83	
Stroke volume (ml/m² of BSA)				
Control group	34±7	32±4	−2±7	0.22
CPC group	35±8	34±8	−1±7	0.31
BMC group	30±9	32±8	+2±7	0.21
P value for all 3 groups	0.08	0.78	0.09	
Left ventricular end-diastolic pressure (mm Hg)				
Control group	14±9	13±6	−3±7	0.15
CPC group	12±7	12±6	0±6	0.84
BMC group	12±8	12±7	0±7	0.91
P value for all 3 groups	0.64	0.61	0.42	

* Plus–minus values are means ±SD. BSA denotes body-surface area.

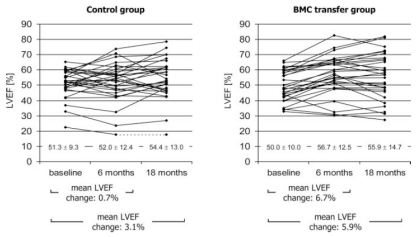
BOOST

Intracoronary Bone Marrow Cell Transfer After Myocardial Infarction

Eighteen Months' Follow-Up Data From the Randomized, Controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) Trial

Gerd P. Meyer, MD*; Kai C. Wollert, MD*; Joachim Lotz, MD; Jan Steffens, BS; Peter Lippolt, MD; Stephanie Fichtner, BS; Hartmut Hecker, MD; Arnd Schaefer, MD; Lubomir Arseniev, MD; Bernd Hertenstein, MD; Arnold Ganser; MD; Helmut Drexler, MD

Circulation 2006;113:1287-1294; originally published online Mar 6, 2006;



The relative improvement in LVEF after infusion of BMC at 6 months was no longer significant at 18 months

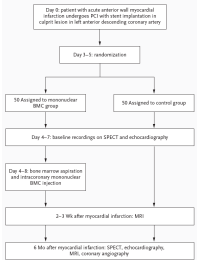
The main effect was an acceleration of recovery

ASTAMI

The NEW ENGLAND JOURNAL of MEDICINE SEPTEMBER 21, 2006

Intracoronary Injection of Mononuclear Bone Marrow Cells in Acute Myocardial Infarction

Ketil Lunde, M.D., Svein Solheim, M.D., Svend Aakhus, M.D., Ph.D., Harald Arnesen, M.D., Ph.D., Michael Abdelnoor, Ph.D., Torstein Egeland, M.D., Ph.D., Knut Endresen, M.D., Ph.D., Arnfinn Ilebekk, M.D., Ph.D., Arild Mangschau, M.D., Ph.D., Jan G. Fjeld, M.D., Ph.D., Hans Jørgen Smith, M.D., Ph.D., Eli Taraldsrud, M.D., Haakon Kiil Gregaard, M.D., Reidar Bjørnerheim, M.D., Ph.D., Magne Brekke, M.D., Carl Müller, M.D., Einar Hopp, M.D., Asgrimur Ragnarsson, M.D., Jan E. Brinchmann, M.D., Ph.D., and Kolbjørn Forfang, M.D., Ph.D.*



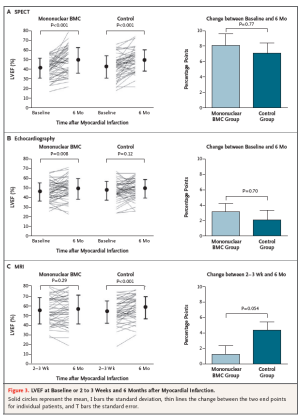


Figure 3. LVEF at Baseline or 2 to 3 Weeks and 4 Months after Myocardial Infarction.
Solid circles represent the mean, I bars the standard deviation, thin lines the change between the two end points for individual patients, and T bars the standard error.

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)

Mobilized bone marrow cells repair the infarcted heart, improving function and survival

Donald Orlic*, Jan Kajstura†, Stefano Chimenti‡, Federica Limana‡, Igor Jakoniuk*, Federico Quaini‡, Bernardo Nadal-Ginard*, David M. Bodine*, Annarosa Leri†, and Piero Anversa*†

*Cardiovascular Research Institute, Department of Medicine, New York Medical College, Valhalla, NY 10595, and †Hematopoiesis Section, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institute of Health, Bethesda, MD 20892

Attempts to repair myocardial infarcts by transplanting cardiomyocytes or skeletal myoblasts have failed to reconstitute healthy myocardium and coronary vessels integrated structurally and functionally with the remaining viable portion of the ventricular wall. The recently discovered growth and transdifferentiation potential of primitive bone marrow cells (BMC) prompted us, in an earlier study, to inject in the border zone of acute infarcts Lin^{-} $c\text{-}kit^{pos}$ BMC from syngeneic animals. These BMC differentiated into myocytes and vascular structures, ameliorating the function of the infarcted heart. Two critical determinants seem to be required for the transdifferentiation of primitive BMC: tissue damage and a high level of pluripotent cells. On this basis, we hypothesized here that BMC, mobilized by stem cell factor and granulocyte-colony stimulating factor, would home to the infarcted region, replicate, differentiate, and ultimately promote myocardial repair. We report that, in the presence of an acute myocardial infarct, cytokine-mediated translocation of BMC resulted in a significant degree of tissue regeneration 27 days later. Cytokine-induced cardiac repair decreased mortality by 68%, infarct size by 40%, cavitary dilation by 26%, and diastolic stress by 70%. Ejection fraction progressively increased and hemodynamics significantly improved as a consequence of the formation of 15×10^6 new myocytes with arterioles and capillaries connected with the circulation of the unaffected ventricle. In conclusion, mobilization of primitive BMC by cytokines might offer a noninvasive therapeutic strategy for the regeneration of the myocardium lost as a result of ischemic heart disease and, perhaps, other forms of cardiac pathology.

10344–10349 | PNAS | August 28, 2001 | vol. 98 | no. 18

STEMMI

Stem Cell Mobilization Induced by Subcutaneous Granulocyte-Colony Stimulating Factor to Improve Cardiac Regeneration After Acute ST-Elevation Myocardial Infarction

Result of the Double-Blind, Randomized, Placebo-Controlled Stem Cells in Myocardial Infarction (STEMMI) Trial

Rasmus Sejersten Ripa, MD; Erik Jørgensen, MD; Yongzhong Wang, MD; Jens Jakob Thune, MD; Jens Christian Nilsson, MD; Lars Søndergaard, MD; Hans Erik Johnsen, MD; Lars Køber, MD; Peer Grande, MD; Jens Kastrup, MD

Background—Phase 1 clinical trials of granulocyte-colony stimulating factor (G-CSF) treatment after myocardial infarction have indicated that G-CSF treatment is safe and may improve left ventricular function. This randomized, double-blind, placebo-controlled trial aimed to assess the efficacy of subcutaneous G-CSF injections on left ventricular function in patients with ST-elevation myocardial infarction.

Methods and Results—Seventy-eight patients (62 men; average age, 56 years) with ST-elevation myocardial infarction were included after successful primary percutaneous coronary stent intervention ~12 hours after symptom onset. Patients were randomized to double-blind treatment with G-CSF (10 μg/kg of body weight) or placebo for 5 days. The primary end point was change in systolic wall thickening from baseline to 6 months determined by cardiac magnetic resonance imaging (MRI). An independent core laboratory analyzed all MRI examinations. Systolic wall thickening improved 17% in the infarct area in the G-CSF group and 17% in the placebo group ($P = 1.0$). Comparable results were found in infarct border and noninfarcted myocardium. Left ventricular ejection fraction improved similarly in the 2 groups measured by both MRI (6.5 versus 8.0; $P = 0.19$) and echocardiography (5.7 versus 3.7; $P = 0.7$). The risk of severe clinical adverse events was not increased by G-CSF. In addition, in-stent late lumen loss and target vessel revascularization rate in the follow-up period were similar in the 2 groups.

Conclusions—Bone marrow stem cell mobilization with subcutaneous G-CSF is safe but did not lead to further improvement in ventricular function after acute myocardial infarction compared with the recovery observed in the placebo group. (*Circulation*. 2006;113:1983-1992.)

Key Words: angiogenesis ■ heart failure ■ magnetic resonance imaging ■ myocardial infarction ■ stem cells

Editorial

The End of Granulocyte Colony-Stimulating Factor in Acute Myocardial Infarction?

Reaping the Benefits Beyond Cytokine Mobilization

Jonathan M. Hill, MA, MBChB, MRCP; Jozef Bartunek, MD, PhD

Circulation April 25, 2006

Cardiac Cell Therapy — Mixed Results from Mixed Cells

Anthony Rosenzweig, M.D.

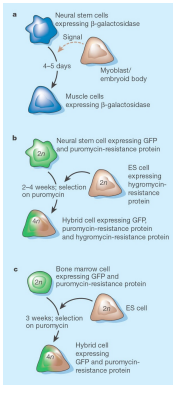
N ENGL J MED 355;12

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[4,9]	PCI after acute myocardial infarction	Randomized trial 30 patients received BMC; 30 received no infusion LVEF assessed by MRI	Approximately 2.5×10^8 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.[8]	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[6]	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[5]	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...



Stem cells

Cell fusion causes confusion

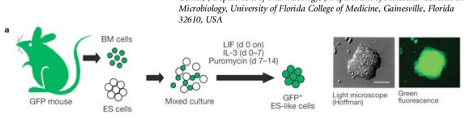
Andrew E. Wurmser and Fred H. Gage

'Transdifferentiation' is a poorly understood process invoked to explain how tissue-specific adult stem cells can generate cells of other tissues. New results challenge its existence.

Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion

Naohiro Terada*†‡, Takashi Hamazaki*, Masahiro Oka*, Masanori Hoki*, Diana M. Mastalerz*, Yuka Nakano‡, Edwin M. Meyer‖, Laurence Morel*, Bryon E. Petersen*† & Edward W. Scott‖§

* *Department of Pathology, † Program in Stem Cell Biology, Shands Cancer Center, ‡ Department of Pharmacology, § Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, Florida 32610, USA*



Stem cells

Lost in translation

Kenneth R. Chien

The potential use of stem cells as agents of repair in human disease makes them the subject of high-profile studies. But we should be wary of prematurely pushing laboratory research into clinical practice.

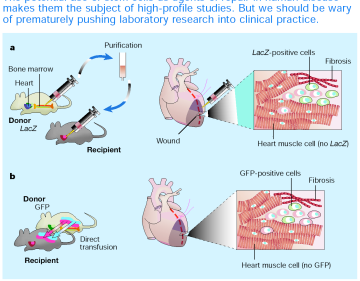


Figure 1 **Two strategies used to show that bone-marrow stem cells do not take on the role of damaged heart cells.** a, Murry *et al.*[2] isolated and purified genetically modified bone-marrow stem cells from mice. The modification 'tagged' the cells (with *LacZ*), enabling them to be detected in the recipient mouse heart, into which the cells were directly injected. Closer inspection of the recipient heart showed that the label could not be detected in heart muscle cells. b, Similar results were shown by Balsam *et al.*[3], although the approach was slightly different. Donor bone-marrow stem cells were transfused directly into the circulation of recipients. Again, the tag (GFP; green fluorescent protein) could not be detected in heart muscle cells of the donor; indeed, the bone-marrow cells continued to differentiate into blood cells while in the heart.

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]

[1]*Departments of Cardiothoracic Surgery,* [2]*Pathology, and* [3]*Developmental Biology, Stanford University School of Medicine, Stanford, California 94305, USA*

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[1], Hisako O. Nakajima[1], Michael Rubart[2], Kishore B. S. Pasumarthi[2,*], Jitka Ismail Virag[1], Stephen H. Bartelmez[1], Veronica Poppa[1], Gillian Bradford[1], Joshua D. Dowell[2], David A. Williams[2,*] & Loren J. Field[2]

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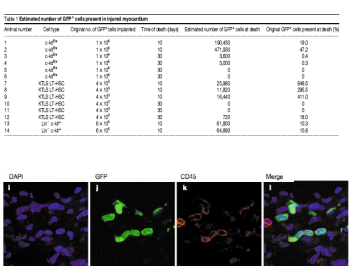
NATURE | VOL 428 | 8 APRIL 2004 | www.nature.com/nature



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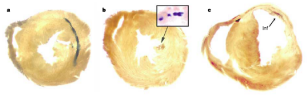
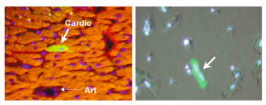


Transgenic mice in which the cardiac-specific MHC promoter drives the expression of a nuclear beta-gal

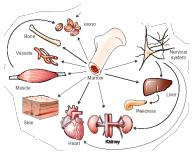


A rare GFP cardiomyocyte in the peri-infarct region, after BMT (MHC staining) and a single rod-shaped enzymatically dispersed cardiomyocyte

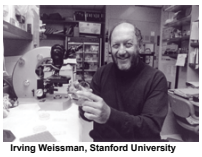
Long-standing biological dogma—that a cell, once committed, can't alter its fate—has been challenged by recent research. But now scientists are taking a more critical look

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

Ahmed Abdel-Latif, MD; Roberto Bolli, MD; Imad M. Tleyjeh, MD, MSc; Victor M. Montori, MD, MSc; Emerson C. Perin, MD; Carlton A. Hornung, PhD, MPH; Ewa K. Zuba-Surma, PhD; Mouaz Al-Mallah, MD; Badkhadeh Dawn, MD

ARCH INTERN MED/VOL 167, MAY 28, 2007

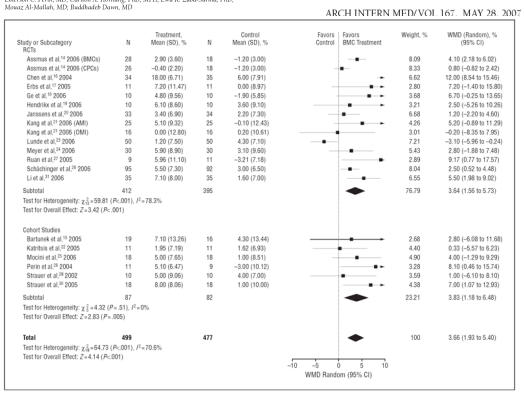


Figure 2. Forest plot of unadjusted difference in mean (with 95% confidence intervals [CIs]) improvement in left ventricular ejection fraction (LVEF) in patients treated with bone marrow–derived cells (BMCs) compared with controls. The figure shows the summary of cohort studies and randomized controlled trials (RCTs). Transplantation with BMCs resulted in a 3.66% (95% CI, 1.93% to 5.40%) increase in mean LVEF. The overall effect was statistically significant in favor of BMC therapy. AMI indicates acute myocardial infarction; CPCs, circulating progenitor cells; OMI, old myocardial infarction; and WMD, weighted mean difference.

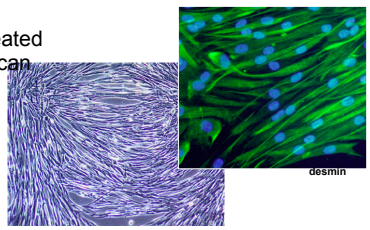
No proof of transdifferentation or myocardial regeneration in human trials !

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

Paracrine effect?

Stem cell therapy

Myoblasts: skeletal mononucleated unipotent progenitor cells that can be expanded in vitro



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

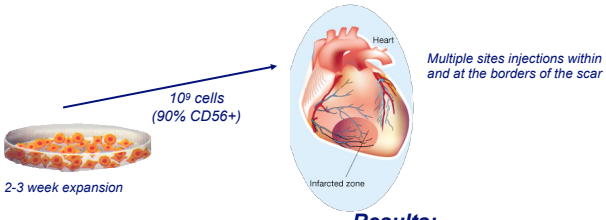
Long-Term Efficacy of Myoblast Transplantation on Regional Structure and Function After Myocardial Infarction

Saïd Ghostine, MD; Claire Carrion, MSc; Luiz César Guarita Souza, MD; Pascal Richard, MD; Patrick Bruneval, MD; Jean-Thomas Vilquin, PhD; Bruno Pouzet, MD; Ketty Schwartz, PhD; Philippe Menasché, MD, PhD; Albert Alain Hagège, MD, PhD

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

The ability of the skeletal myoblasts to differentiate into cardiomyocyte-like cells with intercalated discs and make meaningful electromechanical connections with the host cells is questionable

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



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 :

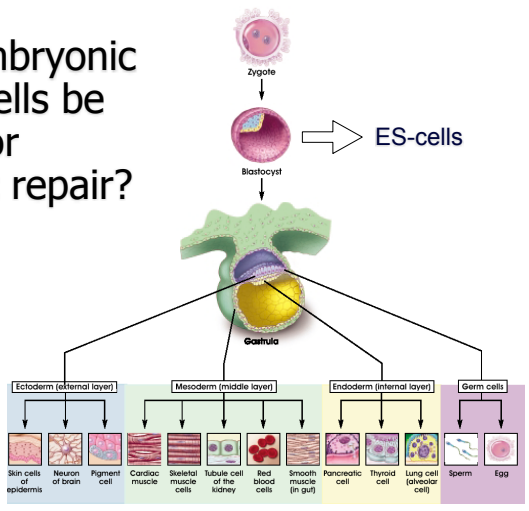
- placebo
- 600 million cells
- 800 million cells

Table 1

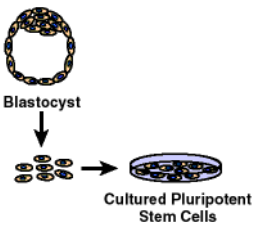
Reference	Adjunct	Source	Patients (*n*)	Cell count	Purity	Sites injected	Results	Complications
Menasche *et al.*	CABG	Autologous	1	800×10^6	65% CD56$^+$	33	Stabilized in NYHA class II LVEF improved to 30% Improved segmental contractility and perfusion	None
Menasche *et al.*	CABG	Autologous	10	871×10^6	86±3% (range 67–97%)	37±3 (range 27–57)	LVEF increase (from 23.8±3.9 to 32.1±7.5%) 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	$300\pm20\times10^6$	82±5%	6±2	Improved regional fractional shortening (9±3 to 20±5%); reduced scar size Improvement in NYHA class	None
Siminiak *et al.*	CABG	Autologous	1	1×10^6	—	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$^+$	3–30	Improved LVEF from 21 to 29% MRI and PET scan showed evidence of viability	None
Sim *et al.*	CABG on beating heart	Autologous	1	3.74×10^8	>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	43 to 97%	3 to 38	Myofiber 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 local hypokinetic regions ^{99m}Tc-tetrofosamine SPECT showed positive dynamics, reduced perfusion defects during exercise and rest	None
Zhang *et al.*	CABG	Autologous	3	—	—	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 ventricle ejection fraction; **MRI**, magnetic resonance imaging; PET, positron emission tomography; VT, ventricular tachycardia.

Can embryonic stem cells be used for cardiac repair?



Establishment in culture of pluripotent cells from mouse embryos

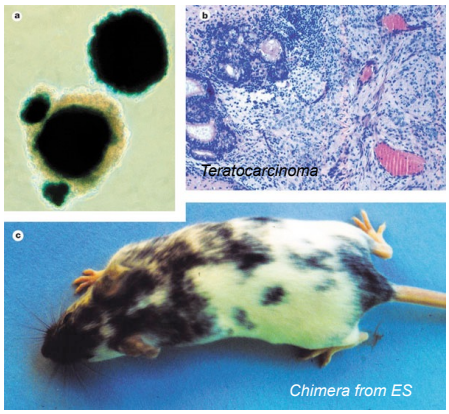


Normal caryotype!
Pluripotency!

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

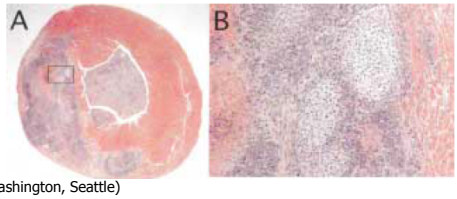
Pluripotency of mouse embryonic stem cells (ES)

Embryoid bodies



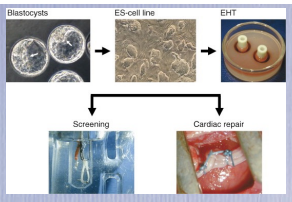
ES cells transplanted into the heart develop into a teratoma:

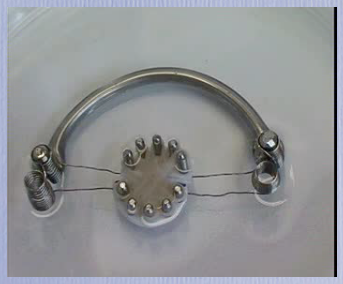
- extensive replacement of the ventricular wall and cavity with tumor, with central necrosis
- Multiple nodules of cartilage (mesodermal) at the interface with host myocardium
- Several poorly differentiated epithelial cells
- Gut epithelium and ciliated respiratory epithelium (endodermal)
- Stratified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

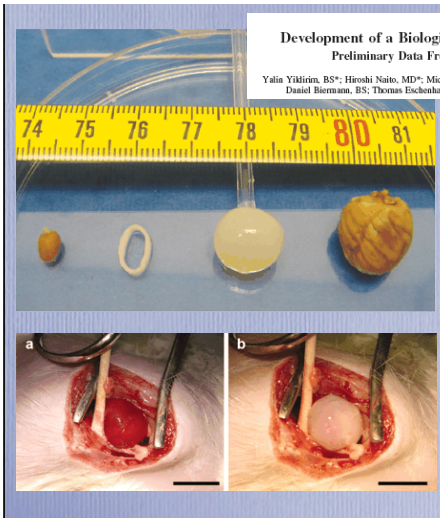


C. Murry (University of Washington, Seattle)

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

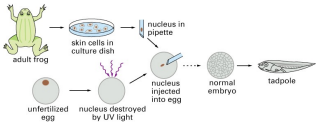
Giugno 2005: 4 referendum per abrogare parte della L40

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

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.

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
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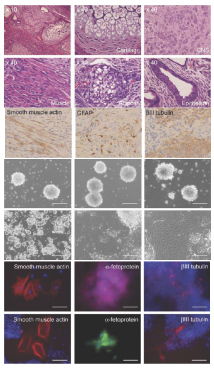
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024
Cell 126, 663–676, August 25, 2006 ©2006 Elsevier Inc.

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], Manhal Habib[1], Aaron Winterstern[1], Amira Gepstein[1], Gil Arbel[1] and Lior Gepstein[1,3,*]
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Received April 4, 2011.
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Abstract

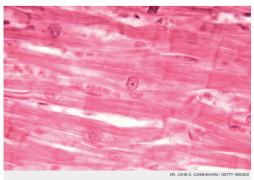
Aims Myocardial cell replacement therapies are hampered by a paucity of sources for human cardiomyocytes and by the expected immune rejection of allogeneic cell grafts. The ability to derive patient-specific human-induced pluripotent stem cells (hiPSCs) may provide a solution to these challenges. We 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

By ALEXANDRA SIFFERLIN @acsifferlin | May 23, 2012



theguardian

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

News › Science › Stem cells

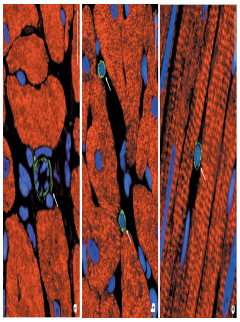
Skin from heart attack patients transformed into beating heart cells

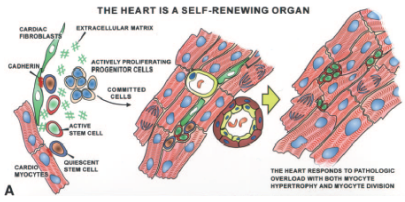
The heart cells created from patients' skin were at the same stage of development as those of a newborn baby

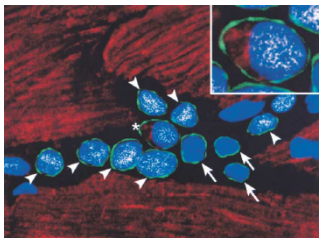
Ian Sample, science correspondent
guardian.co.uk, Wednesday 23 May 2012 00.06 BST
Comments (36)

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

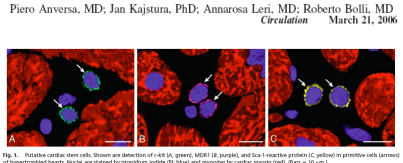
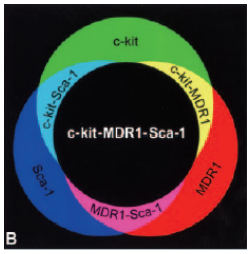
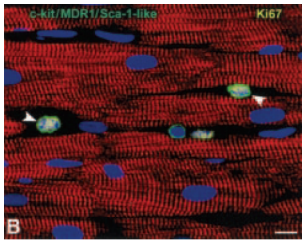
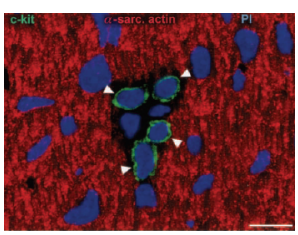


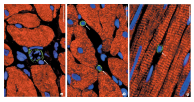
Fig. 1 Putative cardiac stem cells. Shown are detection of c-kit (A, green), MDR1 (B, purple), and Sca-1-reactive protein (C, yellow) in primitive cells (arrows) of hypertrophied hearts. Nuclei are stained by propidium iodide (PI, blue) and myocytes by cardiac myosin (red). (Bars = 10 μm.)







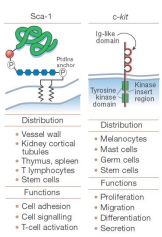
Cardiac stem cells: do they exist?



Supplementary Table 2. C o mparison 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^{++} transients • EC coupling • β-adrenergic response • action potentia l s	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??

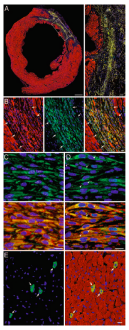
Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function

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

Roberto Bolli, Atul R Chugh, Domenico D'Amario, John H C Loughran, Marcus F Stoddard, Sohail Ikram, Garth M Beache, Stephen G Wagner, Annarosa Leri, Toru Hosoda, Fumihiro Sanada, Julius B Elmore, Polina Goichberg, Donato Cappetta, Naresh K Solankhi, Ibrahim Fahmy, D Gregg Rokosh, Mark S Slaughter, Jan Kajstura, Piero Anversa

Summary

Background c-kit-positive, lineage-negative cardiac stem cells (CSCs) improve post-infarction left ventricular (LV) dysfunction when administered to animals. We undertook a phase 1 trial (Stem Cell Infusion in Patients with Ischemic cardiOmyopathy [SCIPIO]) of autologous CSCs for the treatment of heart failure resulting from ischaemic heart disease.

Methods In stage A of the SCIPIO trial, patients with post-infarction LV dysfunction (ejection fraction [EF] ≤40%) before coronary artery bypass grafting were consecutively enrolled in the treatment and control groups. In stage B, patients were randomly assigned to the treatment or control group in a 2:3 ratio by use of a computer-generated block randomisation scheme. 1 million autologous CSCs were administered by intracoronary infusion at a mean of 113 days (SE 4) after surgery; controls were not given any treatment. Although the study was open label, the echocardiographic analyses were masked to group assignment. The primary endpoint was short-term safety of CSCs and the secondary endpoint was efficacy. A per-protocol analysis was used. This study is registered with ClinicalTrials.gov, number NCT00474461.

Findings This study is still in progress. 16 patients were assigned to the treatment group and seven to the control group; no CSC-related adverse effects were reported. In 14 CSC-treated patients who were analysed, LVEF increased from 30·3% (SE 1·9) before CSC infusion to 38·5% (2·8) at 4 months after infusion (p=0·001). By contrast, in seven control patients, during the corresponding time interval, LVEF did not change (30·1% [2·4] at 4 months after CABG vs 30·2% [2·5] at 4 months after CABG). Importantly, the salubrious effects of CSCs were even more pronounced at 1 year in eight patients (eg, LVEF increased by 12·3 ejection fraction units [2·1] vs baseline, p=0·0007). In the seven treated patients in whom cardiac MRI could be done, infarct size decreased from 32·6 g (6·3) by 7·8 g (1·7; 24%) at 4 months (p=0·004) and 9·8 g (3·5; 30%) at 1 year (p=0·04).

Interpretation These initial results in patients are very encouraging. They suggest that intracoronary infusion of autologous CSCs is effective in improving LV systolic function and reducing infarct size in patients with heart failure after myocardial infarction, and warrant further, larger, phase 2 studies.

www.thelancet.com Vol 378 November 26, 2011

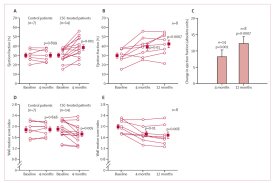


Figure 4: Echocardiographic analysis of CSC-treated patients and controls

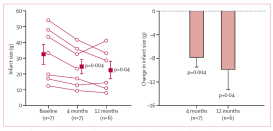
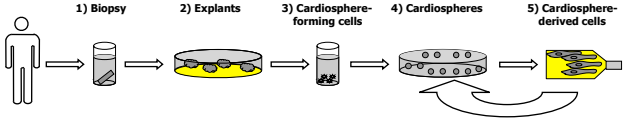
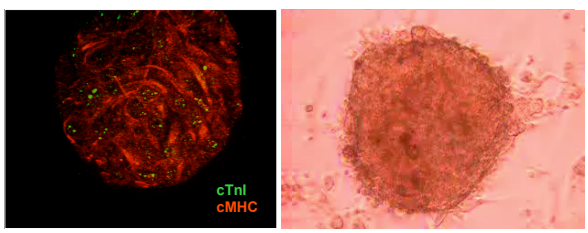


Figure 5: Infarct size and change in infarct size at 4 months and 12 months after baseline in patients administered cardiac stem cells

Cardiospheres





L. Barile

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

Raj R Makkar, Rachel R Smith, Ke Cheng, Konstantinos Malliaras, Louise E J Thomson, Daniel Berman, Lawrence S C Czer, Linda Marbán, Adam Mendizabal, Peter V Johnston, Stuart D Russell, Karl H Schuleri, Albert C Lardo, Gary Gerstenblith, Eduardo Marbán

Summary

Background Cardiosphere-derived cells (CDCs) reduce scarring after myocardial infarction, increase viable myocardium, and boost cardiac function in preclinical models. We aimed to assess safety of such an approach in patients with left ventricular dysfunction after myocardial infarction.

Methods In the prospective, randomised CAdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction (CADUCEUS) trial, we enrolled patients 2–4 weeks after myocardial infarction (with left ventricular ejection fraction of 25–45%) at two medical centres in the USA. An independent data coordinating centre randomly allocated patients in a 2:1 ratio to receive CDCs or standard care. For patients assigned to receive CDCs, autologous cells grown from endomyocardial biopsy specimens were infused into the infarct-related artery 1·5–3 months after myocardial infarction. The primary endpoint was proportion of patients at 6 months who died due to ventricular tachycardia, ventricular fibrillation, or sudden unexpected death, or had myocardial infarction after cell infusion, new cardiac tumour formation on MRI, or a major adverse cardiac event (MACE; composite of death and hospital admission for heart failure or non-fatal recurrent myocardial infarction). We also assessed preliminary efficacy endpoints on MRI by 6 months. Data analysers were masked to group assignment. This study is registered with ClinicalTrials.gov, NCT00893360.

Findings Between May 5, 2009, and Dec 16, 2010, we randomly allocated 31 eligible participants of whom 25 were included in a per-protocol analysis (17 to CDC group and eight to standard of care). Mean baseline left ventricular ejection fraction (LVEF) was 39% (SD 12) and scar occupied 24% (10) of left ventricular mass. Biopsy samples yielded prescribed cell doses within 36 days (SD 6). No complications were reported within 24 h of CDC infusion. By 6 months, no patients had died, developed cardiac tumours, or MACE in either group. Four patients (24%) in the CDC group had serious adverse events compared with one control (13%; p=1·00). Compared with controls at 6 months, MRI analysis of patients treated with CDCs showed reductions in scar mass (p=0·001), increases in viable heart mass (p=0·01) and regional contractility (p=0·02), and regional systolic wall thickening (p=0·015). However, changes in end-diastolic volume, end-systolic volume, and LVEF did not differ between groups by 6 months.

Interpretation We show intracoronary infusion of autologous CDCs after myocardial infarction is safe, warranting the expansion of such therapy to phase 2 study. The unprecedented increases we noted in viable myocardium, which are consistent with therapeutic regeneration, merit further assessment of clinical outcomes.

Funding US National Heart, Lung and Blood Institute and Cedars-Sinai Board of Governors Heart Stem Cell Center.

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

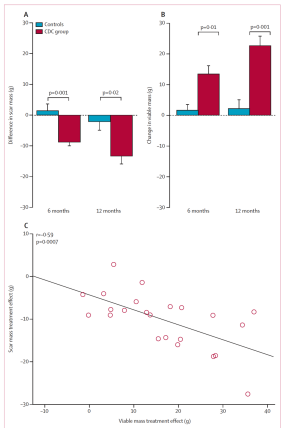
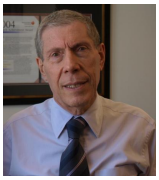


Figure 4: Scar mass and viable left ventricular mass on MRI

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."

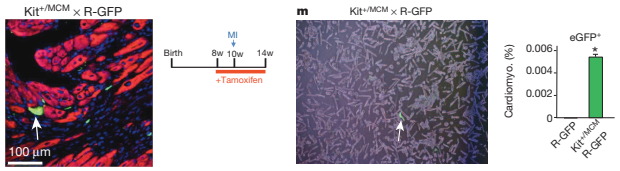
> "There are no stem cells in the heart. Quit trying to publish those results."
> JEFFERY MOLKENTIN, CINCINNATI CHILDREN'S

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.

European Heart Journal (2017) 38, 201–211
doi:10.1093/eurheartj/ehw240

BASIC SCIENCE

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

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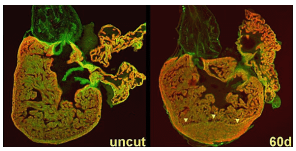
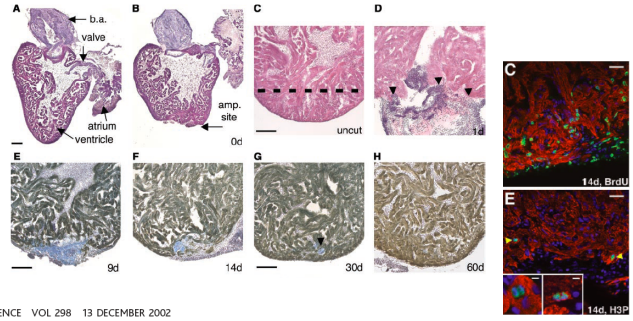
Heart Regeneration in Zebrafish

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

Cardiac injury in mammals and amphibians typically leads to scarring, with minimal regeneration of heart muscle. Here, we demonstrate histologically that zebrafish fully regenerate hearts within 2 months of 20% ventricular resection. Regeneration occurs through robust proliferation of cardiomyocytes localized at the leading epicardial edge of the new myocardium. The hearts of zebrafish with mutations in the Mps1 mitotic checkpoint kinase, a critical cell cycle regulator, failed to regenerate and formed scars. Thus, injury-induced cardiomyocyte proliferation in zebrafish can overcome scar formation, allowing cardiac muscle regeneration. These findings indicate that zebrafish will be useful for genetically dissecting the molecular mechanisms of cardiac regeneration.



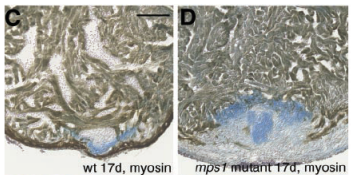


SCIENCE VOL 298 13 DECEMBER 2002

Why do zebrafish respond to cardiac injury with regeneration, whereas fibrosis predominates in other vertebrates?

mps1 mutant zebrafish form normal fibrin clots by day 8, but cardiac myofibers do not penetrate the clot

In these mutants, the ventricular wall cannot be restored; instead, the injured hearts retained fibrin deposits and developed large connective-tissue scars



Mps1 is a mitotic checkpoint kinase that is up-regulated in many proliferative cell types

Scarring might complement regeneration, so that the vigor of myocyte proliferation within a given species would determine the predominant response. According to this model, the inhibition of regeneration would lead to scarring

nature Vol 464 | 25 March 2010 | doi:10.1038/nature08899

LETTERS

Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation

Chris Jopling[1], Eduard Sleep[1,2]†, Marina Raya[1]†, Mercè Martí[1], Angel Raya[1,2,3]† & Juan Carlos Izpisúa Belmonte[1,2,4]

Although mammalian hearts show almost no ability to regenerate, there is a growing initiative to determine whether existing cardiomyocytes or progenitor cells can be coaxed into eliciting a regenerative response. In contrast to mammals, several non-mammalian vertebrate species are able to regenerate their hearts[1–3], including the zebrafish[4,5], which can fully regenerate its heart after amputation of up to 20% of the ventricle. To address directly the source of newly formed cardiomyocytes during zebrafish heart regeneration, we first established a genetic strategy to trace the lineage of cardiomyocytes in the adult fish, on the basis of the Cre/*lox* system widely used in the mouse[6]. Here we use this system to show that regenerated heart muscle cells are derived from the proliferation of differentiated cardiomyocytes. Furthermore, we show that proliferating cardiomyocytes undergo limited dedifferentiation characterized by the disassembly of their sarcomeric structure, detachment from one another and the expression of regulators of cell-cycle progression. Specifically, we show that the gene product of *polo-like kinase 1* (*plk1*) is an essential component of cardiomyocyte proliferation during heart regeneration. Our data provide the first direct evidence for the source of proliferating cardiomyocytes during zebrafish heart regeneration and indicate that stem or progenitor cells are not significantly involved in this process.

Regenerated cardiomyocytes are derived from differentiated, pre-existing cardiomyocytes

has been regenerated by cardiomyocytes. The exact source of these new cardiomyocytes is not yet known definitively. To address this question we developed and successfully implemented the 4-hydroxy-tamoxifen (4-OHT)-inducible Cre/*lox* approach in zebrafish to label regenerating cardiomyocytes genetically (for a detailed description of the lines generated and/or methodologies, see Methods and Supplementary Figs 1–9).

genetically labelled 48 h after fertilization. About 20% of the ventricle was removed, and cardiac regeneration was subsequently assessed at 7, 14 and 30 days after amputation. At 7 days after amputation, the remaining cardiac tissue was uniformly positive for green fluorescent protein (GFP) (Fig. 1a, b), with much of the missing tissue now replaced by a fibrin/collagen clot ($n = 5$ hearts) (Fig. 1c). At 14 days

($n = 7$ hearts; Fig. 1f). These results suggest that the regenerated cardiomyocytes arise from differentiated GFP-positive cardiomyocytes. These findings were substantiated at 30 days after amputation, when regeneration is nearly complete; all of the cardiomyocytes within the

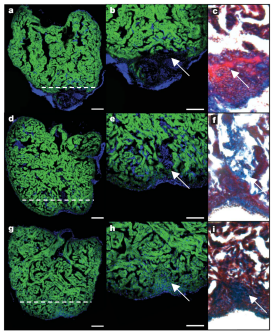


Figure 1 | Regenerated cardiomyocytes are derived from differentiated cardiomyocytes. Cardiomyocytes in transgenic zebrafish (tg-cmlc2a-Cre-Ert2; tg-cmlc2a-LnL–GFP) were genetically labelled at 48 h after fertilization by inducing Cre activity with tamoxifen. These embryos were then grown to adulthood (3 months or sexually mature), at which point the heart was amputated and allowed to regenerate for 7 (**a**–**c**), 14 (**d**–**f**) or 30 (**g**–**i**) days. The dashed white line represents the plane of amputation. At 7 days after amputation (**a**; enlargement in **b**) relatively little regeneration has occurred. Trichromic staining indicates that a fibrin clot has formed adjacent to the wound (**c**). By 14 days after amputation, GFP-positive cardiomyocytes have regenerated a substantial amount of new cardiac tissue (**d**; enlargement in **e**) and the fibrin clot was decreased in size (**f**). At 30 days after amputation, heart regeneration is virtually complete (**g**; enlargement in **h**) and all of the regenerated tissue is composed of GFP-positive cardiomyocytes. The clot has been replaced by a small scar (**h**). Scale bars, 100 μm (**a**, **d**, **g**) and 75 μm (**b**, **e**, **h**). Panels **c**, **f** and **i** are ×2 magnifications of the areas indicated with a white arrow in **b**, **e** and **h**.

Differentiating cardiomyocytes re-enter the cell cycle

We next sought to determine whether GFP-positive cardiomyocytes had re-entered the cell cycle. Adult GFP-positive transgenic zebrafish were treated with bromodeoxyuridine (BrdU) for 7 days after amputation (Fig. 2a–f). Subsequently, at 14 days after amputation, we found a significant increase in the number of BrdU-positive/GFP-positive cardiomyocytes in regenerating hearts compared with non-amputated controls (Fig. 2g). From this we conclude that differentiated GFP-positive cardiomyocytes had re-entered the cell cycle and engaged in DNA replication. We also analysed the position of BrdU-labelled GFP-positive cardiomyocytes within the regenerating heart (Fig. 2h and inset). Whereas most BrdU-positive/GFP-positive labelled cardiomyocytes were concentrated around the wound, a proportion could also be found in regions far from the site of amputation. This suggests that the response to the injury affects the heart in a global manner.

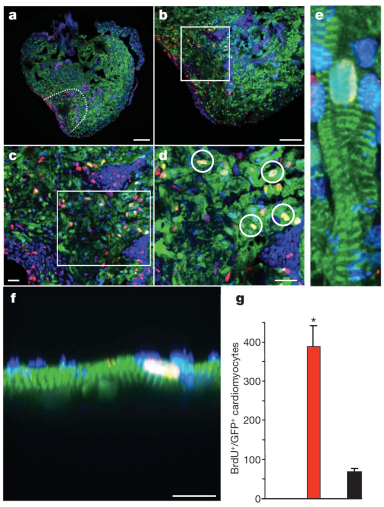


Figure 2 | Differentiated cardiomyocytes re-enter the cell cycle. (**a**–**f**) Transgenic zebrafish (tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL–GFP) genetically labelled at 48 h after fertilization and grown to adulthood underwent cardiac amputation and were then treated with BrdU for 7 days after amputation. Hearts were isolated and processed at 14 days after amputation. Green, GFP-positive cardiomyocytes; red, BrdU-positive cells; blue, 4,6-diamidino-2-phenylindole stain for DNA; yellow, BrdU-positive/GFP-positive cardiomyocytes (white rings in **d**). **a**, Section of the entire heart, with a dashed white line representing the regenerating area. **b**, Enlargement of the regenerating area. **c**, **d**, Enlargements of the boxed areas in **b** and **c**, respectively. **e**, An *XY* reconstruction of an individual BrdU-positive/GFP-positive cardiomyocyte within a regenerating heart 14 days after amputation. **f**, An *XZ* reconstruction of the BrdU-positive/GFP-positive cardiomyocyte shown in **e**. **g**, The average number of BrdU-positive/GFP-positive cardiomyocytes per section (means and s.e.m.). Asterisk, $P < 0.01$ (*t*-test). Amputated (red bar), $n = 17$ sections from seven different animals; control (black bar), $n = 9$ sections from three different animals.

Regenerating cardiomyocyte partially disassemble the contractile apparatus but not revert to an embryonic stage

lineage they regress[7,8]. An increase in the expression of the cardiac-progenitor-associated genes *nkx2.5* and *hand2* during zebrafish heart regeneration has been reported[9]. However, our own *in situ* hybridization analyses failed to detect any significant upregulation of either transcript (data not shown), confirming previous results from our laboratory[5]. Furthermore, genome-wide transcriptome data[10,11] also failed to detect significant changes in the expression of either transcript during zebrafish heart regeneration. These results argue against an extensive dedifferentiation of cardiomyocytes as a prerequisite for their proliferation in the context of heart regeneration.

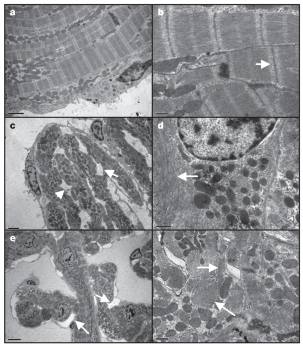


Figure 3 | Cardiomyocytes dedifferentiate, resulting in the disassembly of sarcomeric structure and detachment. Electron microscopy of sections of a control heart (**a**, **b**) and a regenerating heart at 5 days (**c**, **d**) and 7 days (**e**, **f**) after amputation. Cardiomyocytes in unamputated control samples show a tightly organized sarcomeric structure (**a**); at higher magnification (**b**) the Z-lines are clearly visible (arrow). At 5 days after amputation many of the cardiomyocytes have a disorganized sarcomeric structure (**c**) along with the appearance of intercellular spaces (arrows). Closer examination reveals a loss of Z-lines (**d**, arrow). At 7 days after amputation there is a similar loss of structure and appearance of intercellular spaces (**e**, arrows). At higher magnification (**f**) myosin fibres are visible (arrows); however, both longitudinal (upper arrow) and transverse (lower arrow) fibres are present within the same cardiomyocyte, indicating disorganized sarcomeric structure. Scale bars, 0.5 μm (**a**, **b**, **d**) and 2 μm (**c**, **e**, **f**).

In that organism the expression of cardiac sarcomeric genes is down-regulated after amputation; then, as regeneration proceeds, the expression returns to pre-amputation levels[15]. Similar structural changes are also associated with hibernating myocardium in humans after cardiac injury[16]. Hibernating cardiomyocytes typically show a depletion of sarcomeric structure and an expression pattern of structural proteins closely resembling that in fetal heart cells[17]. Although

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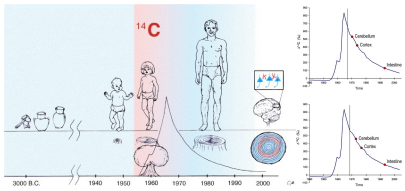
Carbon dating of cardiomyocytes in human hearts indicates a lifetime turnover rate of 50%.

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.

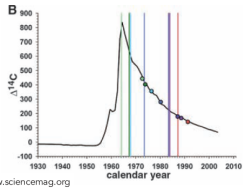


Evidence for Cardiomyocyte Renewal in Humans

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

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

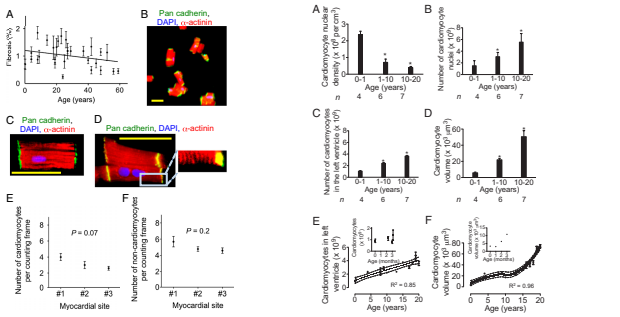
Fewer than 50% of cardiomyocytes are exchanged during a normal life span.



3 APRIL 2009 VOL 324 SCIENCE www.sciencemag.org

Cardiomyocyte proliferation contributes to heart growth in young humans

Mariya Mollova[a,b,1,2], Kevin Bersell[a,c,d,1], Stuart Walsh[a,b], Jainy Savla[a,c,3], Lala Tanmoy Das[a,4], Shin-Young Park[b,e], Leslie E. Silberstein[e,f], Cristobal G. dos Remedios[g], Dionne Graham[a,b], Steven Colan[a,d], and Bernhard Kühn[a,b,h,i,5]



Human cardiomyocytes proliferate and enlarge after birth.

1446–1451 | PNAS | January 22, 2013 | vol. 110 | no. 4

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.**

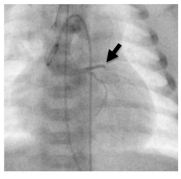
Methods and Results: **Here, we report the case of a newborn child having a severe myocardial infarction due to coronary artery occlusion. The child developed massive cardiac damage as defined by serum markers for cardiomyocyte cell death, electrocardiograms, echocardiography, and cardiac angiography. 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

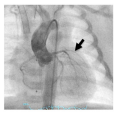
Clinical case

- 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

- 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

Haubner et al., Circulation Research 2016

Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors

Masaki Ieda,[1,2,3,6,*] Ji-Dong Fu,[1,2,3] Paul Delgado-Olguin,[1,2,4] Vasanth Vedantham,[1,5] Yohei Hayashi,[1] Benoit G. Bruneau,[1,2,4] and Deepak Srivastava[1,2,3,*]
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DOI 10.1016/j.cell.2010.07.002
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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.

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