Il concetto di terapia cellulare di rigenerazione tessutale in cardiochirurgia

CLM Biotecnologie Mediche

G. Frati
What is a stem cell?

A single cell that can

replicate itself, or...

differentiate into many cell types.
WORK IN PROGRESS
“Cardiac Literature…”

Results: 1 to 20 of 11936

1. Interaction Between Neuronal NOS Signaling and Temperature Influences SR Ca2+ Leak: Role of Nitroso-Redox Balance.
   Dulce R, Mayo V, Rangel EB, Balkan W, Hare JM.
   PMID: 25326127 [PubMed - as supplied by publisher]
   Related citations

2. Aging and energetics' Top 40 future research opportunities 2010-2013.
   Related citations

3. Cultured Human Bone Marrow-Derived CD31(+) Cells Are Effective for Cardiac and Vascular Repair Through Enhanced Angiogenic, Adhesion, and Anti-Inflammatory Effects.
   Kim SW, Hogue M, Brown M, Davis ME, Yoon YS.
   PMID: 25323256 [PubMed - in process]
   Related citations
2014=570 Clinical Trials on-going....

Source: http://ClinicalTrials.gov
Lo scienziato della Sapienza: così un gruppo di aziende alimenta la speranza

Il metodo Stamina non è una cura

di PAOLO BIANCO

La discussione in Parlamento sui trattamenti a base di cellule staminali, sollecitata anche dall'ecomediativa del «caso Stamina», va letta all'interno di uno scenario internazionale, in cui giocano un ruolo chiave nuovi soggetti economici, interessati a esercitare pressione sui governi per ottenere una de-regolamentazione del settore.

STAMINALI, LA DEREGULATION GIOVA SOLO A CHI VENDE TERAPIE

medici farabutti al soldo delle multinazionali. Quel che la «cura» propone è che un'infusione di cellule ossee (staminali mesenchimali) curi tanti malanni diversi, a prescindere dalla natura del malanno, da quel che le cellule siano in grado di fare, a prescindere dal fatto che le stesse cellule, una volta infuse, rimangano lì o scompaiano. E a prescindere dalla necessità di verificare che sia così. Ma la «cura» coincide con quello che molti nuovi soggetti commerciali propongono. Alcuni di essi emergono dal-
Smoke and mirrors

Italy’s parliament must listen to expert advice before deregulating stem-cell therapies.

Just weeks after the white smoke from the Vatican signalled the election of a new pope, a grimmer pall hangs over the Eternal City — a fog of confusion and misrepresentation about stem-cell therapy. Those who have lit the fire beneath the debate say that they are promoting the translation of stem-cell research into the clinic so that currently incurable diseases can be treated. Nothing could be further from the truth.

The current controversy concerns adult stem cells. These exist in several tissues, but can replace only those particular tissues. Big claims are being made for them, with many trials of therapies under way worldwide for conditions as diverse as Alzheimer’s and heart disease. Some stem-cell therapies are approved by regulatory agencies; others sneak under the radar by exploiting rules allowing compassionate therapy, for example, or by operating in countries such as China or Mexico — and perhaps now Italy — where regulation is less strict.
myocardial infarction

→ Myocyte necrosis and/or apoptosis
→ Scar formation

Carbon-14 assay on heart tissues from individuals born prior to or after the ban on above ground nuclear testing

Some individuals had heart disease, but none had gross hypertrophy

→ 1% turnover per year at age 20, 0.45% at 75

Bergmann et al., Science 2009
Ischemic heart disease

Myocardial infarction

- Loss of cardiac function
- Ventricular remodeling
- Progressive dysfunction

Heart failure

Cardiac Transplantation
Mechanical Device
Regenerative Medicine
What is “regenerative medicine?”

Tissue Engineering and Biomaterials

Cellular Therapies

Medical Devices and Artificial Organs
Cardiac cell therapy: goals

1. Replace scar tissue by living cells
2. Block or reverse ventricular remodeling
3. Replace contractility
4. Restore “contraction synchrony”
5. Promote angiogenesis
Cell Therapy: **Cells**

- Adult/Somatic stem cells
- Umbilical Cord Blood cells
- iPS (induced pluripotent Stem Cells)
- Embryonic stem cells
Translational Research

Stem cell based treatments require GMP (good manufacturing practice) protocols which means a "production and testing practice that helps to ensure a quality product"
A journey into Stem Cell based Therapies...starting from a simple question...

What Makes Clinical Research Ethical?
1) **Value**: enhancements of health or knowledge must be derived from the research;

2) **Scientific validity**: the research must be methodologically rigorous;

3) **Pre-clinical model**: animal models in order to avoid the jump “from bench directly to bedside”

4) **Scientific Publications**: the research must be "unambiguous", available, reproducible;

5) **Respect in the setting of clinical trials** and in enrolling subjects: subjects should have their health protected, the opportunity to withdraw, and their well-being monitored.

6) **Researcher's sense of duty** involved in the care of patients: communication and medical counselling. Avoiding false hoping and expectations from this kind of treatments in patients and their relatives.
15 Years: a long trip across Stem Cells....and is still ongoing...
Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: Implications for myocardium regeneration


In conclusion, our observation that human endothelial cells, such as those derived from the umbilical vein, also share the capacity of generating cardiomyocytes opens perspectives for cell replacement therapy. It may be possible to create an archive of patients’ umbilical cord cells to be used later in life.

these data should set the stage for molecular studies of stem/progenitor plasticity and hence for future clinical applications.
Can Autologous Myoblast Transplantation Decrease Chronic Ischemic Mitral Regurgitation?

Giacomo Frati, Miguel Cortes Morichetti, Emmanuel Messas, Alain Bel, Claire Carrion, Mark Handschumacher, Jean-Thomas Vilquin, Michel Desnos, Patrick Bruneval, Philippe Menasché, Alain Carpentier, Robert A. Levine, Albert A. Hagege, INSERM, Paris, France, Massachusetts General Hospital and Harvard Medical School, Boston, MA, Sapienza University, Rome, Italy

Yes, but...

Skeletal Muscle Tissue in Sheep
2) Scientific validity: the research must be methodologically rigorous
3) Pre-clinical model: animal models in order to avoid the jump “from bench directly to bedside”
Transplantation

The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) Trial

First Randomized Placebo-Controlled Study of Myoblast Transplantation

Philippe Menasché, MD, PhD; Ottavio Alfieri, MD; Stefan Janssens, MD, PhD; William McKenna, MD; Hermann Reichenspurner, MD; Ludovic Trinquart, MSc; Jean-Thomas Vilquin, PhD; Jean-Pierre Marolleau, MD; Barbara Seymour, BS; Jérôme Larghero, PharmD, PhD; Stephen Lake, ScD; Gilles Chatellier, MD, PhD; Scott Solomon, MD; Michel Desnos, MD; Albert A. Hagège, MD, PhD

Background—Phase I clinical studies have demonstrated the feasibility of implanting autologous skeletal myoblasts in postinfarction scars. However, they have failed to determine whether this procedure was functionally effective and arrhythmogenic.

Methods and Results—This multicenter, randomized, placebo-controlled, double-blind study included patients with left ventricular (LV) dysfunction (ejection fraction ≤35%), myocardial infarction, and indication for coronary surgery. Each patient received either cells grown from a skeletal muscle biopsy or a placebo solution injected in and around the scar. All patients received an implantable cardioverter-defibrillator. The primary efficacy end points were the 6-month changes in global and regional LV function assessed by echocardiography. The safety end points comprised a composite index of major cardiac adverse events and ventricular arrhythmias. Ninety-seven patients received myoblasts (400 or 800 million; n=33 and n=34, respectively) or the placebo (n=30). Myoblast transfer did not improve regional or global LV function beyond that seen in control patients. The absolute change in ejection fraction (median [interquartile range]) between 6 months and baseline was 4.4% (0.2; 7.3), 3.4% (−0.3; 12.4), and 5.2% (−4.4; 11.0) in the placebo, low-dose, and high-dose groups, respectively (P=0.95). However, the high-dose cell group demonstrated a significant decrease in LV volumes compared with the placebo group. Despite a higher number of arrhythmic events in the myoblast-treated patients, the 6-month rates of major cardiac adverse events and of ventricular arrhythmias did not differ significantly between the pooled treatment and placebo groups.

Conclusions—Myoblast injections combined with coronary surgery in patients with depressed LV function failed to improve echocardiographic heart function. The increased number of early postoperative arrhythmic events after myoblast transplantation, as well as the capability of high-dose injections to revert LV remodeling, warrants further investigation. (Circulation. 2008;117:1189-1200.)

Key Words: heart failure  ▪  myoblasts  ▪  myocardial infarction  ▪  stem cells  ▪  transplantation
Other cell sources?

 Bone Marrow stem cells?  Resident cardiac stem cells?
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. Mathy, 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*

Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial

Transcoryonary 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.

The strength of plasticity: stem cells for cardiac repair
Donald Orlic PhD
Associate Investigator, Building 37, Room 376A, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA

Transendocardial, Autologous Bone Marrow Cell Transplantation for Severe, Chronic Ischemic Heart Failure
Circulation 2003;107:2294-2302; originally published online Apr 21, 2003; DOI: 10.1161/01.CIR.0000070596.30552.8B
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Copyright © 2003 American Heart Association. All rights reserved. Print ISSN: 0009-7322. Online ISSN: 1524-4539

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 Illebekk, M.D., Ph.D., vild Mariangus, M.D., Ph.D., Jan G. Fjeld, M.D., Ph.D., Hans Jørgen Smith, M.D., Ph.D., Eli Tardalsrud, M.D., Haakon Kil Grogard, M.D., Reidar Bjørnheim, M.D., Ph.D., Magne Brekke, M.D., Carl Müller, M.D., Jar Hoppe, M.D., Asgrimur Ragnarsson, M.D., Jan E. Brinckmann, M.D., Ph.D., and Kolbjørn Forfang, M.D., Ph.D.®
New questions: route of delivery

Surgical: EPICARDIAL
Median sternotomy (MAGIC)

Cell injection
“Wash-out” by myocardial squeezing
Percutaneous approach

1. Intravenous (*systemic*) low cell retention and trapping in other organs, *never!!!*

2. Catheter-based cell delivery
   - Intracoronary
   - Transvenous (coronary sinus)
   - Endomyocardial (endoventricular, trans-endocardial)
Intracoronary route

“Stop Flow” technique for cell transfer
The intracoronary method is preferred in post-acute MI. Retention of cells is suboptimal and unperfused regions of the myocardium are inaccessible. Moreover microvascular obstruction caused by cell sludging may cause microinfarction!!!!!
Clinical Trials performed with BMC by percutaneous delivery 2005

<table>
<thead>
<tr>
<th>Trial or Investigator Group</th>
<th>Setting</th>
<th>Design</th>
<th>No. of Cells Administered in Treatment Group</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOOST&lt;sup&gt;4,9&lt;/sup&gt;</td>
<td>PCI after acute myocardial infarction</td>
<td>Randomized trial</td>
<td>Approximately $2.5 \times 10^9$ unfractonated BMC</td>
<td>At 6 mo: LVEF 6% greater in BMC group than in control group</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 patients received BMC; 30 received no infusion</td>
<td></td>
<td>At 12 mo: no significant difference in LVEF between the 2 groups</td>
</tr>
<tr>
<td>Janssens et al.&lt;sup&gt;8&lt;/sup&gt;</td>
<td>PCI after acute myocardial infarction</td>
<td>Randomized, double-blind trial</td>
<td>Approximately $3 \times 10^8$ Ficoll-separated BMC</td>
<td>At 4 mo: no significant difference in overall LVEF, decreased infarct size and better regional function in BMC group</td>
</tr>
<tr>
<td>TOPCARE-CHD&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Chronic left ventricular dysfunction</td>
<td>Randomized, crossover trial</td>
<td>Approximately $2 \times 10^8$ Ficoll-separated BMC or approximately $2 \times 10^7$ Ficoll-separated, cultured CPC</td>
<td>At 3 mo: greater increase in LVEF (2.9 percentage points) in BMC group than in CPC group or control group</td>
</tr>
<tr>
<td>ASTAMI&lt;sup&gt;7&lt;/sup&gt;</td>
<td>PCI after acute myocardial infarction</td>
<td>Randomized trial</td>
<td>Approximately $7 \times 10^7$ Ficoll-separated BMC</td>
<td>At 6 mo: no significant difference in LVEF between the 2 groups</td>
</tr>
<tr>
<td>REPAIR-AMI&lt;sup&gt;5&lt;/sup&gt;</td>
<td>PCI after acute myocardial infarction</td>
<td>Randomized, double-blind trial</td>
<td>Approximately $2.4 \times 10^8$ Ficoll-separated BMC</td>
<td>At 4 mo: greater absolute increase in LVEF in BMC group than in placebo group (5.5% vs. 3.0%)</td>
</tr>
</tbody>
</table>

*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.

Chronic LV dysfunction (6 yrs post AMI)
“Myoblast is over” and both, surgical direct injection and percutaneous intracoronary delivery of BM cells failed to significantly regenerate myocardium and improve contractile function
Cell survival and distribution after intramyocardial injection.
Cell Distribution after Intramyocardial Injection

- Heart: 29%
- Arterial Blood: 56%
- Veinous Blood: 4%
- Spleen: 3%
- Lung: 3%
- Liver: 2%

First Hour

2nd Week

Zhang, Circulation 2007
Tissue engineering
Tissue engineering is the use of a combination of cells, engineering and materials methods, to improve or replace biological functions.

Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, are typically called scaffolds.

Closely associated with applications that repair or replace portions of or whole tissues.
**Structure**

- **Macro-scale**
- **Random**
- **Alignment**
- **Multi-layer**
- **Nano-scale**
- **Nano fiber**

**Biomaterials**

<table>
<thead>
<tr>
<th>A. Electrospinning</th>
<th>B. Liquid-solid transition</th>
<th>C. Releasing biomolecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA, PGA, PLGA, PCL</td>
<td>Polymeric materials</td>
<td>Growth factors with polymeric carriers</td>
</tr>
<tr>
<td>Collagen, gelatin, alginate, etc.</td>
<td>Hydrogel materials</td>
<td>DNA with viral or non-viral carriers</td>
</tr>
<tr>
<td>Composite with CNT, DNA, and proteins</td>
<td>Composite materials</td>
<td></td>
</tr>
</tbody>
</table>
Tissue grafts could be prepared and delivered in two ways.

Stem cells mixed with extracellular matrix materials can be prepared in vitro as an epicardial **CARDIAC PATCH** which could be grafted on the epicardial face of the injured myocardium.

Stem cells supplemented with extracellular matrix materials could be mixed in **INJECTABLE BIODEGRADABLE GELS** and injected to the site of injured myocardium.

Embedded in bio-engineered tissues, implanted cells would have a **better chance to survive and engraft** in the cardiac microenvironment in comparison to directly exposing the stem cells via infusion or injection.
Biodegradable 3D matrix

**Composition**
- Collagene type I and III
- Micropores (50-100 µm)

**Characteristics**
- Biological and chemical stability
- Plasticity
- Biocompatible: mild immunologic response
- Biodegradable (after 2 months)
- Currently clinically employed (surgical haemosthasys)
3D COLLAGEN MATRIX
Type I/III Collagen Matrix (France)
Type I collagen matrix (size 4x3x1.5 mm) alone

Type I collagen matrix (size 4x3x1.5 mm) seeded with 5 million BM Cells
2) Scientific validity: the research must be methodologically rigorous.

3) Pre-clinical model: animal models in order to avoid the jump “from bench directly to bedside”.

4) Scientific Publications: the research must be...
Cell Based Approaches for Myocardial Regeneration and Artificial Myocardium

Jorge Genovese¹, Miguel Cortes-Morichetti², Emmanuel Chachques¹, Giacomo Frati², Amit Patel¹ and Juan C. Chachques*²

¹University of Pittsburgh Medical Center and McGowan Institute for Regenerative Medicine, USA
²European Hospital Georges Pompidou, University of Paris, France

Abstract: Ischemic myocardial disease, the main cause of heart failure, is a major public health and economic problem. Given the aging population, heart failure is becoming an increasing clinical issue and a substantial financial burden. Thus, research in heart failure is of relevant interest and importance, involving specialties such as cellular and molecular biology, tissue engineering, genetics, biophysics and electrophysiology. Stem cell-based regenerative therapy is undergoing experimental and clinical trials in order to limit the consequences of decreased contractile function and compliance of damaged ventricles following myocardial infarction or in patients presenting non-ischemic dilated cardiomyopathies. This biological approach is particularly attractive due to the potential for myocardial regeneration with a variety of myogenic and angiogenic cell types. The development of a bio-artificial myocardium using biological or synthetic matrix is a new challenge.
Association Between a Cell-Seeded Collagen Matrix and Cellular Cardiomyoplasty for Myocardial Support and Regeneration

MIGUEL CORTES-MORICHETTI, M.D.,¹ GIACOMO FRATI, M.D.,² OLIVIER SCHUSSLER, M.D., Ph.D.,¹ JEAN-PAUL DUONG VAN HUYEN, M.D.,¹ EVELYNE LAURET, Ph.D.,³ JORGE A. GENOVESE, M.D.,⁴ ALAIN F. CARPENTIER, M.D., Ph.D.,¹ and JUAN C. CHACHQUES, M.D., Ph.D.¹

ABSTRACT

The objective of cellular cardiomyoplasty is to regenerate the myocardium using implantation of living cells. Because the extracellular myocardial matrix is deeply altered in ischemic cardiomyopathies, it could be important to create a procedure aiming at regenerating both myocardial cells and the extracellular matrix. We evaluated the potential of a collagen matrix seeded with cells and grafted onto infarcted ventricles. A myocardial infarction was created in 45 mice using coronary artery ligation. Animals were randomly assigned to 4 local myocardial treatment groups. Group I underwent sham treatment (injection of cell culture medium). Group II underwent injection of human umbilical cord blood mononuclear cells (HUCBCs). Group III underwent injection of HUCBCs and fixation onto the epicardium of a collagen matrix seeded with HUCBCs. Group IV underwent fixation of collagen matrix (without cells) onto the infarct. Echocardiography was performed on postoperative days 7 and 45, followed by histological studies. Echocardiography showed that the association between the cell-loaded matrix and the intramurcal cell implants was the most efficient approach to limiting postschismic ventricular dilation and remodeling. Ejection fraction improved in both cell-treated groups. The collagen matrix alone did not improve left ventricular (LV) function and remodeling. Histology in Group III showed fragments of the collagen matrix thickening and protecting the infarct scars. Segments of the matrix were consistently aligned along the LV wall, and cells were assembled within the collagen fibers in large populations. Intramyocardial injection of HUCBCs preserves LV function following infarction. The use of a cell-seeded matrix combined with cell injections prevents ventricular wall thinning and limits postschismic remodeling. This tissue engineering approach seems to improve the efficiency of cellular cardiomyoplasty and could emerge as a new therapeutic tool for the prevention of adverse remodeling and progressive heart failure.
Surgical approach: CELL THERAPY + TISSUE ENGINEERING
BIOARTIFICIAL MYOCARDIUM « MAGNUM » Clinical Trial

Myocardial Assistance by Grafting a New bioartificial Upgraded Myocardium
Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM Trial): Clinical Feasibility Study

MIGUEL CORTES-MORICHETTI, M.D.,1 GIACOMO FRATI, M.D.,2 OLIVIER SCHUSSLER, M.D., Ph.D.,1 JEAN-PAUL DUONG VAN HUYEN, M.D.,1 EVELYNE LAURET, Ph.D.,3 JORGE A. GENOVESE, M.D.,4 ALAIN F. CARPENTIER, M.D., Ph.D.,1 and JUAN C. CHACHQUES, M.D., Ph.D.1

Department of Cardiovascular Surgery, Pompidou Hospital, Paris, France; and Departments of Cardiology and Cardiovascular Surgery, Avellaneda Hospital, Buenos Aires, Argentine

Background. Cell transplantation for the regeneration of ischemic myocardium is limited by poor graft viability and low cell retention. In ischemic cardiomyopathy, the extracellular matrix is deeply altered; therefore, it could be important to associate a procedure aiming at regenerating myocardial cells and restoring the extracellular matrix function. We evaluated the feasibility and safety of intrainfarct cell therapy associated with a cell-seeded collagen scaffold grafted onto infarcted ventricles.

Methods. In 20 consecutive patients presenting with left ventricular postischemic myocardial scars and indication for coronary artery bypass graft surgery, bone marrow cells were implanted during surgery. In the last 10 patients, we added a collagen matrix seeded with bone marrow cells, placed onto the scar.

Results. There was no mortality and any related adverse events (follow-up 10 ± 3.5 months). New York Heart Association functional class improved in both groups from 2.3 ± 0.5 to 1.3 ± 0.5 (matrix, p = 0.0002) versus 2.4 ± 0.5 to 1.5 ± 0.5 (no matrix, p = 0.001). Left ventricular end-diastolic volume evolved from 142.4 ± 24.5 mL to 112.9 ± 27.3 mL (matrix, p = 0.02) versus 138.9 ± 36.1 mL to 148.7 ± 41 mL (no matrix, p = 0.57), left ventricular filling deceleration time improved significantly in the matrix group from 162 ± 7 ms to 198 ± 9 ms (p = 0.01) versus the no-matrix group (from 159 ± 5 ms to 167 ± 8 ms, p = 0.07). Scar area thickness progressed from 6 ± 1.4 to 9 mm ± 1.1 mm (matrix, p = 0.005) versus 5 ± 1.5 mm to 6 ± 0.8 mm (no matrix, p = 0.09). Ejection fraction improved in both groups, from 25.3% ± 7.3% to 32% ± 5.4% (matrix, p = 0.03) versus 27.2% ± 6.9% to 34.6% ± 7.3% (no matrix, p = 0.031).

Conclusions. This tissue-engineered approach is feasible and safe and appears to improve the efficiency of cellular cardiomyoplasty. The cell-seeded collagen matrix increases the thickness of the infarct scar with viable tissue and helps to normalize cardiac wall stress in injured regions, thus limiting ventricular remodeling and improving diastolic function.

Clinical Collagen Matrix Implantation

Matrix seeded with HBMC

LITA to LAD
Conclusion II

In case of BM derived Stem Cell, surgical delivery of engineered scaffolds modifies cardiac remodelling but poorly influence the functional results...even if Bm acquire Cardiac Stem Cells properties Other cell sources???
Isolation and Expansion of Adult Cardiac Stem Cells From Human and Murine Heart

Elisa Messina, Luciana De Angelis, Giacomo Frati, Stefania Morrone, Stefano Chimenti, Fabio Fiordaliso, Monica Salio, Massimo Battaglia, Michael V.G. Latronico, Marcello Coletta, Elisabetta Vivarelli, Luigi Frati, Giulio Cossu and Alessandro Giacomello

_Circ. Res._ 2004;95:911-921; originally published online Oct 7, 2004;
DOI: 10.1161/01.RES.0000147315.71699.51

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http://circres.ahajournals.org/cgi/content/full/95/9/911

Owner: University of Rome “Sapienza” Teritorial covering: Italy, Europe, U.S.A., Canada, China


International patent number: WO2005012510, 2005-02-10
Cardiospheres...

Human cardiospheres 5/6 days after harvest

Poly-D-lysine coat
bFGF, EGF, CT-1, Thrombin
Low serum
Serum
CSp characterization

Human CSps

Co-cultured CSps

GFP/hoechst

cTnI/hoechst

GFP/cTnI/hoechst

10 µm

GFP/hoechst

Cx43/hoechst

GFP/Cx43/hoechst

10 µm

Repetitive action potentials recorded from CSc -50mV
2) Scientific validity: the research must be methodologically rigorous

3) Pre-clinical model: animal models in order to avoid the jump “from bench directly to bedside”

4) Scientific Publications: the research must be.....
Matrix seeded with Cells by centrifugation (800 Rpm/5 minutes)
SCID N° 3174
IMA T2
Matrix+CS
Ligation LAD + Matrix (nod/scid mice)

Epicardium from human adult atrium

MRI: Function

Short Term: Large MI

Long Term: Small MI
Xenogen® in vivo imaging system
Fate and engraftment luciferase
Border line of the infarct

Matrix

1 Week

1 Week
Border line of the infarct

Matrix

Sacrifice

Sacrifice
Operative mortality: 14.5%

**Functional Outcomes**

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDV1 l</th>
<th>LVEDV2 l</th>
<th>LVEF1 %</th>
<th>LVEF2 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.4</td>
<td>88.8</td>
<td>30.2</td>
<td>24.7</td>
</tr>
<tr>
<td>Matrix + CS</td>
<td>45.8</td>
<td>54.3</td>
<td>32.1</td>
<td>42.9</td>
</tr>
</tbody>
</table>
Cardiospheres and tissue engineering for myocardial regeneration: potential for clinical application

Roberto Gaetani a, b, *, Giuseppe Rizzitelli c, Isotta Chimenti a, Lucio Barile d, Elvira Forte a, Vittoria Ionta a, Francesco Angelini a, Joost P.G. Sluijter b, e, Andrea Barbetta c, Elisa Messina a, Giacomo Frati a, f

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b Department of Cardiology, HLCU, University Medical Center Utrecht, Utrecht, The Netherlands
c Department of Chemistry, University of Rome ‘Sapienza’, Rome, Italy
d Department of Biotechnology and Bioscience, University of Rome ‘Sapienza’, Rome, Italy
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Received: March 29, 2010; Accepted: April 25, 2010
Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial


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 CArdiosphere-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.
Cardiospheres and CDCs seems to dramatically improve cardiac function.

Tissue engineering seems to boost retention and increase engraftment of transplanted cells.
“from bedside to bench and return...”

...and in Italy???
Tracciabilità

Cell and Tissue Bank Dir 2004/23/EC

Cell Factory GMP authorized Reg. 2007/1394/EC

Point of use Hospitals

Sui cittadini. La richiesta che sale in Italia dal pubblico di liberalizzare per legge le terapie compassionevoli (cioè non sperimentate né approvate) coincide dunque con interessi commerciali, ben diversi dalle motivazioni del pubblico. Nelle stesse case italiane...
THROMBIN AND THROMBIN-DERIVED PEPTIDES PROMOTE PROLIFERATION OF CARDIAC PROGENITOR CELLS IN THE FORM OF CARDIOSPHERES WITHOUT AFFECTING THEIR DIFFERENTIATION POTENTIAL


1Dep. of Anatomy, Histology, Forensic Medicine and Orthopedics, “Sapienza” University, Rome, Italy; 2Dep. of Biothecnology and Medical-Surgical Sciences, “Sapienza” University, Latina, Italy; 3Dep. of Molecular Medicine, Pasteur Institute, Cenci-Bolognetti Foundation, “Sapienza” University, Rome.

Many studies demonstrated that human adult cardiac progenitor cells in the form of cardiospheres (CSPs) could represent a powerful candidate for cardiac cell therapy. To achieve the clinical translation of this biotechnological product, the development of well-defined culture conditions is required to optimize their proliferation and differentiation. Thrombin, a serine protease acting through the protease-activated receptor 1 (PAR-1) signalling to modulate many cellular functions such as proliferation and differentiation in several cell types, is one of the factors included in the CSPs medium. Therefore, the assessment of the effective dependence of the thrombin-related cellular effects from PAR-signalling is strategic both for understanding the biological potential of these cells and for the GMP translation of the medium formulation, using synthetised analogs. In this study the effects of thrombin on human CSPs and their potential relationship with the specific proteolytic activation of PAR-1 have been investigated in different culture conditions, including thrombin inhibitor hirudin and PAR-1 agonist/antagonist peptides TFLLR and MUMB2. In this study we show that, in the presence of thrombin and TFLLR, CSPs, in which PAR-1 expression was evidenced by immunofluorescence and western blot analysis, increase their proliferative activity (BrdU assay). Such increased proliferative rate was consistently associated with a higher phosphorylation level of the cell cycle inhibitor GSK3. Concerning the assessment of the potential effects of thrombin and its agonist on differentiation, both western blot and real-time PCR analysis for “stemness”, cardiac and vascular markers (such as cKit, cx43 and KDR) showed that CSPs commitment was substantially unaffected, except for GATA4 mRNA, whose transcription was down-regulated in the presence of the natural protease, but not after treatment with TFLLR. In conclusion, activation of PAR-1-dependent signalling is important to support CSPs proliferative potential, keeping unaltered or at best stable their differentiation properties. The availability of thrombin agonists, such as TFLLR, able to guaranty the required growth effect without affecting CSPs lineage commitment, could represent a technological improvement for cost-effective, easy-to-handle and GMP-translatable synthetic media.
Serum and supplement optimization for EU GMP-compliance in cardiospheres cell culture

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Received: July 3, 2013; Accepted: November 15, 2013

Abstract

Cardiac progenitor cells (CPCs) isolated as cardiospheres (CSs) and CS-derived cells (CDCs) are a promising tool for cardiac cell therapy in heart failure patients, having CDCs already been used in a phase I/II clinical trial. Culture standardization according to Good Manufacturing Practices (GMPs) is a mandatory step for clinical translation. One of the main issues raised is the use of xenogenic additives (e.g. FBS, foetal bovine serum) in cell culture media, which carries the risk of contamination with infectious viral/prion agents, and the possible induction of immunizing effects in the final recipient. In this study, B27 supplement and sera requirements to comply with European GMPs were investigated in CSs and CDCs cultures, in terms of process yield/efficiency and final cell product gene expression levels, as well as phenotype. B27– free CS cultures produced a significantly reduced yield and a 10-fold drop in c-kit expression levels versus B27+ media. Moreover, autologous human serum (aHS) and two different commercially available GMP AB HSs were compared with standard research-grade FBS. CPCs from all HSs explants had reduced growth rate, assumed a senescent-like morphology with time in culture, and/or displayed a significant shift towards the endothelial phenotype. Among three different GMP gamma-irradiated FBSs (gFBSs) tested, two provided unsatisfactory cell yields, while one performed optimally, in terms of CPCs yield/phenotype. In conclusion, the use of HSs for the isolation and expansion of CSs/CDCs has to be excluded because of altered proliferation and/or commitment, while media supplemented with B27 and the selected gFBS allows successful EU GMP-complying CPCs culture.

Keywords: adult stem cells • cardiac cell therapy • cardiospheres • Good Manufacturing Practice compliance • human sera
Optimization of the isolation and expansion method of human mediastinal–adipose tissue derived mesenchymal stem cells with virally inactivated GMP-grade platelet lysate

Camilla Siciliano · Mohsen Ibrahim · Gaia Scafetta · Chiara Napoletano · Giorgio Mangino · Luca Pierelli · Giacomo Frati · Elena De Falco

Abstract Mesenchymal stem cells (MSCs) are adult multipotent cells currently employed in several clinical trials due to their immunomodulating, angiogenic and repairing features. The adipose tissue is certainly considered an eligible source of MSCs. Recently, putative adipose tissue derived MSCs (ADMSCs) have been isolated from the mediastinal depots. However, very little is known about the properties, the function and the potential of human mediastinal ADMSCs (hmADMSCs). However, the lack of standardized methodologies to culture ADMSCs prevents comparison across. Herein for the first time, we report a detailed step by step description to optimize the isolation and the expansion methodology of hmADMSCs using a virally inactivated good manufacturing practice (GMP)-grade platelet lysate, highlighting the critical aspects of the procedure and providing useful troubleshooting suggestions. Our approach offers a reproducible system which could provide standardization across laboratories. Moreover, our system is time and cost effective, and it can provide a reproducible source of adipose stem cells to enable future studies to unravel new insights regard this promising stem cell population.

Giacomo Frati and Elena De Falco have contributed equally to this work.

C. Siciliano · G. Scafetta · G. Mangino · G. Frati · E. De Falco (✉)
Department of Medical-Surgical Science and Biotechnologies, Faculty of Pharmacy and Medicine, University of Rome “Sapienza”, V. le Regina Elena 324, 00161 Rome, Italy
THE CD133⁺ CELL AS ADVANCED MEDICINAL PRODUCT FOR MYOCARDIAL AND LIMB ISCHEMIA

Dario Bongiovanni¹², Beatrice Bassetti¹, Elisa Gambini¹, Giuseppe Gaipa³, Daniela Belotti³, Frati Giacomo⁴⁷, Paolo Scacciatella², MC Capogrossi⁵, and Giulio Pompilio¹⁶

Title PHASE I TRIAL OF ENDOCavitARY INJECTION OF BONE-MARROW-DERIVED CD133⁺ CELLS IN ISCHEMIC REFRACtORY CARDIOMYOPATHY (RECARDIO Trial)

RECARDIO Trial
Studio clinico di Fase I di iniezione endocavitaria intramiocardica di cellule CD133⁺ derivate da midollo per il trattamento della cardiomiopatia ischemica refrattaria (RECARDIO Trial)
Future Directions...
A Preview of the Future:

1) Minimally invasive delivery by new biomaterials

2) Left Ventricular Assist Devices and Stem Cells

3) “Second Hand Heart” Reconditioning
Hydrogel polymers
Thermal properties of hydrogel polymers

Hydrogels are thermosensitive. The hydrogel polymer solutions are liquid at 4 C and formed solid hydrogel at 37 C

They can be easily injected through a 30-gauge needle, a typical size for heart injection. When transferred into a 37 C water bath, the hydrogel solutions quickly gelled to form solid hydrogels. All the solutions regardless of collagen addition had a gelation time < 5 s.

All the hydrogels were highly flexible at body temperature
Hydrogel eco-guided injection in infarcted area???
Pre-Hydrogel  Post-Hydrogel

Frati et al. unpublished data
The FMT-XCT aims to combine X-ray computed tomography (XCT) and fluorescence molecular tomography (FMT) into a hybrid, quantitative imaging system. FMT provides a quantifiable measurement of fluorochrome concentration at any depth in the subject.
Magnitude and Time Course of Changes Induced by Continuous-Flow Left Ventricular Assist Device Unloading in Chronic Heart Failure
Insights Into Cardiac Recovery

Letters To The Editor | September 2013

Letter to the Editor: Left ventricular assist devices in chronic heart failure: *more questions than answers?*

*(Letter of comment on Drakos et al, J Am Coll Cardiol 2013;61:1985-94) ONLINE FIRST*

Antonino G.M. Marullo, MD, PhD; Mariangela Peruzzi, MD, PhD; Elena Cavarretta, MD, PhD; Giuseppe Biondi-Zocca, MD; Giacomo Frati, MD

*J Am Coll Cardiol.* 2013();. doi:10.1016/j.jacc.2013.06.060

Conclusions
Continuous-flow LVAD unloading induced in a subset of patients, both ischemic and nonischemic, early improvement in myocardial structure and systolic and diastolic function that was largely completed within 6 months, with no evidence of subsequent regression. *(J Am Coll Cardiol 2013;61:1985-94) © 2013 by the American College of Cardiology Foundation*
Molecular Changes After Left Ventricular Assist Device Support for Heart Failure

Emma J. Birks

Abstract—Heart failure is associated with remodeling that consists of adverse cellular, structural, and functional changes in the myocardium. Until recently, this was thought to be unidirectional, progressive, and irreversible. However, irreversibility has been shown to be incorrect because complete or partial reversal can occur that can be marked after myocardial unloading with a left ventricular assist device (LVAD). Patients with chronic advanced heart failure can show near-normalization of nearly all structural abnormalities of the myocardium or reverse remodeling after LVAD support. However, reverse remodeling does not always equate with clinical recovery. The molecular changes occurring after LVAD support are reviewed, both those demonstrated with LVAD unloading alone in patients bridged to transplantation and those occurring in the myocardium of patients who have recovered enough myocardial function to have the device removed. Reverse remodeling may be attributable to a reversal of the pathological mechanisms that occur in remodeling or the generation of new pathways. A reduction in cell size occurs after LVAD unloading, which does not necessarily correlate with improved cardiac function. However, some of the changes in both the cardiac myocyte and the matrix after LVAD support are specific to myocardial recovery. In the myocyte, increases in the cytoskeletal proteins and improvements in the Ca\(^{2+}\) handling pathway seem to be specifically associated with myocardial recovery. Changes in the matrix are complex, but excessive scarring appears to limit the ability for recovery, and the degree of fibrosis in the myocardium at the time of implantation may predict the ability to recover. ([Circ Res. 2013;113:777-791.]

Key Words: heart failure □ left ventricular assist device □ remodeling □ reverse remodeling
Molecular changes during LVAD support

1. LVAD
2. Myocyte size
3. Apoptosis
4. Calcium handling
5. Cytoskeletal proteins
6. The extracellular matrix (ECM)
7. Metabolic enzymes
8. The immune system
9. The sympathetic nervous system

BRIDGE TO RECOVERY
Bridge to recovery

Abstract
Purpose. Despite the remarkable advances with the use of ventricular assist devices (VAD) in adults, pneumatic pulsatile support in children is still limited. We report on our

Left Ventricular Assist Device and Resident Cardiac Stem Cells in Heart Failure: Human Heart’s Potential Matter

Mariangela Peruzzi, MD, Giuseppe Biondi-Zoccai, MD, Ernesto Greco, MD, Antonino G.M. Marullo, MD, PhD, Antonio Barretta, MD, Piergiusto Vitulli, MD, Giulio Pompilio, MD, Giacomo Frati, MD

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4Department of Cardiac Surgery, Laboratory of Vascular Biology and Regenerative Medicine, Centro Cardiologico Monzino, IRCCS, Milan, Italy
1. Si prevede di utilizzare n. **40 animali Mini-Pig**

2. Tipo di animale **Sumu**


5. Fornitore **Centro Ricerche Sperimentali - Università Cattolica del S. Cuore, Roma**

6. Titolo ed obiettivo del progetto di ricerca:

**Utilizzo di cellule staminali cardiache in associazione ad un sistema di assistenza ventricolare intracorporeo per il trattamento di pazienti in scompenso cardiaco terminale: creazione di un modello sperimentale pre-clinico**

L'obiettivo di questo progetto sarà quello di valutare l'effetto della terapia cellulare in associazione all'impianto di sistemi di assistenza ventricolare sulla funzione globale e regionale del ventricolo sinistro in un modello sperimentale animale (Mini-Pig) di infarto miocardico

7. Durata della sperimentazione (massimo trentasei mesi) **36**

8. Personale che attende alla esecuzione degli esperimenti e/o al controllo degli animali (compreso il responsabile degli esperimenti)

<table>
<thead>
<tr>
<th>Cognome e nome</th>
<th>Giacomo Frati</th>
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<tr>
<td>Cognome e nome</td>
<td>Antonio Amodeo</td>
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<td>Titolo di studio</td>
<td>Laurea in Medicina e Chirurgia</td>
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<td>Qualifica</td>
<td>Dirigente Medico</td>
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<td>Dipartimento</td>
<td>Medicina Cardiovascolare, Università Cattolica del S. Cuore, Roma</td>
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Research Article

A Novel Closed-Chest Porcine Model of Chronic Ischemic Heart Failure Suitable for Experimental Research in Cardiovascular Disease

Giuseppe Biondi-Zoccai, Elena De Falco, Mariangela Peruzzi, Elena Cavarretta, Massimo Mancone, Omar Leoni, Maria Emiliana Caristo, Marzia Lotriente, Antonino G. M. Marullo, Antonio Amodeo, Luca Pacini, Antonella Calogero, Vincenzo Petrozza, Isotta Chimenti, Fabrizio D’Ascenzo, and Giacomo Frati

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Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart

Tobias C Ott, Thomas S Matthiesen, Saik-Kia Goh, Lauren D Black, Stefan M Krenn, Theoden I Netoff, & Doris A Taylor

About 3,000 individuals in the United States are awaiting a donor heart worldwide. Every 2,2 million individuals are living with heart failure. A bioartificial heart is a theoretical alternative to transplantation or mechanical support. Generating a bioartificial heart requires engineering of cardiac architecture, appropriate cellular constituents and pump function. We decellularized hearts by coronary perfusion with detergents, preserved the underlying extracellular matrix, and prepared decellularized, perfusable vascular architectures, competent aortic valves and intact chamber geometry. To mimic cardiac cell composition, we seeded these constructs with cardiac or endothelial cells. To establish function, we maintained eight constructs for up to 28 days by coronary perfusion in a bioventricle that simulated cardiac physiology. By day 4, we observed macroscopic contractions. By day 8, under physiological load and electrical stimulation, constructs could generate contractile function (equivalent to about 2% of adult or 25% of 16-week fetal heart function) in a modified working heart preparation.

In the United States alone, nearly 8 million people live with heart failure and about 300,000 new cases are diagnosed annually. Heart transplantation remains the definitive treatment for end-stage heart disease. However, the supply of donor organs is limited. Once a heart is transplanted, individuals face lifelong immunosuppression and often late rejections. Heart transplantation, however, is a global issue, with patients living with heart failure who are on waiting lists in countries around the world.

RESULTS

Perfusion decellularization of cardiac hearts

To develop a valid perfusion decellularization protocol, we carried out antegrade coronary perfusion of 140 cadaveric rat hearts on a modified Langendorff apparatus and compared the degree of decellularization (that is, removal of DNA and intracellular structural proteins) that resulted from the use of three detergent solutions (Fig. 1). The use of SDS (Fig. 1c) gave better results than did polyethylene glycol (PEG, Fig. 1a) or other detergent protocols (data not shown) for full removal of cellular constituents. Antegrade coronary SDS perfusion over 12 h (Fig. 1c) yielded a fully decellularized construct. Histological evaluation revealed no remaining nuclei or contractile elements (Fig. 1g). DNA content decreased to less than 4% of that in cadaveric hearts (Supplementary Fig. 1 online), whereas the glycogen/mycline content was unchanged. After perfusion with Triton-X100 (Fig. 1e) and washing, SDS levels in the decellularized myocardium could not be differentiated from those in a quantitative assay (Supplementary Fig. 1).

Properties of the decellularized construct

Cells that had been cultured in the decellularized matrix (Fig. 2a) remained viable within the decellularized, decellularized matrix. The fiber composition (woven, striated cells) and orientation of the myocardial ECM were preserved, whereas cardiac cells were removed (Fig. 2b). Staining of compressed constructs. Within the retained ventricular ECM, we saw intact vascular basal membranes without endothelial or...
Supplementary Figure 3. Adult swine heart before (top) and after (bottom) complete SDS-perfusion-decellularization depicting intact 4-chamber geometry, retention of great vessel conduits and maintenance of cardiac architecture.
Il metodo Stamina non è una cura

di PAOLO BIANCO

La discussione in Parlamento sui trattamenti a base di cellule staminali, sollecitata anche dall'eco mediatica del caso Stamina i ministri, legifera d'urgenza il Parlamento. Se si debba o no praticare la cura definita trapianto di cellule staminali è divenuto materia di giurisprudenza e non di medicina; perché che davvero di cura si tratti è dato assurdamente per scontato. Così assumono i magistrati, i decreti ministeriali, e anche la stampa, a sua volta senza cercare verifiche dirette.

«STAMINALI, LA DEREGULATION

sui cittadini. La richiesta che sale in Italia dai pubblici di liberalizzare per legge le terapie compassionevoli (cioè non sperimentate né approvate) coincide dunque con interessi commerciali, ben diversi dalle motivazioni del pubblico. Nello stesso caso italiano

Un mercato in cui si vende non un bene tangibile industrialmente prodotto come la pasticca d'antan, ma un bene immateriale commercialmente valorizzato: si vende la speranza e la parola staminali, veicolo seducente e pegno di virtù taumaturgiche. I go-
I died waiting for embryonic stem cell research to find a cure. What about you?

I was the embryo.
FAITH-BASED MEDICAL SCIENCE

TAKE TWO ASPIRIN AND PRAY FOR A PARKINSON'S CURE IN THE MORNING.
Stem cell therapy: from evidence-based medicine to emotion-based medicine? The long Italian way for a scientific regulation

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