HHS Public Access

Author manuscript

Circulation. Author manuscript; available in PMC 2018 August 15.

Published in final edited form as:

Circulation. 2017 August 15; 136(7): 680–686. doi:10.1161/CIRCULATIONAHA.117.029343.

Cardiomyocyte Regeneration: A Consensus Statement

Thomas Eschenhagen, MD[1,*], **Roberto Bolli, MD**[2], **Thomas Braun, MD**[3], **Loren J. Field, PhD**[4], **Bernd K. Fleischmann, MD**[5], **Jonas Frisén, MD, PhD**[6], **Mauro Giacca, MD**[7], **Joshua M. Hare, MD**[8], **Steven Houser, PhD**[9,*], **Richard T. Lee, MD**[10], **Eduardo Marbán, MD**[11], **James F. Martin, MD, PhD**[12], **Jeffery D. Molkentin, PhD**[13], **Charles E. Murry, MD, PhD**[14], **Paul R. Riley, PhD**[15], **Pilar Ruiz-Lozano, PhD**[16], **Hesham A. Sadek, MD, PhD**[17,*], **Mark A. Sussman, PhD**[18], and **Joseph A. Hill, MD, PhD**[17,*]

[1]Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg Eppendorf, Martinistraße 52, 20246 Hamburg, Germany, and DZHK (German Center for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Germany

[2]Institute of Molecular Cardiology, University of Louisville, Louisville, KY 40292 USA

[4]Max-Planck-Institute for Heart and Lung Research, Ludwigstrasse 43, 61231 Bad Nauheim, Germany and University of Giessen, Department of Internal Medicine II, Klinikstr.33, 35392 Giessen, Germany, member of the German Center for Cardiovascular Research (DZHK), member of the German Center for Lung Research (DZL)

[4]Krannert Institute of Cardiology & Wells Center for Pediatric Research Indiana University School of Medicine

[5]Institute of Physiology I, Life and Brain Center, Medical Faculty, University of Bonn Sigmund-Freudstr. 25 D-53127 Bonn

Correspondence: Thomas Eschenhagen, Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg Eppendorf, Martinistraße 52, 20246 Hamburg, Germany, and DZHK (German Center for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Germany, Tel: +49-40-7410-52180; Fax: +49-40-7410-54876, teschenhagen@uke.de, Joseph A. Hill, Division of Cardiology, Departments of Internal Medicine and Molecular Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX, USA, 75390-8573, Tel: 1-214-648-1400; Fax: 1-214-648-1450, joseph.hill@utsouthwestern.edu.

*Served in the role of discussion moderator

DISCLOSURES

TE is co-founder of EHT-Technologies GmbH, a company providing instrumentation for the generation of engineered heart tissue.
RB has no disclosures to report.
TB has no disclosures to report.
LJF has no disclosures to report.
BKF is a stockholder in Axiogenesis
JF is co-founder and has significant ownership in Spatial Transcriptomics AB
MG has no disclosures to report.
JMH is a stockholder in Vestion, Inc., Heart Genomics, Biscayne Pharma, and Longeveron, LLC.
SH has no disclosures to report.
RTL is a consultant to Mesoblast and founder of ProteoThera.
EM has significant ownership in Capricor.
JFM has no disclosures to report.
JDM has no disclosures to report.
CEM is a scientific founder and equity holder in BEAT Biotherapeutics.
PRR a cofounder of OxStem Cardio an Oxford University spin-out which seeks to exploit therapeutic strategies stimulating endogenous repair in cardiovascular regenerative medicine.
PR-L is a shareholder of Regencor Inc.
HAS has no disclosures to report.
MAS is co-founder and Chief Scientific Officer for Cardiocreate Inc. and holds a significant interest in the company.
JAH has no disclosures to report.

[6]Department of Cell and Molecular Biology, Karolinska Institute, SE-171 77 Stockholm, Sweden

[7]International Centre for Genetic Engineering and Biotechnology (ICGEB) AREA Science Park, Padriciano 99, 34149 Trieste, Italy

[8]Donald Soffer Endowed Program in Regenerative Medicine Miller School of Medicine and adjunct Professor of Medicine at Johns Hopkins University School of Medicine

[9]Department of Physiology, Lewis Katz School od Medicine, Temple University, Philadelphia, PA

[10]Department of Stem Cell and Regenerative Biology, Harvard University

[11]The Heart Institute Cedars-Sinai Heart Institute

[12]Cardiomyocyte Renewal Laboratory, Texas Heart Institute, Houston, Texas 77030. Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030

[13]Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, USA

[14]Departments of Pathology, Bioengineering and Medicine/Cardiology; Institute for Stem Cell and Regenerative Medicine; Center for Cardiovascular Biology, University of Washington, Seattle WA 98109

[15]University of Oxford, Department of Physiology, Anatomy and Genetics, South Parks Road, Oxford OX1 3PT, United Kingdom

[16]Regencor, Inc., Los Altos, CA 94022

[17]Departments of Internal Medicine (Division of Cardiology) and Molecular Biology, UT Southwestern Medical Center, Dallas, Texas, USA

[18]SDSU Heart Institute Integrated Regenerative Research Institute, San Diego State University, Biology Department

Background

Cell therapy is an exciting option for repairing the injured heart, one which has attracted considerable interest over the past 15 years. Consensus exists that the injection/infusion or tissue-based implantation of various cell types may exert therapeutic effects[1–3], and there is general agreement that additional molecular, translational and clinical studies are required to define the optimal cell source, method of delivery, and underlying mechanism(s) of action.

One of the remaining questions in this field pertains to cardiomyocyte turnover under normal and diseased conditions and its contribution to the beneficial effects of cell therapy. While results published in the literature have not been consistent, we believe that time is ripe to formulate a consensus regarding many of the pertinent questions.

It is important to emphasize that the focus of this consensus statement is on cardiomyocyte renewal; it is not on cell therapy in general. Whereas we touch on some aspects of therapeutic strategies based on delivery of exogenous cells, our intent here is to define areas

of agreement, and areas requiring further elucidation related to the regenerative potential of the myocardium itself.

We have included references to the scientific literature throughout the document. Whereas it is impossible for us to include all publications in this expansive field, representative studies that corroborate statements herein have been cited.

Central questions

1. Definition of cardiomyocyte renewal

In this consensus statement, the term “cardiomyocyte renewal” is defined as the ability to replace lost cardiomyocytes by new ones. It is distinct from the turnover of cardiac proteins or the generation of polyploid cardiomyocytes (i.e. those harboring more than two sets of chromosomes), either by nuclear division giving rise to multinucleation or by duplication of DNA without nuclear division resulting in polyploid nuclei.

2. Naturally occurring cardiomyocyte renewal and proliferation

- During normal mammalian development
  - **i.** Growth of the heart during embryonic and fetal development involves an absolute increase in the number of cardiomyocytes and is brought about by differentiation of precursor cells and by division of relatively immature cardiomyocytes.
  - **ii.** The rodent heart continues to grow by means of cardiomyocyte proliferation (hyperplastic growth) in the early postnatal period.[4] During a brief postnatal window of 7 days in rodents, myocardial injury induces a regenerative response resulting in replacement of lost cardiomyocytes by new ones.[5] Fate mapping studies suggest that this type of myocardial regeneration is mediated primarily by cardiomyocyte proliferation.[5] It remains unclear whether this regenerative window exists in large animals or in humans.
  - **iii.** While cardiomyocytes appear to continue to renew throughout life, the quantitatively dominant mechanism of growth in the mammalian postnatal heart is an increase in cardiomyocyte size (reviewed in Heineke et al[6]).
  - **iv.** In the healthy, uninjured adult human and murine heart, the total number of cardiomyocytes remains essentially stable, and cardiomyocyte turnover is currently estimated at 0.5–2% per year in both species.[7–9]
- Following cardiac injury in adult mammals
  - **i.** Cardiomyocyte renewal rates may be higher after injury than under normal conditions.[9]

ii. The experimental determination of cardiomyocyte turnover after cardiac injury can be challenging owing to inflammation, proliferation of stromal and vascular cells, and scar formation.

- After heart or bone marrow transplantation (chimerism)

  i. Sex-mismatched heart transplantation in patients with end-stage heart failure or sex-mismatched bone marrow transplantation provide opportunities to ascertain experimentally cardiomyocyte renewal deriving from extra-cardiac sources.

  ii. While data are not completely consistent, the preponderance of studies suggest that the level of cardiomyocyte chimerism after sex-mismatched transplantation is <1%[10–12], and may arise at least partially from fusion events[10].

  iii. Insufficient data are available to determine the time course within which such chimerism develops.

3. Mechanisms of endogenous cardiomyocyte renewal

There is no infallible means of tracking cell renewal in any organ system. However, in preclinical models of cardiomyocyte renewal (e.g. mouse and fish), genetic fate mapping studies provide the strongest level of scientific evidence. Critical biological issues such as promoter fidelity (leakiness and sensitivity), inefficient reporter expression (Cre recombinase activity), and cellular fusion or transfer of reporter proteins are relevant and must be considered in the interpretation of the findings. Further, appropriate control studies are essential to assess for deleterious consequences of haploinsufficiency which could result from genetic manipulation of an endogenous gene locus.

- Cardiomyocyte proliferation

  i. The majority of studies suggest that cardiomyocyte renewal in the uninjured adult heart derives from a modest level of pre-existing cardiomyocyte mitosis.[13–15] Support for this interpretation derives from experiments in zebrafish[1617], newts[18] and other species[19] in which cardiomyocyte renewal occurs more robustly than in mammals.

- Resident stem/progenitor cells

  i. Resident stem/progenitor cells contribute to multiple cell types within the ventricle, including cardiomyocytes. However, in terms of adult myocardial homeostasis in mice, current evidence suggests that their contribution under basal conditions or after cardiac injury is low (estimates in rodents based on genetic fate-mapping experiments suggest a rate of <0.01% per year).[20, 21]

- Extracardiac stem/progenitor cells

  i. The contribution of extracardiac stem or progenitor cells to cardiomyocyte renewal has been studied largely with chimeric mice, in which the bone marrow is genetically labeled, and in parabiotic mice,

in which the circulation of a genetically labeled mouse is experimentally linked to another unlabeled mouse. Cell fusion and transdifferentiation events have been evaluated using genetic lineage tracing, and the findings are largely concordant. In humans, the role of extracardiac stem/progenitor cells in cardiomyocyte renewal has been studied by sex-mismatched heart and bone marrow transplantation.

ii. Homing of extracardiac bone marrow-derived cells to the uninjured heart is a rare event of uncertain physiologic relevance.

iii. Extracardiac bone marrow-derived cells enter the injured heart at a higher rate. The majority of these cells are of hematopoietic origin.

iv. A small fraction of cardiomyocytes within injured rodent hearts carry the genetically determined label of bone marrow cells (estimates in rodents based on genetic fate-mapping experiments suggest a rate of <0.2%[22, 23]). Most studies suggest that the majority of these cells originate from cell fusion, and <1% derive from transdifferentiation (estimates in rodents based on genetic fate-mapping experiments suggest a rate of <0.002% in total[23]).

4. Therapeutic manipulation of cardiomyocyte renewal

i. Most studies suggest that the infusion, injection or tissue-based implantation of cells of various origins confer therapeutic benefits to the injured heart.

ii. Cell-based therapies may affect endogenous cardiomyocyte renewal and/or directly generate new cardiomyocytes from the transplanted cells.

iii. The degree of new cardiomyocyte formation depends on the cell type, as well as on retention and survival of those cells within the heart. Retention of unselected bone marrow cells in the heart is low (a study in patients determined a rate of <3% for unselected bone marrow cells and approximately 10-fold higher with $CD34^+$ cells 1 hour after coronary infusion[24]). It may be higher following cell injection into the myocardium.[25] Co-injection of scaffolding materials and use of tissue engineering approaches may increase this rate.[26]

iv. The degree of engraftment and differentiation of transplanted cells into cardiomyocytes does not appear to match the extent of functional improvement, suggesting that other mechanisms account for at least part of the beneficial effects of cell therapy.[27]

v. Mechanisms of benefit of cellular transplantation experiments remain obscure but may involve paracrine actions, including exosome-derived effects on pre-existing cardiac tissue[28, 29], as well as cell-specific post-translational protein modifications.[30]

vi. Transplantation of cardiomyocytes derived from pluripotent stem cells can generate new myocardium that beats in synchrony with the host myocardium and

may contribute to systolic force generation, although the extent of this contribution has not been precisely determined.

- Bone marrow-derived cells
  - **i.** Prevailing evidence suggests that unfractionated bone marrow-derived cells do not become cardiomyocytes when infused or injected into the heart.[31–33]
  - **ii.** Fractionated bone marrow populations consisting of c-kit$^+$ cells or mesenchymal stem cells may confer structural and/or functional benefits primarily by indirect biological activities that may promote cardiomyocyte renewal.[3435, 36]
  - **iii.** Initial studies with bone marrow-derived mesenchymal cells are promising[37] and phase 3 trials are underway.
  - **iv.** Evidence for the ultimate fate of mesenchymal cells after infusion or injection into the heart is inconsistent, but some studies report unmanipulated mesenchymal cells can transdifferentiate into cardiomyocytes at low rates.[38, 39]
- Cardiac-derived stem/progenitor cells
  - **i.** Most experiments have been performed with c-kit$^+$, cardiosphere-derived cells, or Sca1$^+$ cells isolated from heart biopsies and cultured in vitro.
  - **ii.** These cells can emerge as cells expressing cardiomyocyte markers when cultured in vitro under specific conditions, and they can also express some cardiomyocyte markers in vivo.[4041] Co-culturing cardiac c-kit$^+$ cells with mesenchymal stem cells enhances their lineage commitment towards a cardiac myocyte fate.[42]
  - **iii.** The degree of functional improvement following in vivo delivery of cardiac-derived stem/progenitor cells cannot be explained solely by new cardiomyocyte formation from transplanted cells, which is very low.[43, 44]
  - **iv.** Genetic or ex vivo manipulation of transplanted cardiac-derived stem/progenitor cells enhances engraftment as well as structural and functional recovery of uninjured myocardium in preclinical animal models.[45, 46]
- Pluripotent cells
  - **i.** Pluripotent stem cells (embryonic stem cells [ESCs] or induced pluripotent stem cells [iPSCs]) proliferate in an undifferentiated state indefinitely, and upon exposure to specific culture conditions can differentiate into almost all cell types of the organism including cardiomyocytes.

ii. The efficiency of differentiation of pluripotent stem cells into immature cardiomyocytes in vitro can exceed 80%.[47–51]

iii. Undifferentiated pluripotent stem cells can form teratomas when injected into the heart of immunocompromised organisms.[52]

iv. Pluripotent stem cell-derived cardiomyocytes successfully engraft, generating new myocardium when injected into the injured or uninjured heart of immunosuppressed animals.53–58[a] Long-term engraftment (> 3 months) of these cells has not been studied.

v. Pluripotent stem cell-derived cardiomyocytes can couple electrically with host cardiomyocytes, beating in synchrony, although evidence for proarrhythmic effects has been reported.[54, 58]

vi. Although direct force generation deriving from the injected myocytes may explain some of the functional improvement, it is not clear whether the degree of emergence of new myocardium entirely accounts for the degree of contractile improvement; paracrine signalling events may contribute as well.

- Stimulation of endogenous cardiomyocyte proliferation[b]

i. The normal turnover of cardiomyocytes can be stimulated as a therapeutic strategy to achieve regeneration.

ii. Endogenous cardiomyocyte proliferation can be enhanced by manipulation of cell cycle regulators[59, 60], redox regulators[61–63], growth factors acting through cell surface receptors[6430] or through the transfer of nucleic acids acting intracellularly[176566].

5. Important questions remaining to be answered

i. Identify mechanisms of endogenous cardiomyocyte renewal in mammals as a target for therapy, including mechanisms of cardiomyocyte proliferation and characterization of populations of proliferative cardiomyocytes.

ii. Define the relative roles of progenitor cell differentiation versus cardiomyocyte proliferation in regenerating the injured myocardium.

iii. Unveil mechanism(s) of benefit deriving from cell-based therapy, including the contribution of new cardiomyocytes, angiogenesis, anti-inflammatory actions, anti-fibrotic actions, anti-apoptotic actions, or other effects.

iv. Define the paracrine mechanisms or host immune response signals that mediate many of the beneficial effects of cell therapy.

v. Improve the efficiency of cell therapy with regard to modes of delivery, enhancement of engraftment, and differentiation.

[a] M.S. expressed concerns regarding use of the term “new myocardium” in this sentence.
[b] M.S. cited efficacy and feasibility concerns for “therapeutic strategy” implementation and caution regarding discrimination of “proliferation” from cell cycle induction without mitosis.

vi. Explore new therapeutic options that provide the same beneficial effects as cellular transplantation, either through exosomes, selected paracrine factors, or induction of the innate and adaptive sterile immune responses in the heart.

vii. Define the risk/benefit aspects for genetically modified stem cells, pluripotent stem cell-based therapies, and cell combination strategies.

Acknowledgments

SOURCES OF FUNDING

Work by RB was supported by grants from the NIH (HL-113530 and HL-78825).

Research in the TB laboratory was mainly funded by the Max Planck Society (MPS), the Deutsche Forschungsgemeinschaft (DFG), the German Bundesministerium für Bildung und Forschung (BMBF) ERA-CVD, the Deutsches Zentrum for Herz- und Kreislaufforschung (DZHK), the Deutsches Zentrum für Lungenforschung (DZL), and the Foundation Leducq (Cardiostemnet).

The work of TE in this field was supported by research grants from the DZHK (German Centre for Cardiovascular Research), the German Ministry of Research and Education (BMBF), the European Research Council (ERC-AG IndivuHeart), the German Research Foundation (Es 88/12-1), the British Heart Foundation (Regenerative Medicine Centre) and the EU (EU FP7 Biodesign).

Work by LJF was supported by a grant from the NIH (HL132927).

Work by BF was supported by grants from Deutsche Forschungsgemeinschaft (FL 276/7-2 and Research Training Group 1873).

Work by MG was supported by grants from the Leducq Foundation Transatlantic Network of Excellence (14CVD04) and the Italian Ministry of Health (RF-2011-02348164).

Work by JMH was funded by grants from the NIH/NHLBI (R01 HL107110, 1R01HL134558-01, 4R01HL084275-10, and 5R01HL116899-04); the NHLBI (HHSN268201600012I); the NIH CCTRN (4UM1HL113460-05); the NIH/NCI (5R01CA136387-07); and the Marcus Foundation, Inc. (Grant ID 2164, and Grant ID 2248).

Work by RTL was funded by grants from the NIH (HL119230 and HL117986).

Work by EM was supported by grants from the NIH (R01HL124074-01), the DoD (PR150620), and CIRM (RB4-06215).

Work by JFM was supported by grants from the NIH (DE 023177, HL 127717, HL 130804, and HL 118761), the Vivian L. Smith Foundation, and the LeDucq Foundation Transatlantic Networks of Excellence in Cardiovascular Research Award (14CVD01).

Work by CM was supported byu grants from the NIH (P01HL094374, P01GM081619, and R01HL12836) and a grant from the Fondation Leducq Transatlantic Network of Excellence (CEM).

Work by PRR was supported by grants from the British Heart Foundation (RG/13/9/30269 and CH/11/1/28798) and from the BHF Oxbridge Regenerative Medicine Centre (RM/13/3/30159).

Work by HAS was supported by grants from the NIH (R01HL115275 and R01HL131778), NASA (NNX15AE06G), the AHA (16EIA27740034), and CPRIT (RP160520).

Work by MAS was supported by grants from the NIH (R01HL067245, R37HL091102, R01HL105759, R01HL113647, R01HL117163, P01HL085577, and R01HL122525), as well as an award from the Fondation Leducq.

Work by JAH was supported by grants from the NIH (HL-120732; HL-100401; HL-128215), American Heart Association (14SFRN20510023; 14SFRN20670003), Fondation Leducq (11CVD04), and Cancer Prevention and Research Institute of Texas (RP110486P3).

Work by SH was supported by a grant from the NIH (HL33921).

References

1. Hare JM, DiFede DL, Rieger AC, Florea V, Landin AM, El-Khorazaty J, Khan A, Mushtaq M, Lowery MH, Byrnes JJ, Hendel RC, Cohen MG, Alfonso CE, Valasaki K, Pujol MV, Golpanian S, Ghersin E, Fishman JE, Pattany P, Gomes SA, Delgado C, Miki R, Abuzeid F, Vidro-Casiano M, Premer C, Medina A, Porras V, Hatzistergos KE, Anderson E, Mendizabal A, Mitrani R, Heldman AW. Randomized Comparison of Allogeneic Versus Autologous Mesenchymal Stem Cells for Nonischemic Dilated Cardiomyopathy: POSEIDON-DCM Trial. J Am Coll Cardiol. 2017; 69:526–537. [PubMed: 27856208]
2. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marban L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marban E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet. 2012; 379:895–904. [PubMed: 22336189]
3. Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet. 2011; 378:1847–1857. [PubMed: 22088800]
4. Soonpaa MH, Field LJ. Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. Am J Physiol. 1997; 272:H220–H226. [PubMed: 9038941]
5. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA. Transient regenerative potential of the neonatal mouse heart. Science. 2011; 331:1078–1080. [PubMed: 21350179]
6. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. Nat Rev Mol Cell Biol. 2006; 7:589–600. [PubMed: 16936699]
7. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabe-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisen J. Evidence for cardiomyocyte renewal in humans. Science. 2009; 324:98–102. [PubMed: 19342590]
8. Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, Sjostrom SL, Szewczykowska M, Jackowska T, Dos Remedios C, Malm T, Andra M, Jashari R, Nyengaard JR, Possnert G, Jovinge S, Druid H, Frisen J. Dynamics of Cell Generation and Turnover in the Human Heart. Cell. 2015; 161:1566–1575. [PubMed: 26073943]
9. Hsieh PC, Segers VF, Davis ME, MacGillivray C, Gannon J, Molkentin JD, Robbins J, Lee RT. Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. Nat Med. 2007; 13:970–974. [PubMed: 17660827]
10. Laflamme MA, Myerson D, Saffitz JE, Murry CE. Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. Circ Res. 2002; 90:634–640. [PubMed: 11934829]
11. Muller P, Pfeiffer P, Koglin J, Schafers HJ, Seeland U, Janzen I, Urbschat S, Bohm M. Cardiomyocytes of noncardiac origin in myocardial biopsies of human transplanted hearts. Circulation. 2002; 106:31–35. [PubMed: 12093766]
12. Hocht-Zeisberg E, Kahnert H, Guan K, Wulf G, Hemmerlein B, Schlott T, Tenderich G, Korfer R, Raute-Kreinsen U, Hasenfuss G. Cellular repopulation of myocardial infarction in patients with sex-mismatched heart transplantation. Eur Heart J. 2004; 25:749–758. [PubMed: 15120885]
13. Kimura W, Xiao F, Canseco DC, Muralidhar S, Thet S, Zhang HM, Abderrahman Y, Chen R, Garcia JA, Shelton JM, Richardson JA, Ashour AM, Asaithamby A, Liang H, Xing C, Lu Z, Zhang CC, Sadek HA. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. Nature. 2015; 523:226–230. [PubMed: 26098368]
14. Senyo SE, Steinhauser ML, Pizzimenti CL, Yang VK, Cai L, Wang M, Wu TD, Guerquin-Kern JL, Lechene CP, Lee RT. Mammalian heart renewal by pre-existing cardiomyocytes. Nature. 2013; 493:433–436. [PubMed: 23222518]
15. Ali SR, Hippenmeyer S, Saadat LV, Luo L, Weissman IL, Ardehali R. Existing cardiomyocytes generate cardiomyocytes at a low rate after birth in mice. Proc Natl Acad Sci U S A. 2014; 111:8850–8855. [PubMed: 24876275]

16. Kikuchi K, Holdway JE, Werdich AA, Anderson RM, Fang Y, Egnaczyk GF, Evans T, Macrae CA, Stainier DY, Poss KD. Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. Nature. 2010; 464:601–605. [PubMed: 20336144]
17. Aguirre A, Montserrat N, Zacchigna S, Nivet E, Hishida T, Krause MN, Kurian L, Ocampo A, Vazquez-Ferrer E, Rodriguez-Esteban C, Kumar S, Moresco JJ, Yates JR 3rd, Campistol JM, Sancho-Martinez I, Giacca M, Izpisua Belmonte JC. In Vivo Activation of a Conserved MicroRNA Program Induces Mammalian Heart Regeneration. Cell Stem Cell. 2014; 15:589–604. [PubMed: 25517466]
18. Laube F, Heister M, Scholz C, Borchardt T, Braun T. Re-programming of newt cardiomyocytes is induced by tissue regeneration. J Cell Sci. 2006; 119:4719–4729. [PubMed: 17077121]
19. Godwin JW, Pinto AR, Rosenthal NA. Macrophages are required for adult salamander limb regeneration. Proc Natl Acad Sci U S A. 2013; 110:9415–9420. [PubMed: 23690624]
20. van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin SC, Middleton RC, Marban E, Molkentin JD. c-kit+ cells minimally contribute cardiomyocytes to the heart. Nature. 2014; 509:337–341. [PubMed: 24805242]
21. Sultana N, Zhang L, Yan J, Chen J, Cai W, Razzaque S, Jeong D, Sheng W, Bu L, Xu M, Huang GY, Hajjar RJ, Zhou B, Moon A, Cai CL. Resident c-kit(+) cells in the heart are not cardiac stem cells. Nat Commun. 2015; 6:8701. [PubMed: 26515110]
22. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest. 2001; 107:1395–1402. [PubMed: 11390421]
23. Wu JM, Hsueh YC, Ch'ang HJ, Luo CY, Wu LW, Nakauchi H, Hsieh PC. Circulating cells contribute to cardiomyocyte regeneration after injury. Circ Res. 2015; 116:633–641. [PubMed: 25398235]
24. Hofmann M, Wollert KC, Meyer GP, Menke A, Arseniev L, Hertenstein B, Ganser A, Knapp WH, Drexler H. Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation. 2005; 111:2198–2202. [PubMed: 15851598]
25. Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET, Yeung AC, Johnstone BH, Yock PG, March KL. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation. 2005; 112:I150–I156. [PubMed: 16159808]
26. Wall ST, Yeh CC, Tu RY, Mann MJ, Healy KE. Biomimetic matrices for myocardial stabilization and stem cell transplantation. J Biomed Mater Res A. 2010; 95:1055–1066. [PubMed: 20878934]
27. Loffredo FS, Steinhauser ML, Gannon J, Lee RT. Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. Cell Stem Cell. 2011; 8:389–398. [PubMed: 21474103]
28. Khan M, Nickoloff E, Abramova T, Johnson J, Verma SK, Krishnamurthy P, Mackie AR, Vaughan E, Garikipati VN, Benedict C, Ramirez V, Lambers E, Ito A, Gao E, Misener S, Luongo T, Elrod J, Qin G, Houser SR, Koch WJ, Kishore R. Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction. Circ Res. 2015; 117:52–64. [PubMed: 25904597]
29. Sahoo S, Losordo DW. Exosomes and cardiac repair after myocardial infarction. Circ Res. 2014; 114:333–344. [PubMed: 24436429]
30. Wei K, Serpooshan V, Hurtado C, Diez-Cunado M, Zhao M, Maruyama S, Zhu W, Fajardo G, Noseda M, Nakamura K, Tian X, Liu Q, Wang A, Matsuura Y, Bushway P, Cai W, Savchenko A, Mahmoudi M, Schneider MD, van den Hoff MJ, Butte MJ, Yang PC, Walsh K, Zhou B, Bernstein D, Mercola M, Ruiz-Lozano P. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. Nature. 2015; 525:479–485. [PubMed: 26375005]
31. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature. 2004; 428:664–668. [PubMed: 15034593]
32. Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J, Taneera J, Fleischmann BK, Jacobsen SE. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low

frequency through cell fusion, but not transdifferentiation. Nat Med. 2004; 10:494–501. [PubMed: 15107841]

33. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature. 2004; 428:668–673. [PubMed: 15034594]
34. Quijada P, Toko H, Fischer KM, Bailey B, Reilly P, Hunt KD, Gude NA, Avitabile D, Sussman MA. Preservation of myocardial structure is enhanced by pim-1 engineering of bone marrow cells. Circ Res. 2012; 111:77–86. [PubMed: 22619278]
35. Karantalis V, Hare JM. Use of mesenchymal stem cells for therapy of cardiac disease. Circ Res. 2015; 116:1413–1430. [PubMed: 25858066]
36. Dawn B, Tiwari S, Kucia MJ, Zuba-Surma EK, Guo Y, Sanganalmath SK, Abdel-Latif A, Hunt G, Vincent RJ, Taher H, Reed NJ, Ratajczak MZ, Bolli R. Transplantation of bone marrow-derived very small embryonic-like stem cells attenuates left ventricular dysfunction and remodeling after myocardial infarction. Stem Cells. 2008; 26:1646–1655. [PubMed: 18420834]
37. Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, Tracy M, Ghersin E, Johnston PV, Brinker JA, Breton E, Davis-Sproul J, Schulman IH, Byrnes J, Mendizabal AM, Lowery MH, Rouy D, Altman P, Wong Po Foo C, Ruiz P, Amador A, Da Silva J, McNiece IK, Heldman AW, George R, Lardo A. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA. 2012; 308:2369–2379. [PubMed: 23117550]
38. Hatzistergos KE, Quevedo H, Oskouei BN, Hu Q, Feigenbaum GS, Margitich IS, Mazhari R, Boyle AJ, Zambrano JP, Rodriguez JE, Dulce R, Pattany PM, Valdes D, Revilla C, Heldman AW, McNiece I, Hare JM. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circ Res. 2010; 107:913–922. [PubMed: 20671238]
39. Behfar A, Terzic A. Derivation of a cardiopoietic population from human mesenchymal stem cells yields cardiac progeny. Nat Clin Pract Cardiovasc Med. 2006; 3(Suppl 1):S78–S82. [PubMed: 16501637]
40. Zaruba MM, Soonpaa M, Reuter S, Field LJ. Cardiomyogenic potential of C-kit(+)-expressing cells derived from neonatal and adult mouse hearts. Circulation. 2010; 121:1992–2000. [PubMed: 20421520]
41. Jesty SA, Steffey MA, Lee FK, Breitbach M, Hesse M, Reining S, Lee JC, Doran RM, Nikitin AY, Fleischmann BK, Kotlikoff MI. c-kit+ precursors support postinfarction myogenesis in the neonatal, but not adult, heart. Proc Natl Acad Sci U S A. 2012; 109:13380–13385. [PubMed: 22847442]
42. Hatzistergos KE, Saur D, Seidler B, Balkan W, Breton M, Valasaki K, Takeuchi LM, Landin AM, Khan A, Hare JM. Stimulatory Effects of Mesenchymal Stem Cells on cKit+ Cardiac Stem Cells Are Mediated by SDF1/CXCR4 and SCF/cKit Signaling Pathways. Circ Res. 2016; 119:921–930. [PubMed: 27481956]
43. Keith MC, Bolli R. "String theory" of c-kit(pos) cardiac cells: a new paradigm regarding the nature of these cells that may reconcile apparently discrepant results. Circ Res. 2015; 116:1216–1230. [PubMed: 25814683]
44. Malliaras K, Zhang Y, Seinfeld J, Galang G, Tseliou E, Cheng K, Sun B, Aminzadeh M, Marban E. Cardiomyocyte proliferation and progenitor cell recruitment underlie therapeutic regeneration after myocardial infarction in the adult mouse heart. EMBO molecular medicine. 2013; 5:191–209. [PubMed: 23255322]
45. Mohsin S, Khan M, Toko H, Bailey B, Cottage CT, Wallach K, Nag D, Lee A, Siddiqi S, Lan F, Fischer KM, Gude N, Quijada P, Avitabile D, Truffa S, Collins B, Dembitsky W, Wu JC, Sussman MA. Human cardiac progenitor cells engineered with Pim-I kinase enhance myocardial repair. J Am Coll Cardiol. 2012; 60:1278–1287. [PubMed: 22841153]
46. Kulandavelu S, Karantalis V, Fritsch J, Hatzistergos KE, Loescher VY, McCall F, Wang B, Bagno L, Golpanian S, Wolf A, Grenet J, Williams A, Kupin A, Rosenfeld A, Mohsin S, Sussman MA, Morales A, Balkan W, Hare JM. Pim1 Kinase Overexpression Enhances ckit+ Cardiac Stem Cell Cardiac Repair Following Myocardial Infarction in Swine. J Am Coll Cardiol. 2016; 68:2454–2464. [PubMed: 27908351]

47. Burridge PW, Thompson S, Millrod MA, Weinberg S, Yuan X, Peters A, Mahairaki V, Koliatsos VE, Tung L, Zambidis ET. A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability. PLoS One. 2011; 6:e18293. [PubMed: 21494607]
48. Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, Kennedy M, Henckaerts E, Bonham K, Abbott GW, Linden RM, Field LJ, Keller GM. Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. Nature. 2008; 453:524–528. [PubMed: 18432194]
49. Burridge PW, Keller G, Gold JD, Wu JC. Production of de novo cardiomyocytes: human pluripotent stem cell differentiation and direct reprogramming. Cell Stem Cell. 2012; 10:16–28. [PubMed: 22226352]
50. Mummery CL, Zhang J, Ng ES, Elliott DA, Elefanty AG, Kamp TJ. Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview. Circ Res. 2012; 111:344–358. [PubMed: 22821908]
51. Palpant NJ, Pabon L, Friedman CE, Roberts M, Hadland B, Zaunbrecher RJ, Bernstein I, Zheng Y, Murry CE. Generating high-purity cardiac and endothelial derivatives from patterned mesoderm using human pluripotent stem cells. Nat Protoc. 2017; 12:15–31. [PubMed: 27906170]
52. Nussbaum J, Minami E, Laflamme MA, Virag JA, Ware CB, Masino A, Muskheli V, Pabon L, Reinecke H, Murry CE. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. FASEB J. 2007; 21:1345–1357. [PubMed: 17284483]
53. Shiba Y, Fernandes S, Zhu WZ, Filice D, Muskheli V, Kim J, Palpant NJ, Gantz J, Moyes KW, Reinecke H, Van Biber B, Dardas T, Mignone JL, Izawa A, Hanna R, Viswanathan M, Gold JD, Kotlikoff MI, Sarvazyan N, Kay MW, Murry CE, Laflamme MA. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. Nature. 2012; 489:322–325. [PubMed: 22864415]
54. Chong JJ, Yang X, Don CW, Minami E, Liu YW, Weyers JJ, Mahoney WM, Van Biber B, Cook SM, Palpant NJ, Gantz JA, Fugate JA, Muskheli V, Gough GM, Vogel KW, Astley CA, Hotchkiss CE, Baldessari A, Pabon L, Reinecke H, Gill EA, Nelson V, Kiem HP, Laflamme MA, Murry CE. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature. 2014; 510:273–277. [PubMed: 24776797]
55. Riegler J, Tiburcy M, Ebert A, Tzatzalos E, Raaz U, Abilez OJ, Shen Q, Kooreman NG, Neofytou E, Chen VC, Wang M, Meyer T, Tsao PS, Connolly AJ, Couture LA, Gold JD, Zimmermann WH, Wu JC. Human Engineered Heart Muscles Engraft and Survive Long Term in a Rodent Myocardial Infarction Model. Circ Res. 2015; 117:720–730. [PubMed: 26291556]
56. Weinberger F, Breckwoldt K, Pecha S, Kelly A, Geertz B, Starbatty J, Yorgan T, Cheng KH, Lessmann K, Stolen T, Scherrer-Crosbie M, Smith G, Reichenspurner H, Hansen A, Eschenhagen T. Cardiac repair in guinea pigs with human engineered heart tissue from induced pluripotent stem cells. Sci Transl Med. 2016; 8:363ra148.
57. Kawamura T, Miyagawa S, Fukushima S, Maeda A, Kashiyama N, Kawamura A, Miki K, Okita K, Yoshida Y, Shiina T, Ogasawara K, Miyagawa S, Toda K, Okuyama H, Sawa Y. Cardiomyocytes Derived from MHC-Homozygous Induced Pluripotent Stem Cells Exhibit Reduced Allogeneic Immunogenicity in MHC-Matched Non-human Primates. Stem Cell Reports. 2016; 6:312–320. [PubMed: 26905198]
58. Shiba Y, Gomibuchi T, Seto T, Wada Y, Ichimura H, Tanaka Y, Ogasawara T, Okada K, Shiba N, Sakamoto K, Ido D, Shiina T, Ohkura M, Nakai J, Uno N, Kazuki Y, Oshimura M, Minami I, Ikeda U. Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. Nature. 2016; 538:388–391. [PubMed: 27723741]
59. Pasumarthi KB, Nakajima H, Nakajima HO, Soonpaa MH, Field LJ. Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice. Circ Res. 2005; 96:110–118. [PubMed: 15576649]
60. Chen J, Huang ZP, Seok HY, Ding J, Kataoka M, Zhang Z, Hu X, Wang G, Lin Z, Wang S, Pu WT, Liao R, Wang DZ. mir-17-92 cluster is required for and sufficient to induce cardiomyocyte proliferation in postnatal and adult hearts. Circ Res. 2013; 112:1557–1566. [PubMed: 23575307]

61. Nakada Y, Canseco DC, Thet S, Abdisalaam S, Asaithamby A, Santos CX, Shah AM, Zhang H, Faber JE, Kinter MT, Szweda LI, Xing C, Hu Z, Deberardinis RJ, Schiattarella G, Hill JA, Oz O, Lu Z, Zhang CC, Kimura W, Sadek HA. Hypoxia induces heart regeneration in adult mice. Nature. 2017; 541:222–227. [PubMed: 27798600]

62. Canseco DC, Kimura W, Garg S, Mukherjee S, Bhattacharya S, Abdisalaam S, Das S, Asaithamby A, Mammen PP, Sadek HA. Human ventricular unloading induces cardiomyocyte proliferation. J Am Coll Cardiol. 2015; 65:892–900. [PubMed: 25618530]

63. Tao G, Kahr PC, Morikawa Y, Zhang M, Rahmani M, Heallen TR, Li L, Sun Z, Olson EN, Amendt BA, Martin JF. Pitx2 promotes heart repair by activating the antioxidant response after cardiac injury. Nature. 2016; 534:119–123. [PubMed: 27251288]

64. D'Uva G, Aharonov A, Lauriola M, Kain D, Yahalom-Ronen Y, Carvalho S, Weisinger K, Bassat E, Rajchman D, Yifa O, Lysenko M, Konfino T, Hegesh J, Brenner O, Neeman M, Yarden Y, Leor J, Sarig R, Harvey RP, Tzahor E. ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. Nat Cell Biol. 2015; 17:627–638. [PubMed: 25848746]

65. Eulalio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, Giacca M. Functional screening identifies miRNAs inducing cardiac regeneration. Nature. 2012; 492:376–381. [PubMed: 23222520]

66. Tian Y, Liu Y, Wang T, Zhou N, Kong J, Chen L, Snitow M, Morley M, Li D, Petrenko N, Zhou S, Lu M, Gao E, Koch WJ, Stewart KM, Morrisey EE. A microRNA-Hippo pathway that promotes cardiomyocyte proliferation and cardiac regeneration in mice. Sci Transl Med. 2015; 7:279ra38.