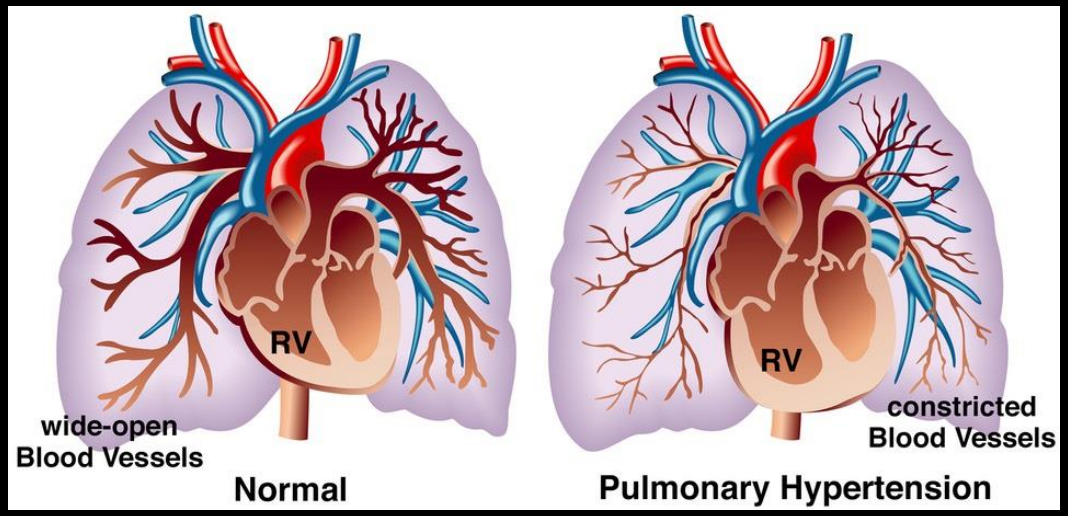
Stem Cell Therapies in Pulmonary hypertension

Olga Tura Ceide

BRN Seminars

27/09/2018

Pulmonary Hypertension



Serious progressive disorder

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Characterised by increased pulmonary arterial pressure and vascular remodeling

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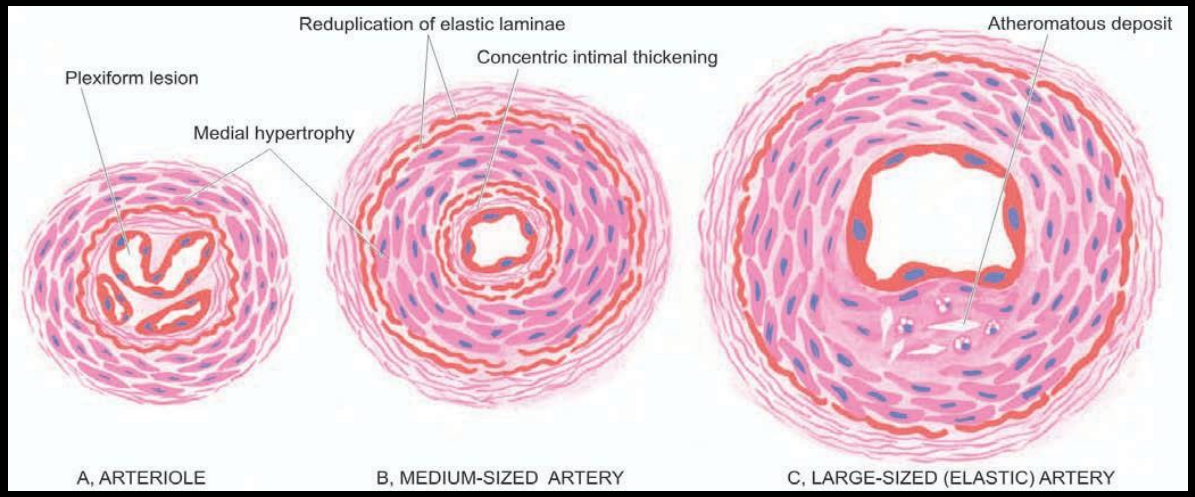
Right heart failure
death

Pulmonary Hypertension



Endothelial dysfunction

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Dysregulation of smooth muscle cell and endothelial cell funtion

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Increased proliferation, perivascular inflammation and decreased vessel luminal diameter

Pulmonary Hypertension

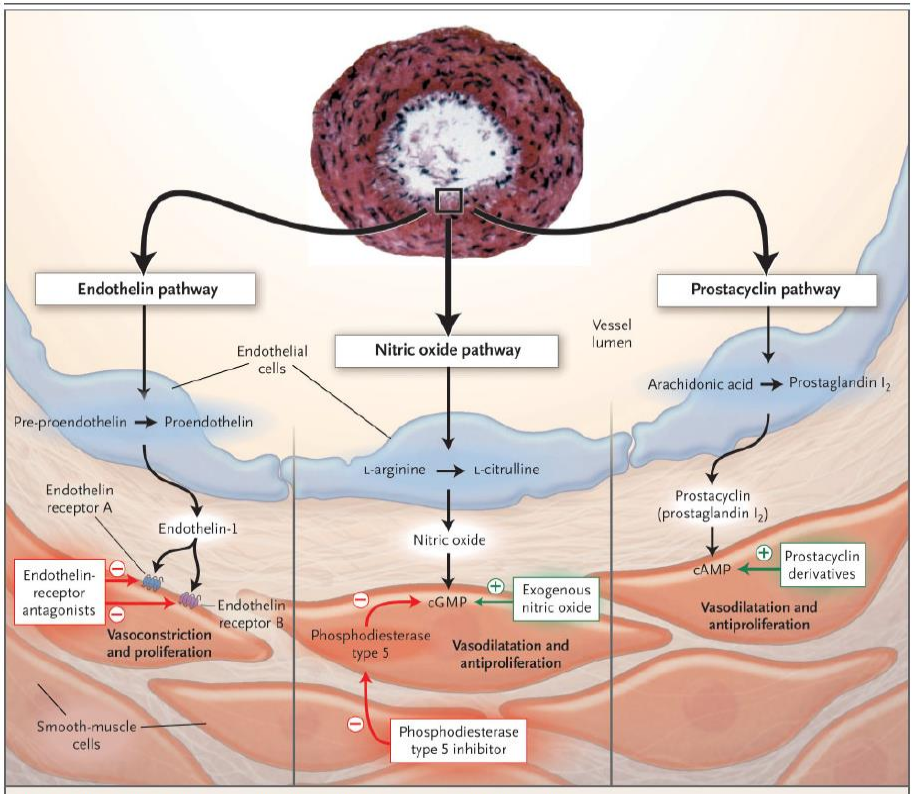


Figure 1. Targets for Current or Emerging Therapies in Pulmonary Arterial Hypertension.

Three major pathways involved in abnormal proliferation and contraction of the smooth-muscle cells of the pulmonary artery in patients with pulmonary arterial hypertension are shown. These pathways correspond to important therapeutic targets in this condition and play a role in determining which of four classes of drugs — endothelin-receptor antagonists, nitric oxide, phosphodiesterase type 5 inhibitors, and prostacyclin derivatives — will be used. At the top of the figure, a transverse section of a small pulmonary artery (<500 μm in diameter) from a patient with severe pulmonary arterial hypertension shows intimal proliferation and marked medial hypertrophy. Dysfunctional pulmonary-artery endothelial cells (blue) have decreased production of prostacyclin and endogenous nitric oxide, with an increased production of endothelin-1 — a condition promoting vasoconstriction and proliferation of smooth-muscle cells in the pulmonary arteries (red). Current or emerging therapies interfere with specific targets in smooth-muscle cells in the pulmonary arteries. In addition to their actions on smooth-muscle cells, prostacyclin derivatives and nitric oxide have several other properties, including antiplatelet effects. Plus signs denote an increase in the intracellular concentration; minus signs blockage of a receptor, inhibition of an enzyme, or a decrease in the intracellular concentration; and cGMP cyclic guanosine monophosphate.

Despite advances in the treatment:

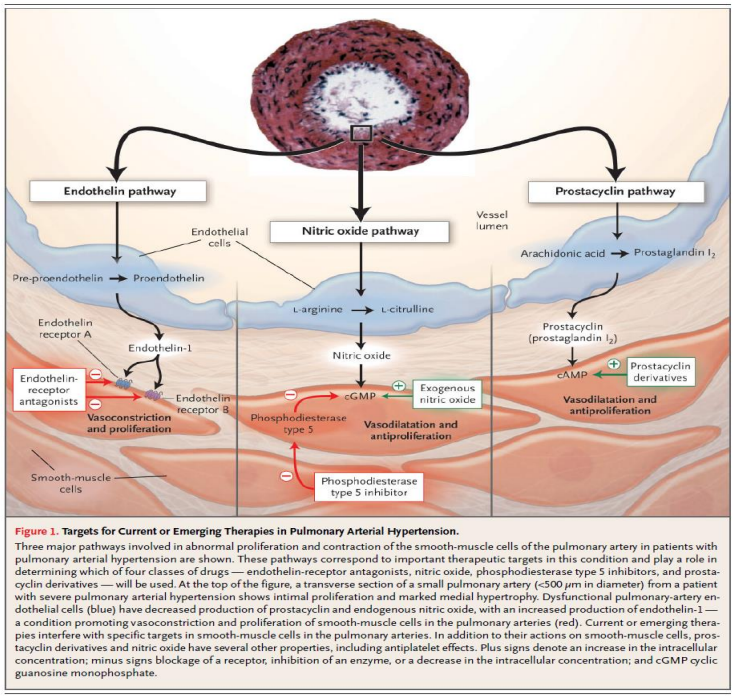
1) Prostacyclin and analogs
2) Endothelin receptor antagonists
3) Phosphodiesterase type 5 inhibitors

↓

Modest improvements measured by:

1) Exercise capacity
2) Quality of life measurements

Pulmonary Hypertension



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3) Phosphodiesterase type 5 inhibitors

↓

Modest improvements measured by:

1) Exercise capacity
2) Quality of life measurements

- PH remains a fatal disease
- Patients refractory to pharmacologic therapy-- lung trasplantation
- Limited availability of organs, postoperative infection, graft rejection--limited patient survival.

Facing obstacles

- Stem cell therapy have been reported to have a beneficial effects in experimental models of PH.
- Encouraging results (feasibility, safety and efficency of cell therapy).

Facing obstacles

Stem cell therapy have been reported to have a beneficial effects in experimental models of PH.

Encouraging results (feasibility, safety and efficency of cell therapy).

However:

- Different types of stem cells
- Different types of PH
- Different administration routes

- Difficult to compare results as the exact nature of delivered cells remains undetermined.
- Urgent need to move to a standardized stem cell identification before use.
- Few studies *in vitro* designed to elucidate potential their potential mechanisms of action.

Different types of stem cells

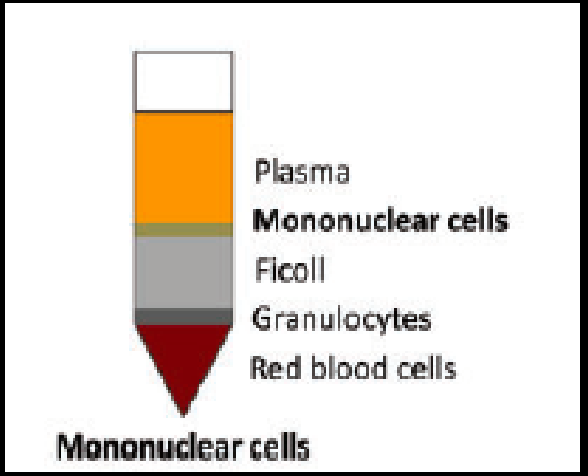
- Mononuclear stem cells (MNCs)
- Mesenchymal stromal cells (MSCs)
- Endothelial progenitor cells (EPCs)
- Adipose-derived stem cells (ADSc)
- iPS derived cells (iPS)
- Cardiac progenitor cells (CPCs)

Different types of stem cells

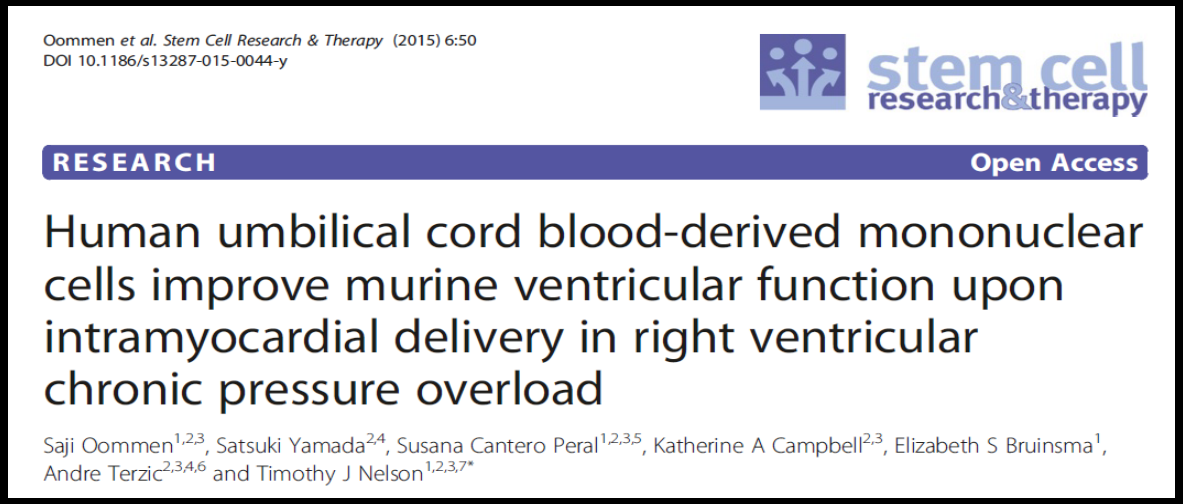
- Mononuclear stem cells (MNCs)
  - Umbilical cord blood PBMNCs

  Advantatges
  - Pro-angiogenic
  - Readely available
  - Easy to obtain

  Disadvantatges
  - comprises different cell types
  - Allogenic source (most of the times)



Different types of stem cells



Oommen *et al. Stem Cell Research & Therapy* (2015) 6:50
DOI 10.1186/s13287-015-0044-y

stem cell research&therapy

RESEARCH Open Access

Human umbilical cord blood-derived mononuclear cells improve murine ventricular function upon intramyocardial delivery in right ventricular chronic pressure overload

Saji Oommen[1,2,3], Satsuki Yamada[2,4], Susana Cantero Peral[1,2,3,5], Katherine A Campbell[2,3], Elizabeth S Bruinsma[1], Andre Terzic[2,3,4,6] and Timothy J Nelson[1,2,3,7*]

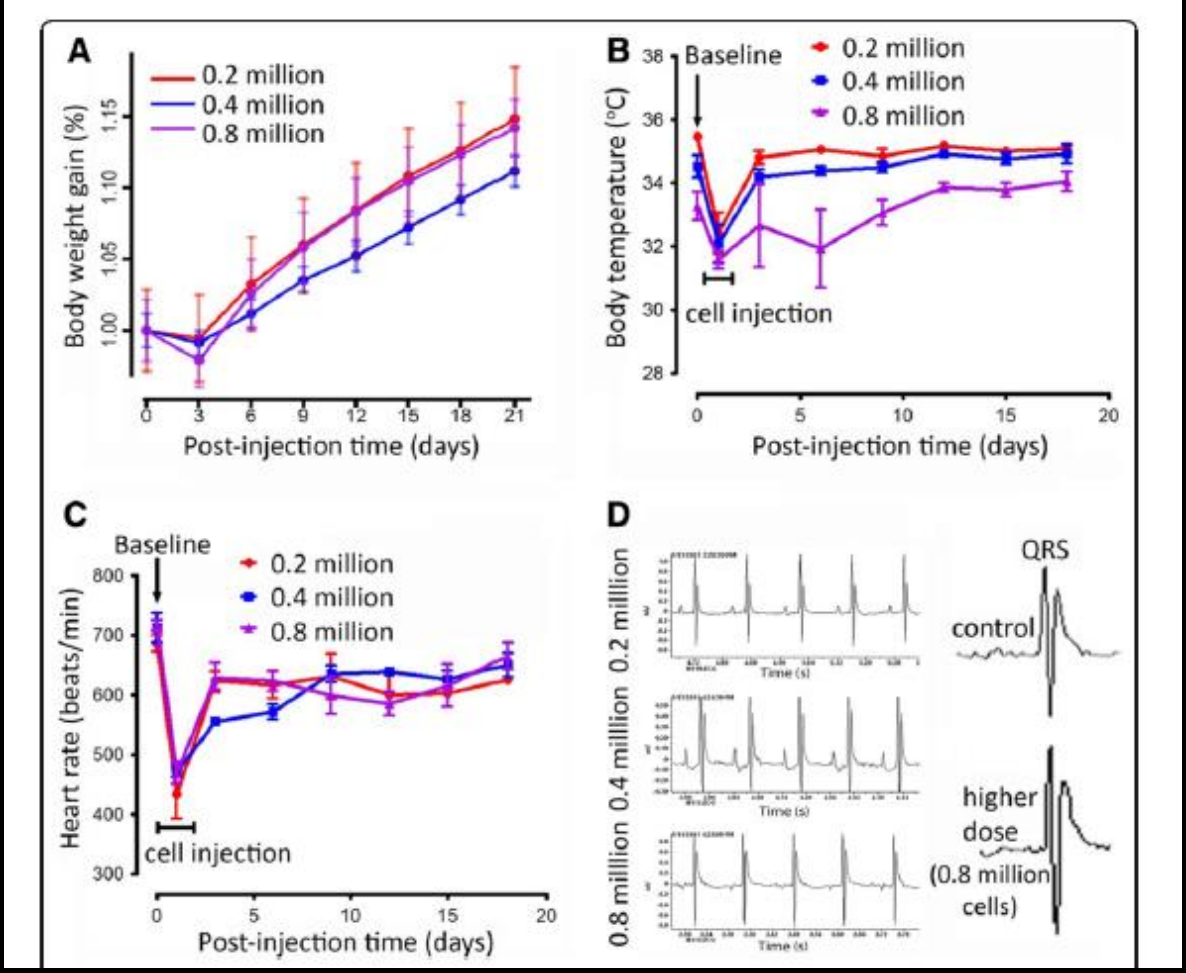
Using a pulmonary artery banding athymic murine model (PAB), UCB-MNCs injections led to improvement in:

- RV function, structure
- Reduction in RV fibrosis
- Increase in angiogenic biomarkers.

Oommen *et al*. Stem Cell Research and Therapy (2015)

Mononuclear stem cells_Cord blood

UCB-MNCs transplantation is safe (0.4-0.8 million cells/heart) without altering QT or ST-segments or electrocardiograph waves morphology



Intramyocardial injections to the right ventricle (5 injections 2,5 ul each)

Oommen *et al*. Stem Cell Research and Therapy (2015)

Mononuclear stem cells_Cord blood

Myocardial delivery of UCB-MNCs improves RV

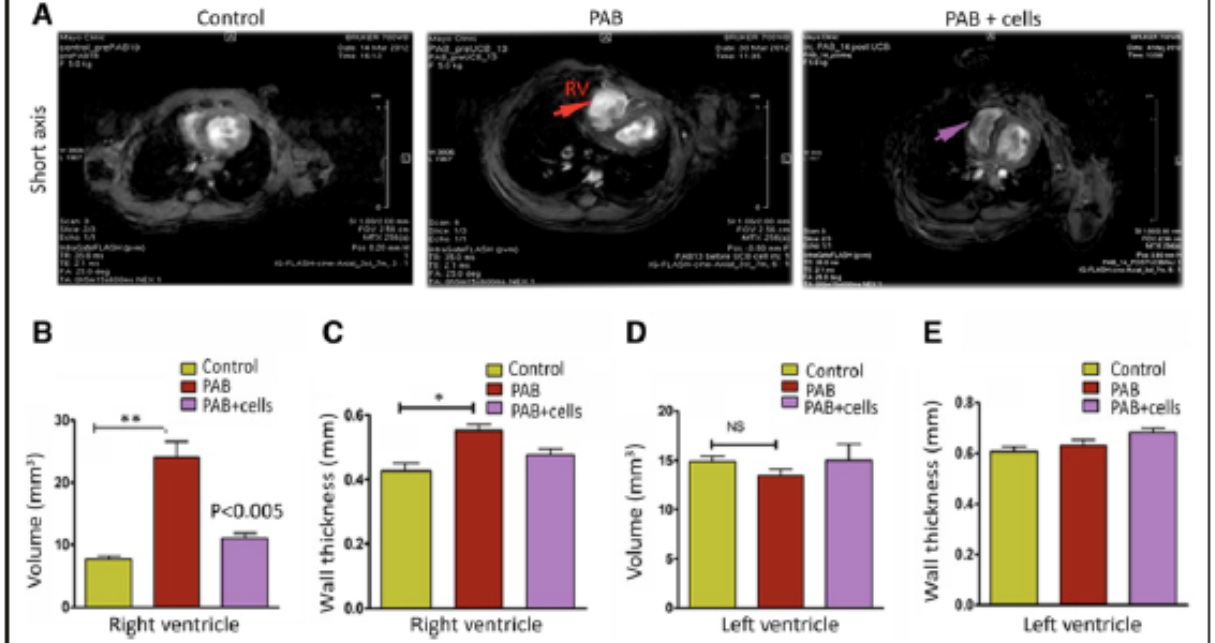


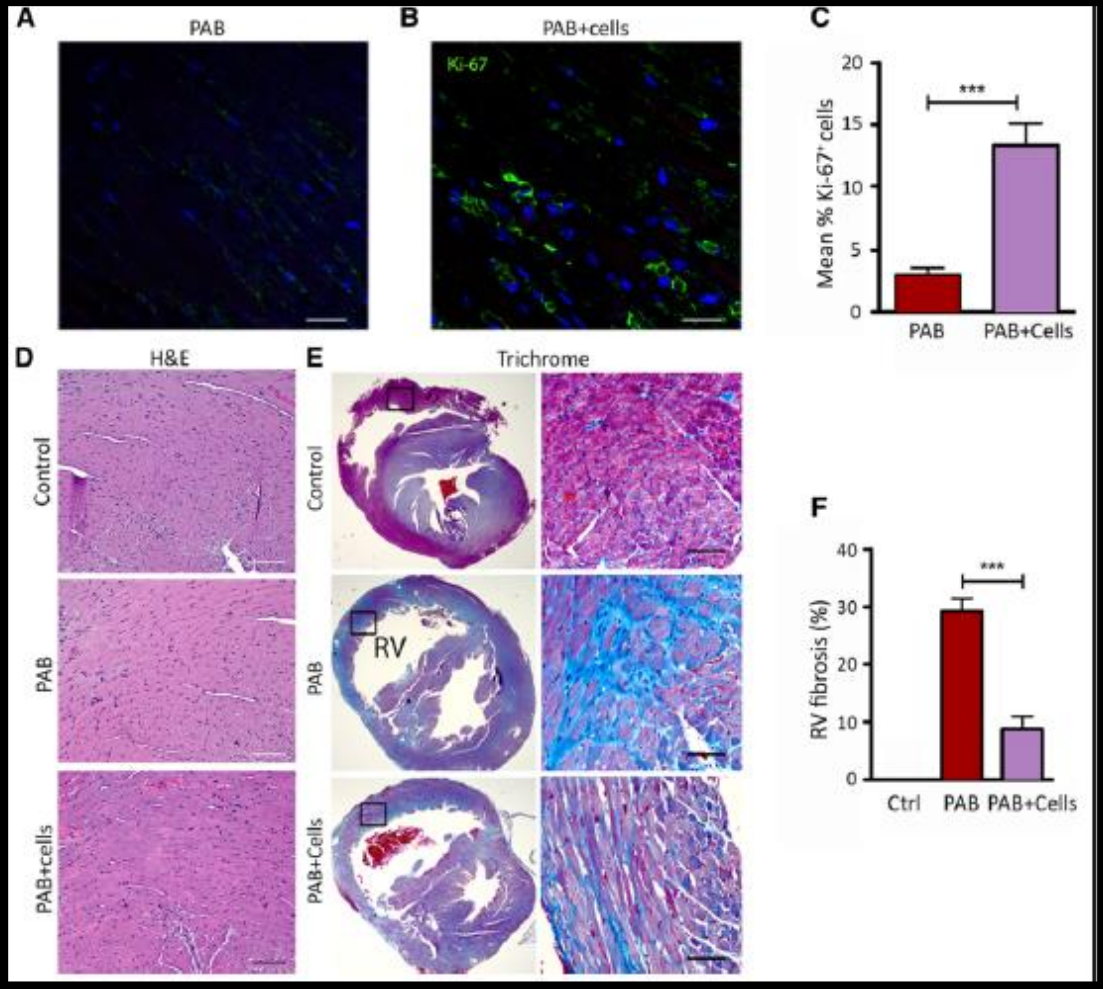
Figure 5 Myocardial delivery of UCB-MNCs improves RV function and favorable RV remodeling after pulmonary artery banding. **(A)** Short-axis image obtained from magnetic resonance imaging in the three experimental groups. Pulmonary artery banding (n = 6) produced severe RV dilation and ventricular dysfunction when compared with control (sham) animals (n = 6). Four weeks after cell transplantation, there was a less-pronounced RV dilation in the UCB-MNCs transplanted group (n = 6). **(B)** Magnetic resonance imaging-derived right ventricular volumes in control, PAB and cell transplanted group. The PAB only group demonstrated an increase in RV volume (** *P*-value <0.005 versus control). Intramyocardial delivery of UCB-MNCs indicated a reduction in RV volume (*P*-value <0.005 versus PAB). **(C)** Right ventricular wall thickness was compared among groups; the PAB-only animals showed a significant increase in RV wall thickness (* *P*-value <0.05); however, the cell transplanted group demonstrated a smaller reduction of the RV wall thickness. **(D, E)** No significant changes in LV functions were noticed between the experimental groups. LV, left ventricle; PAB, pulmonary artery banding; RV, right ventricle; UCB-MNCs, umbilical cord blood-mononuclear cells.

For weeks after CB-MNCs injection, MRI revealed a recovery of right ventricular chamber size and volume

Oommen *et al*. Stem Cell Research and Therapy (2015)

Mononuclear stem cells_Cord blood

CB-MNCs transplantation augments myocardial cell proliferation and attenuates myocardial fibrosis.



Oommen *et al*. Stem Cell Research and Therapy (2015)

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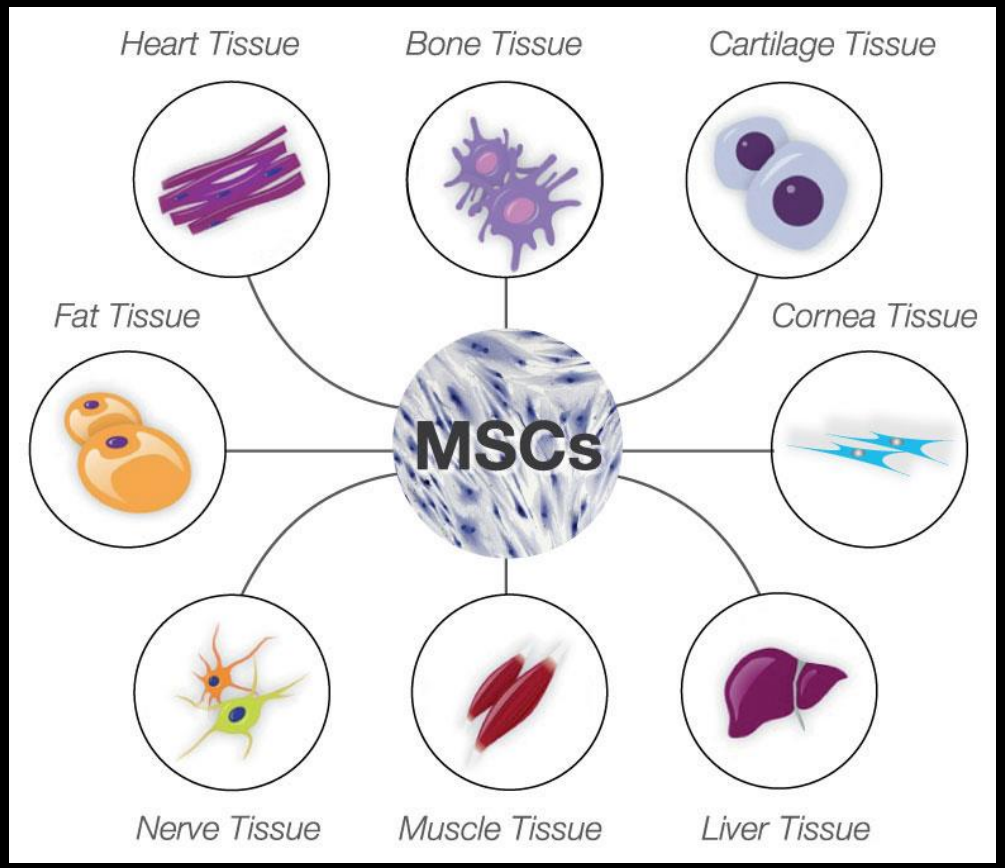
Mesenchymal stromal cells (MSCs)

- Isolated from: Bone marrow, wharton´s jelly, placenta, adipose tissue and muscle
- Multipotent stem cells
- Proangiogenic
- Cytoprotective
- Immune tolerance
- Easy to isolate
- Rapidly amplified



Mesenchymal stromal cells (MSCs)

Am J Physiol Heart Circ Physiol 297: H1606–H1616, 2009.
First published September 25, 2009; doi:10.1152/ajpheart.00590.2009.

TRANSLATIONAL PHYSIOLOGY

Allogenic stem cell therapy improves right ventricular function by improving lung pathology in rats with pulmonary hypertension

Soban Umar,[1] Yvonne P. de Visser,[2] Paul Steendijk,[1] Cindy I. Schutte,[1] El Houari Laghmani,[2] Gerry T. M. Wagenaar,[2] Wilhelmina H. Bax,[1] Eleni Mantikou,[1] Daniel A. Pijnappels,[1] Douwe E. Atsma,[1] Martin J. Schalij,[1] Ernst E. van der Wall,[1] and Arnoud van der Laarse[1]
Departments of [1]Cardiology and [2]Pediatrics, Leiden University Medical Center, Leiden, The Netherlands

ORIGINAL ARTICLE
Cardiovascular Disorders

JKMS

http://dx.doi.org/10.3346/jkms.2015.30.5.576 • *J Korean Med Sci 2015; 30: 576-585*

The Effect of Umbilical Cord Blood Derived Mesenchymal Stem Cells in Monocrotaline-induced Pulmonary Artery Hypertension Rats

- They concluded that MSCs reduced RV pressure overload, RV dysfunction and lung pathology.
- Autologous MSCs may alleviate cardiac and pulmonary symptoms in PH patients.

Umar et al. Am.J. Physiol. Heart Circ. Physiol 297 (2009)

Mesenchymal stromal cells (MSCs)

Intravenous injection of BM and UCB-derived MSCs improved RV hypertrophy and RV ejection fraction of MCT-induced PAH rats.

Table 3. *RV hemodynamic data of rats in the control, MCT60, and MCT + MSC groups*

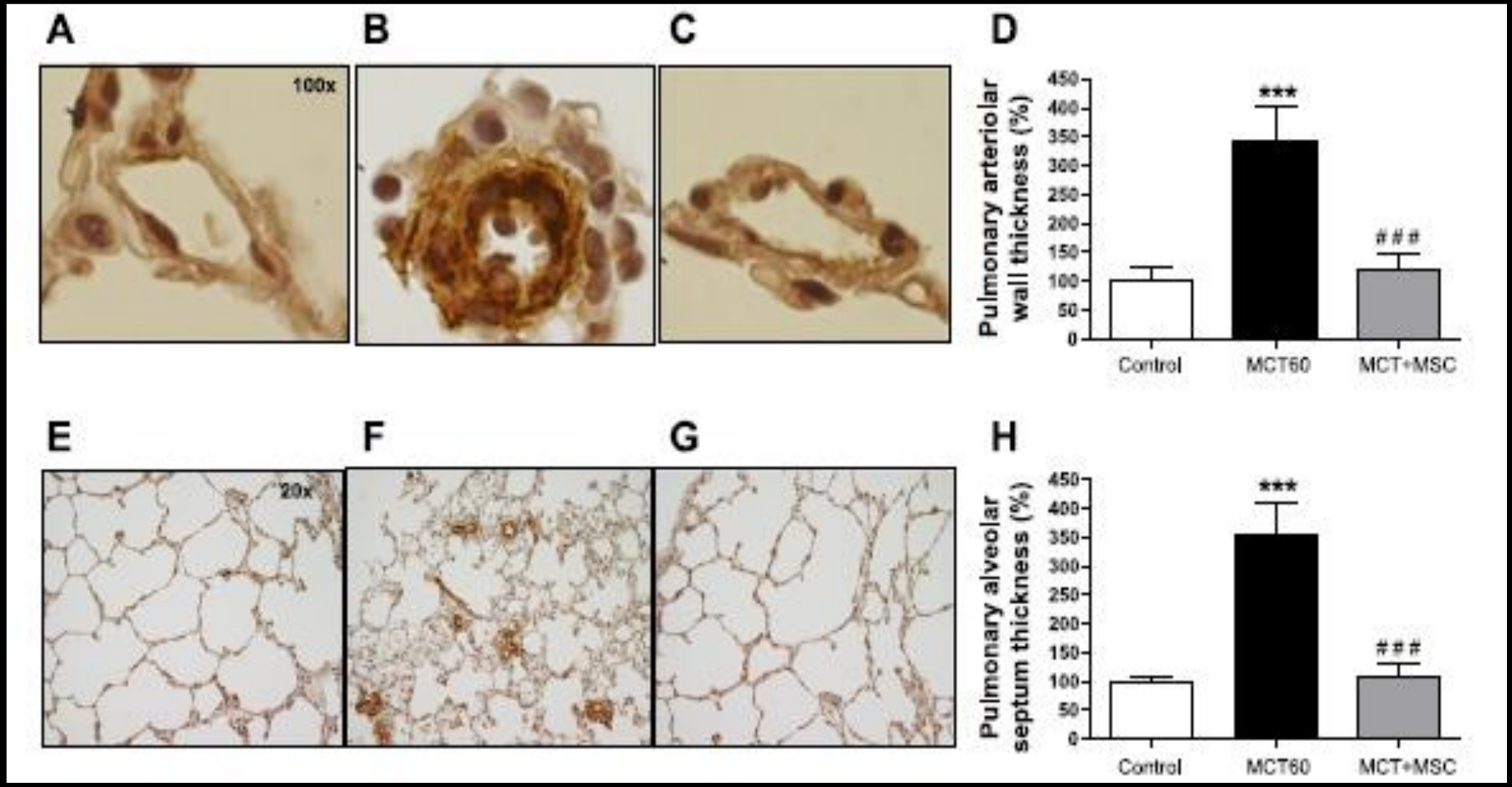
	Control	MCT60	MCT + MSC
Heart rate, beats/min	330±26	341±36	327±26
Stroke volume, μl	228±50	234±64	287±37
Cardiac output, ml/min	74±13	79±20	93±10*
Ejection fraction, %	56.2±11.2	42.8±6.2†	52.1±5.2
End-systolic volume, μl	200±103	323±132	270±75
End-diastolic volume, μl	427±150	556±183	557±99
End-systolic pressure, mmHg	24±5	38±15*	28±4
End-diastolic pressure, mmHg	1.3±1.2	3.9±1.8†	1.9±0.9‡
Peak systolic pressure, mmHg	27.2±4.9	41.5±16.9*	30.7±4.4
dP/dt_{max}, mmHg/s	1,565±383	2,215±1,040	1,832±455
Negative dP/dt_{min}, mmHg/s	1,334±385	1,912±860	1,675±493
Stroke work, mmHg·μl	5,071±1,415	7,044±2,238	6,682±1,117
Relaxation time constant, ms	13.7±3.8	14.0±2.9	12.9±4.7
Arterial elastance (afterload), mmHg/μl	0.11±0.04	0.19±0.12	0.10±0.03
End-systolic elastance, mmHg/μl	0.19±0.17	0.17±0.20	0.10±0.07
End-diastolic elastance, mmHg/μl	0.008±0.005	0.010±0.004	0.007±0.004
Preload recruitable stroke work, mmHg	21±7	25±15	19±5

Values are means ± SD. Data were collected 28 days after MCT (or control) treatment. *$P < 0.05$ and †$P < 0.01$ vs. the control group; ‡$P < 0.05$ vs. the MCT60 group.

Umar et al. Am.J. Physiol. Heart Circ. Physiol 297 (2009)

Mesenchymal stromal cells (MSCs)

MSC therapy drecreased the arterioral thickness and medial hypertrophy



Umar et al. Am.J. Physiol. Heart Circ. Physiol 297 (2009)

Mesenchymal stromal cells (MSCs)

Implantation of Mesenchymal Stem Cells Overexpressing Endothelial Nitric Oxide Synthase Improves Right Ventricular Impairments Caused by Pulmonary Hypertension

Sachiko Kanki-Horimoto, MD, PhD; Hitoshi Horimoto, MD, PhD; Shigetoshi Mieno, MD, PhD; Kenji Kishida, MD; Fusao Watanabe, PhD; Eisuke Furuya, PhD; Takahiro Katsumata, MD, PhD

Intravenously administered gene-transduced MSC overexpressing eNOS in MCT-induced PAH rats.

↙ ↘

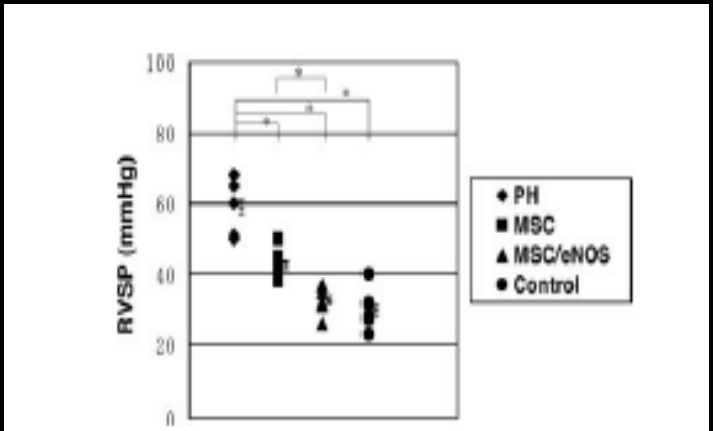
↑↑ Increase endothelial cells
↑↑ Secretion of NO

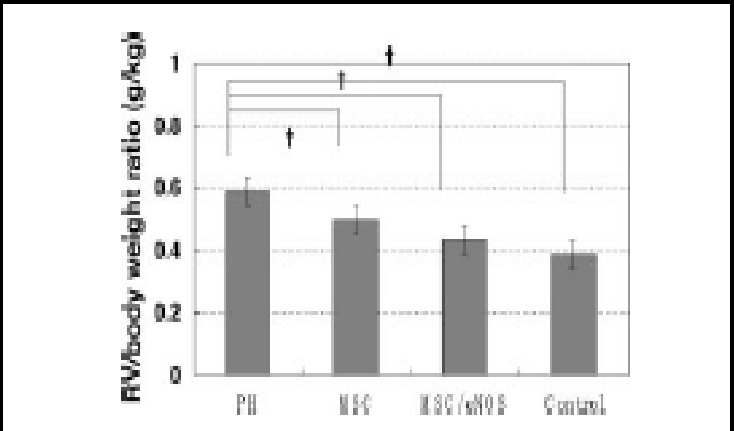
↓

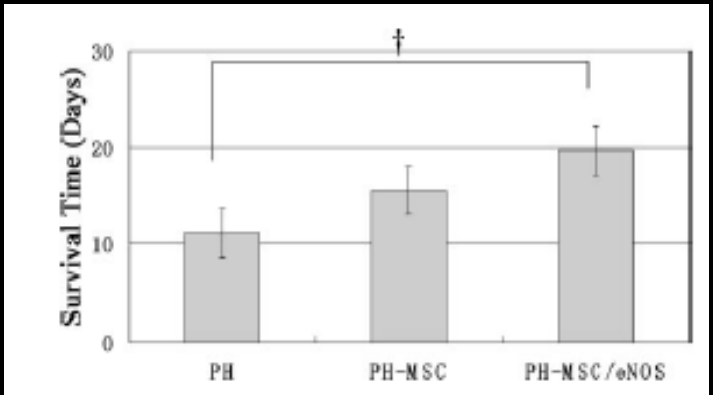
↓↓↓ Constriction of pulmonar arteries
↓↓↓ Resistance
↓↓↓ Pulmonary hypertension

Kanki-Horimoto et al. Circulation. 2006;114

Mesenchymal stromal cells (MSCs)







Kanki-Horimoto et al. Circulation. 2006;114

- Both MSC and MSC/eNOS groups significantly inhibited RVSP progression and RV/body weight ratio compared to nontreated MCT-PH rats.
- MSC/eNOS group significantly prolonged survival time compared to non-treated PH group
- MSC overexpresing eNOS offers therapeutic effects for PH-induced RV impairment.

Mesenchymal stromal cells (MSCs)

STEM CELLS

TISSUE-SPECIFIC STEM CELLS

Mesenchymal Stromal Cells Expressing Heme Oxygenase-1 Reverse Pulmonary Hypertension

OLIN D. LIANG, S. ALEX MITSIALIS, MUN SEOG CHANG, ELENI VERGADI, CHANGJIN LEE, MUHAMMAD ASLAM, ANGELES FERNANDEZ-GONZALEZ, XIANLAN LIU, RAJIV BAVEJA, STELLA KOUREMBANAS

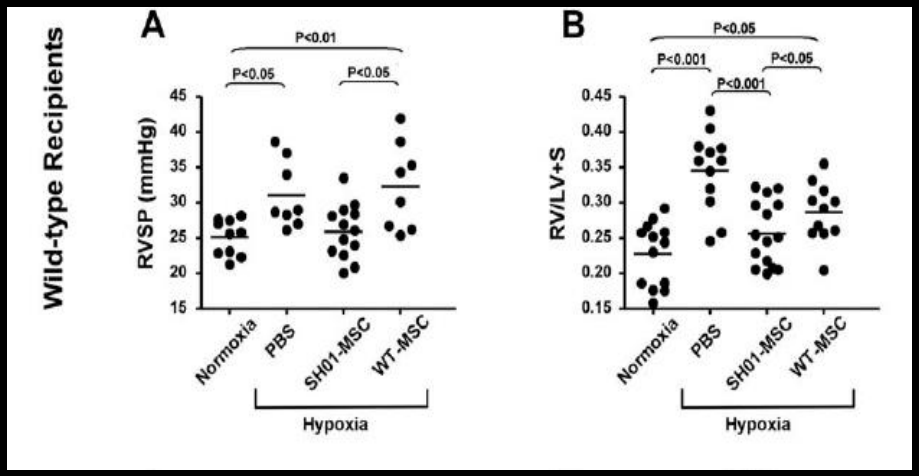
Division of Newborn Medicine, Children's Hospital Boston and Harvard Medical School, Boston, Massachusetts, USA

- Heme oxygenase-1 isoform (HO-1) activity restores homostasis by exerting antiinflammatory, antiapoptotic and antiproliferative effects on diverse cell types.
- It has been shown that upregulation of endogenous HO-1 can prevent hypoxia-induced PAH in rat.

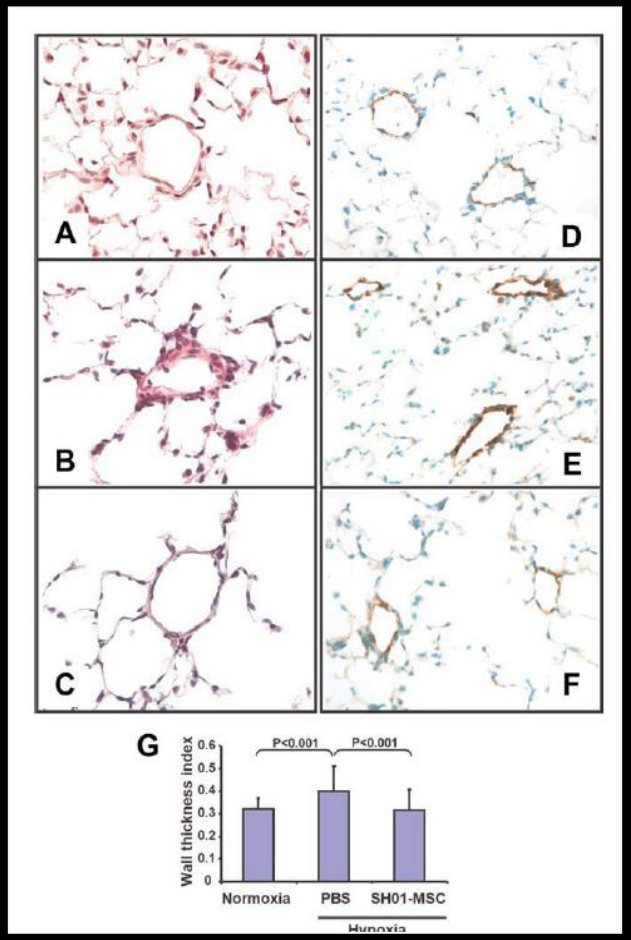
Liang *et al*. STEM CELLS 2011;29:99–107

Mesenchymal stromal cells (MSCs)

MSC transplantation reverses established PAH



MSC transplantation reverses lung vascular remodelling

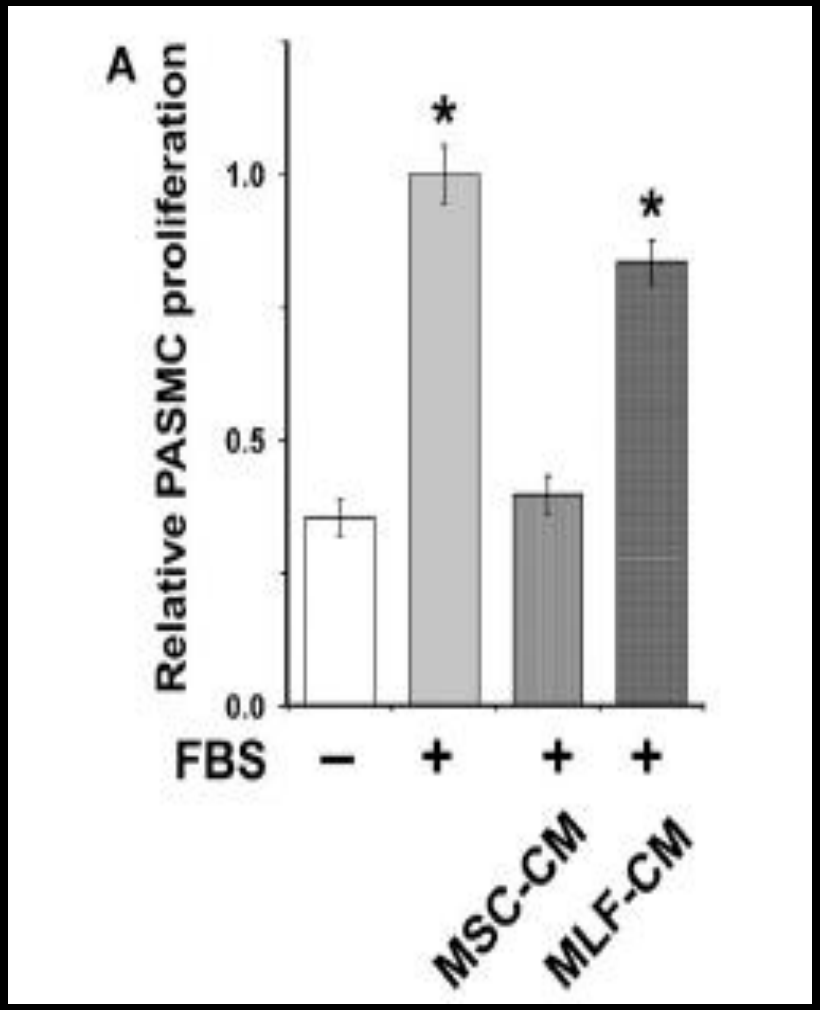


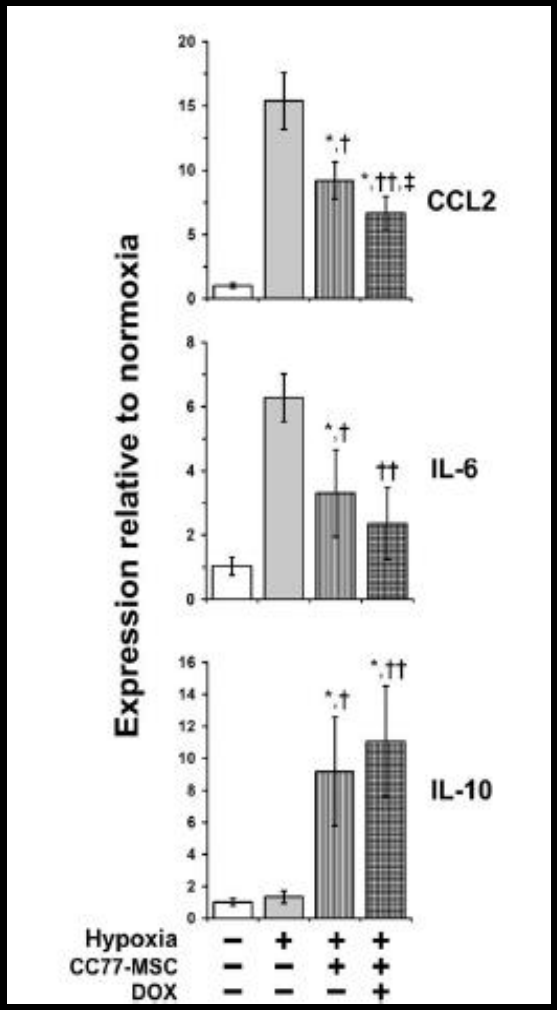
Intravenous injection of MSCs expressing HO-1 in a chronic hypoxia induced PAH model resulted in nomal RVSP and a significant reduction of RVH.

Mesenchymal stromal cells (MSCs)

MSC-CM inhibits pulmonary artery smooth muscle cell proliferation

MSC transplantation modulates the hypoxia-induced lung inflammation





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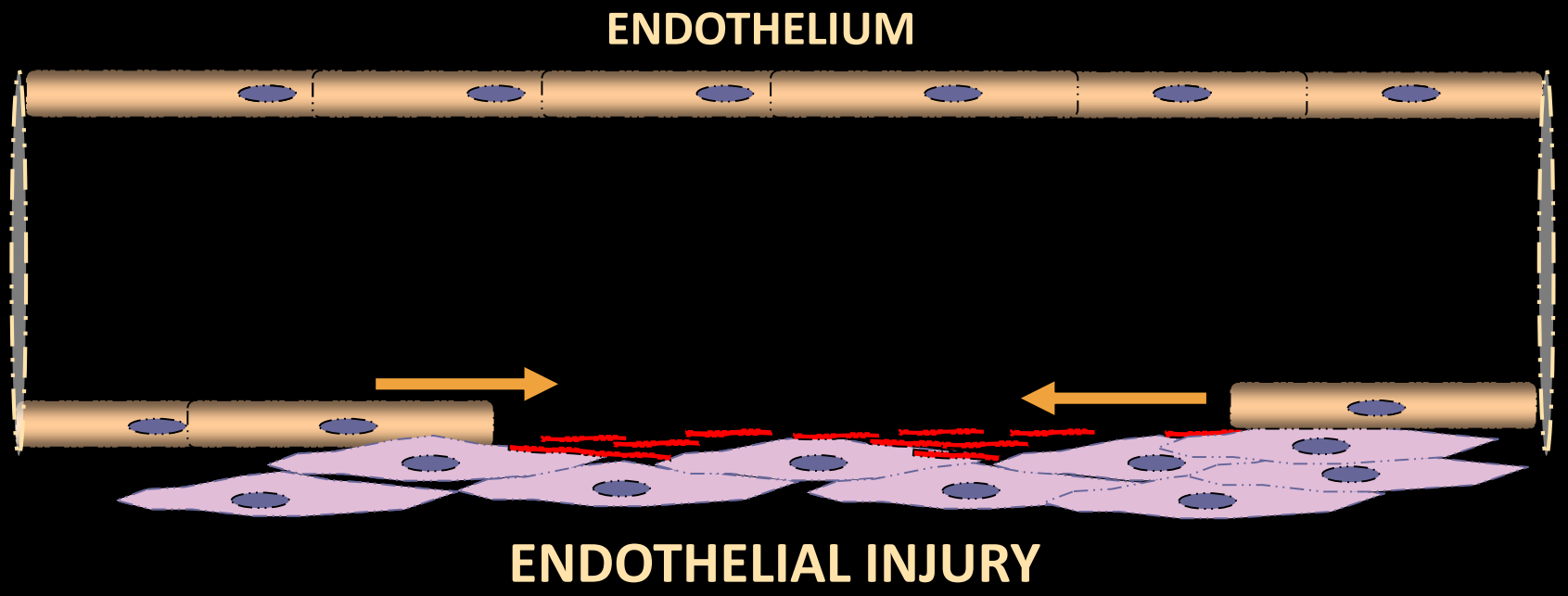
ANGIOGENESIS; PARADIGM



It was believed that the vasculature was only repaired locally by outgrowth from existing vessels (angiogenesis).

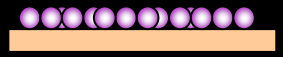
TAKAYUKI ASAHARA- DISCOVERY OF ENDOTHELIAL PROGENITOR CELLS IN THE ADULT.

Science
AAAS

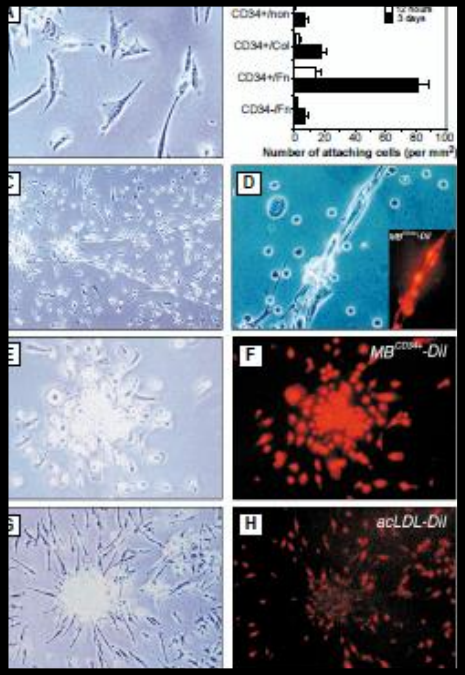
Isolation of Putative Progenitor Endothelial Cells for Angiogenesis
Takayuki Asahara *et al.*
Science **275**, 964 (1997);
DOI: 10.1126/science.275.5302.964

MONONUCLEAR CELLS

MAGNETIC SEPARATION
CD34+ & KDR+



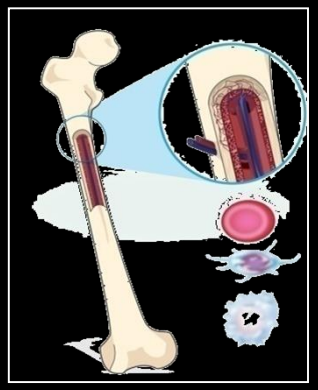
FIBRONECTIN PLATES



Endothelial progenitor cells isolated from the bone marrow could differentiate into mature endothelial cells and form new blood vessels *in vitro*.

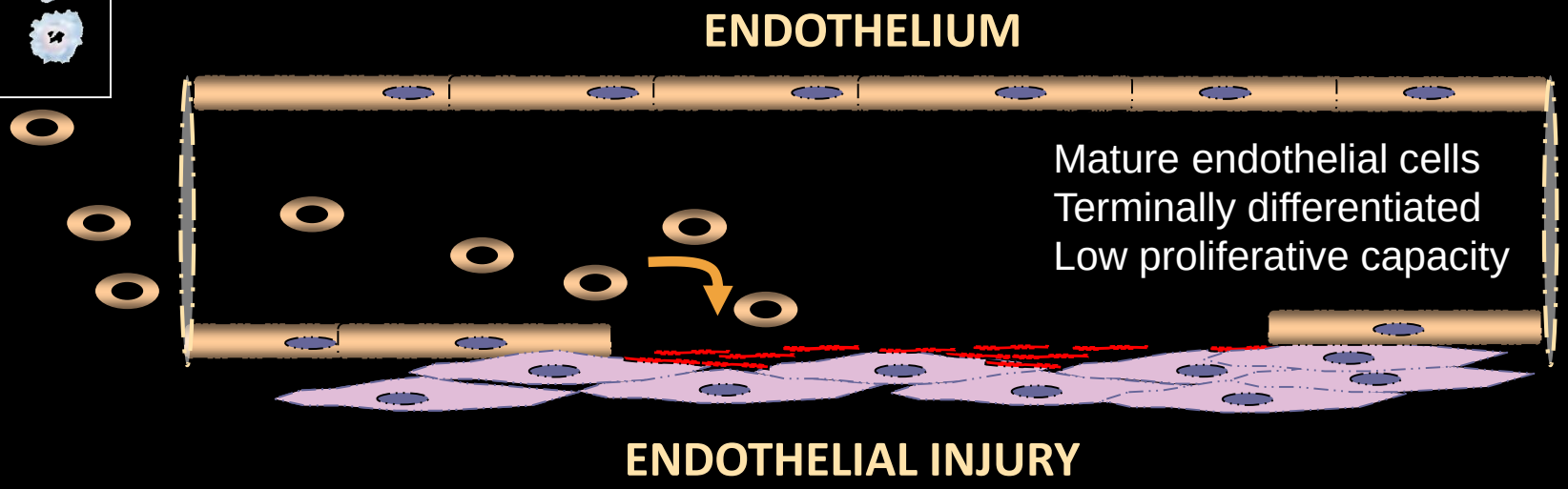
Asahara *et al*, Science; 275:964-7

•NEO-VASCULOGENESIS



BONE MARROW

Endothelial progenitor cells
Home to site of injury
Facilitate revascularisation



It is now believed that vascular repair could be initiated remotely from a recirculating progenitor cell (vasculogenesis), such as occurs in the developing foetus, and which might home to ischaemic tissue and initiate new vessel growth.

CLINICAL INTEREST

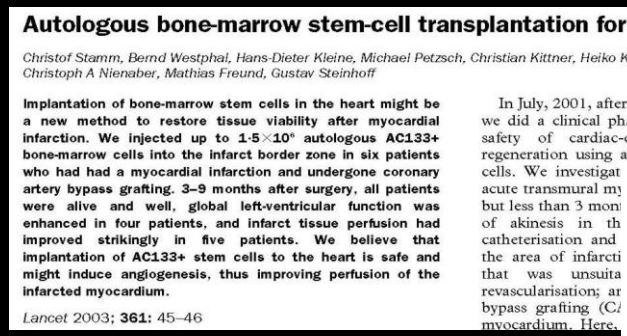
Lancet 2002: 360; 427

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



Autologous bone-marrow stem-cell transplantation for myocardial regeneration

Christof Stamm, Bernd Westphal, Hans-Dieter Kleine, Michael Petzsch, Christian Kittner, Heiko Klinge, Carl Schümichen, Christoph A Nienaber, Mathias Freund, Gustav Steinhoff

Implantation of bone-marrow stem cells in the heart might be a new method to restore tissue viability after myocardial infarction. We injected up to 1·5×10⁸ autologous AC133+ bone-marrow cells into the infarct border zone in six patients who had had a myocardial infarction and undergone coronary artery bypass grafting. 3–9 months after surgery, all patients were alive and well, global left-ventricular function was enhanced in four patients, and infarct tissue perfusion had improved strikingly in five patients. We believe that implantation of AC133+ stem cells to the heart is safe and might induce angiogenesis, thus improving perfusion of the infarcted myocardium.

Lancet 2003; **361:** 45–46

In July, 2001, after approval by the local ethics committee, we did a clinical phase-1 trial to assess the feasibility and safety of cardiac-cell transplantation for myocardial regeneration using autologous, bone-marrow-derived stem cells. We investigat
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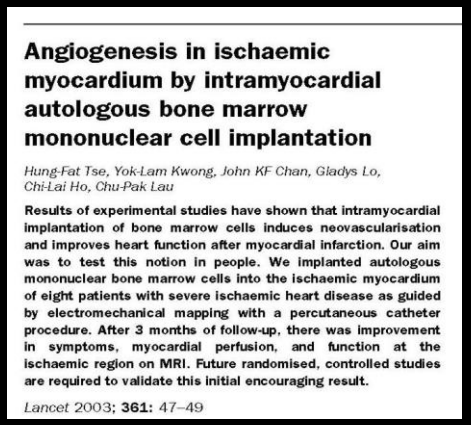
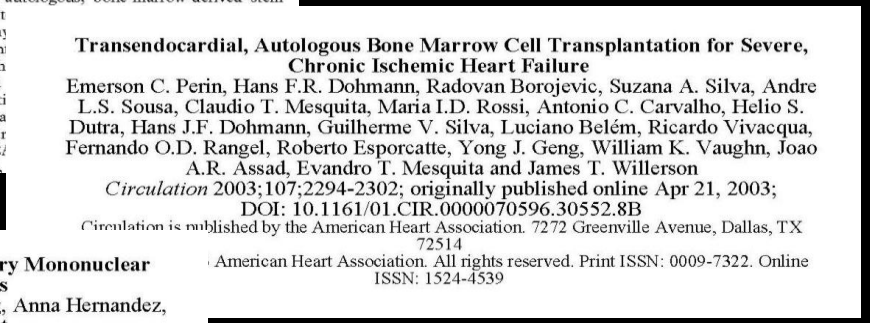


Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation

Hung-Fat Tse, Yok-Lam Kwong, John KF Chan, Gladys Lo, Chi-Lai Ho, Chu-Pak Lau

Results of experimental studies have shown that intramyocardial implantation of bone marrow cells induces neovascularisation and improves heart function after myocardial infarction. Our aim was to test this notion in people. We implanted autologous mononuclear bone marrow cells into the ischaemic myocardium of eight patients with severe ischaemic heart disease as guided by electromechanical mapping with a percutaneous catheter procedure. After 3 months of follow-up, there was improvement in symptoms, myocardial perfusion, and function at the ischaemic region on MRI. Future randomised, controlled studies are required to validate this initial encouraging result.

Lancet 2003; **361:** 47–49



Transendocardial, Autologous Bone Marrow Cell Transplantation for Severe, Chronic Ischemic Heart Failure

Emerson C. Perin, Hans F.R. Dohmann, Radovan Borojevic, Suzana A. Silva, Andre L.S. Sousa, Claudio T. Mesquita, Maria I.D. Rossi, Antonio C. Carvalho, Helio S. Dutra, Hans J.F. Dohmann, Guilherme V. Silva, Luciano Belém, Ricardo Vivacqua, Fernando O.D. Rangel, Roberto Esporcatte, Yong J. Geng, William K. Vaughn, Joao A.R. Assad, Evandro T. Mesquita and James T. Willerson

Circulation 2003;107;2294-2302; originally published online Apr 21, 2003;
DOI: 10.1161/01.CIR.0000070596.30552.8B

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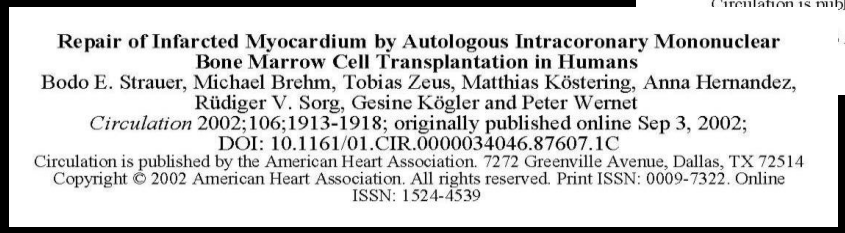

Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans

Bodo E. Strauer, Michael Brehm, Tobias Zeus, Matthias Köstering, Anna Hernandez, Rüdiger V. Sorg, Gesine Kögler and Peter Wernet

Circulation 2002;106;1913-1918; originally published online Sep 3, 2002;
DOI: 10.1161/01.CIR.0000034046.87607.1C

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The discovery of circulating endothelial progenitor cells has stimulated clinical interest in the potential of these cells to promote vascular regeneration in patients with ischaemic heart disease or peripheral vascular disease.

Eriko Tateishi-Yuyama, *et al* THE LANCET • Vol 360 • August 10, 2002

Lancet 2002: 360; 427

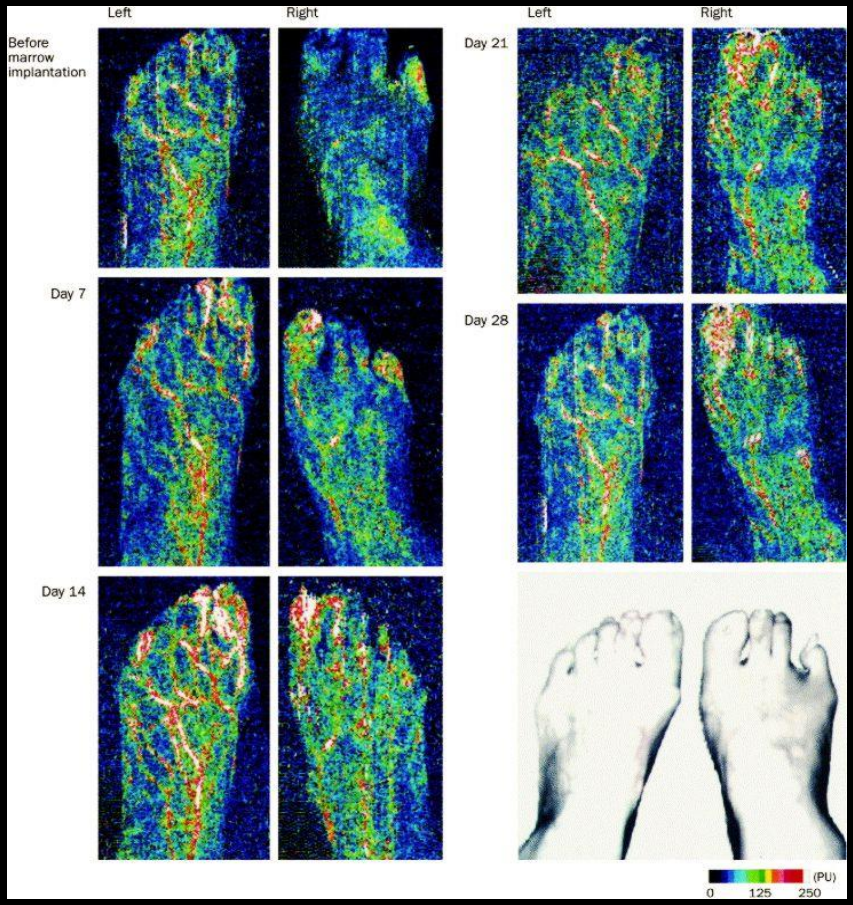
ARTICLES

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

Patients with bilateral leg ischaemia were randomly injected with bone marrow mononuclear cells in one leg, and saline in the other as a control.



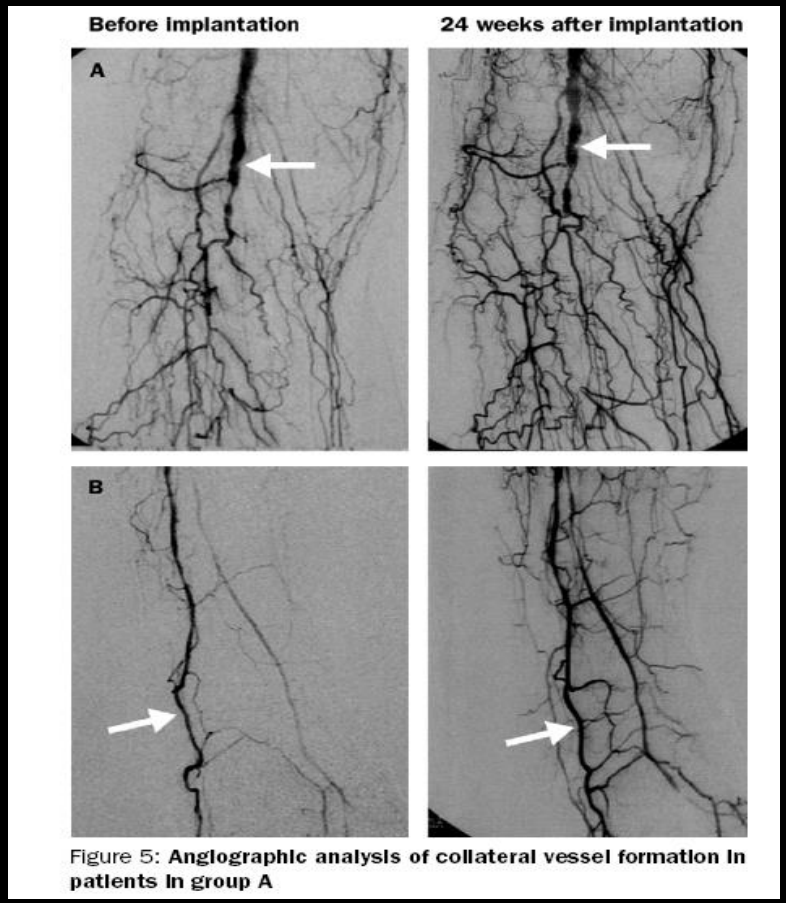


Figure 5: **Angiographic analysis of collateral vessel formation in patients in group A**

Laser Doppler blood perfusion. Right leg was implanted with bone marrow-mononuclear cells (red to white colour shows enriched perfusion). Left leg injected with saline.
Right digit IV was amputated.

CLINICAL INTEREST-PH

Models	Cell type	Route of administration	Pulmonary effect	Direct / indirect RV effect	Found cell engraftment	Study primarily designed for RV effects	Authors
Pre clinical study MCT rats	EPC	Intravenous	↓connexin43, eNOS expression, ↑ alveolar sacs, ↑ small lung arterioles	Indirect. ↓RVSP, ↓ RVH, ↓ connexin43, eNOS expression	In pulmonary arterioles	No	Yip et al., 2008
			↑ n small lung arterioles, ↓ pulmonary arteriole muscularization	Indirect. ↓RVSP, ↓ RVH	In small pulmonary arterioles	No	Xia et al., 2009
			↓ pulmonary arteriole muscularization	Indirect. ↓RVSP, ↓ RVH	In lung tissue after 15 min but not 24h after injection	No	Ormiston et al., 2009
			ø	ø	ø	No	Mirsky et al., 2011
	EPC producing prostacyclin	Intravenous	↓ pulmonary vessel wall thickening, ↓ cell proliferation in pulmonary vessel wall	Indirect. ↓RVSP, ↓ RVH	In lungs up to 25 days	No	Zhou et al., 2013
	eNOS transduced EPC	Intravenous	↓ pulmonary arteriole muscularization, ↑ microvascular perfusion	Indirect. ↓RVSP, ↓ RVH	In distal arterioles	No	Zhao et al., 2005
	EPC + sildenafil	Intravenous	↑ alveolar sacs, ↑ small lung arterioles, ↓ apoptic and inflammatory biomarkers	Indirect. ↓ apoptic and inflammatory biomarkers, ↓RVSP, ↓ RV weigh	ø	No	Yen et al., 2013
	EPC + cilostazol	Intravenous	↑ alveolar sacs, ↑ small lung arterioles	Indirect. ↓ connexin43, eNOS expression, ↓RVSP, ↓ RV weigh	In pulmonary arterioles	No	Sun et al., 2009

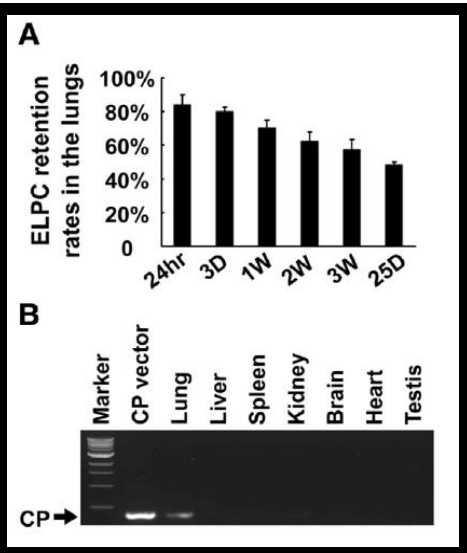
Over the years, there have been many studies involving administration of autologous EPC as a treatment for PH

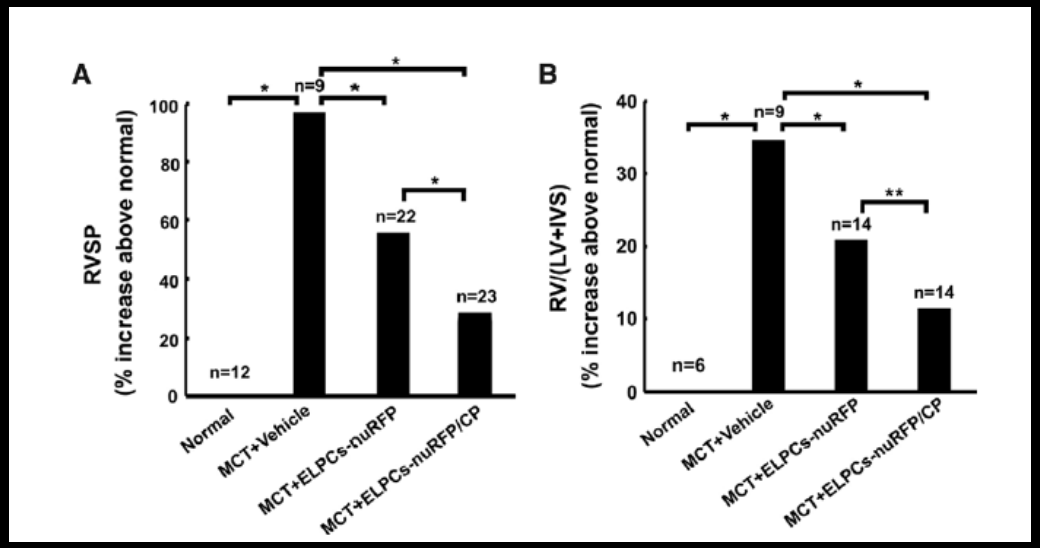
Endothelial progenitor cells

Hypertension

Endothelial-Like Progenitor Cells Engineered to Produce Prostacyclin Rescue Monocrotaline-Induced Pulmonary Arterial Hypertension and Provide Right Ventricle Benefits

Lei Zhou, MD, PhD; Zhiqiang Chen, MD; Peter Vanderslice, PhD; Shui-Ping So, PhD; Ke-He Ruan, MD, PhD; James T. Willerson, MD; Richard A.F. Dixon, PhD



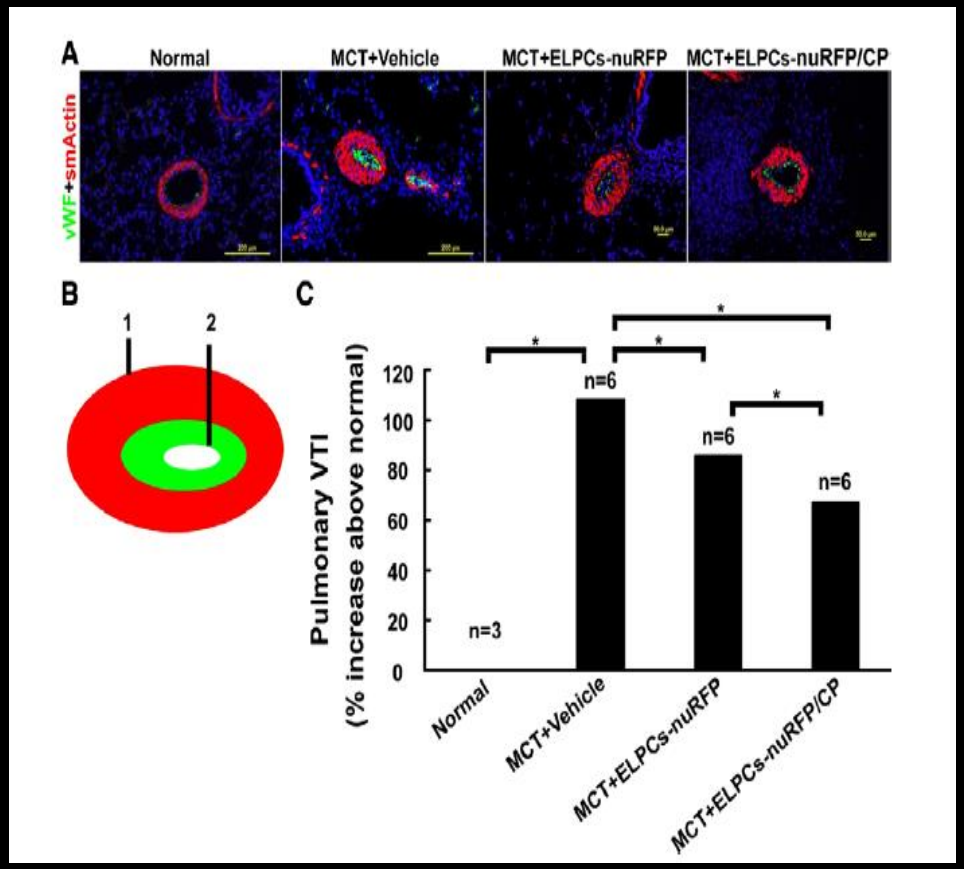


-Cyclooxygenase isoform 1-prostacyclin synthase–expressing ELPCs reversed MCT-induced PAH.

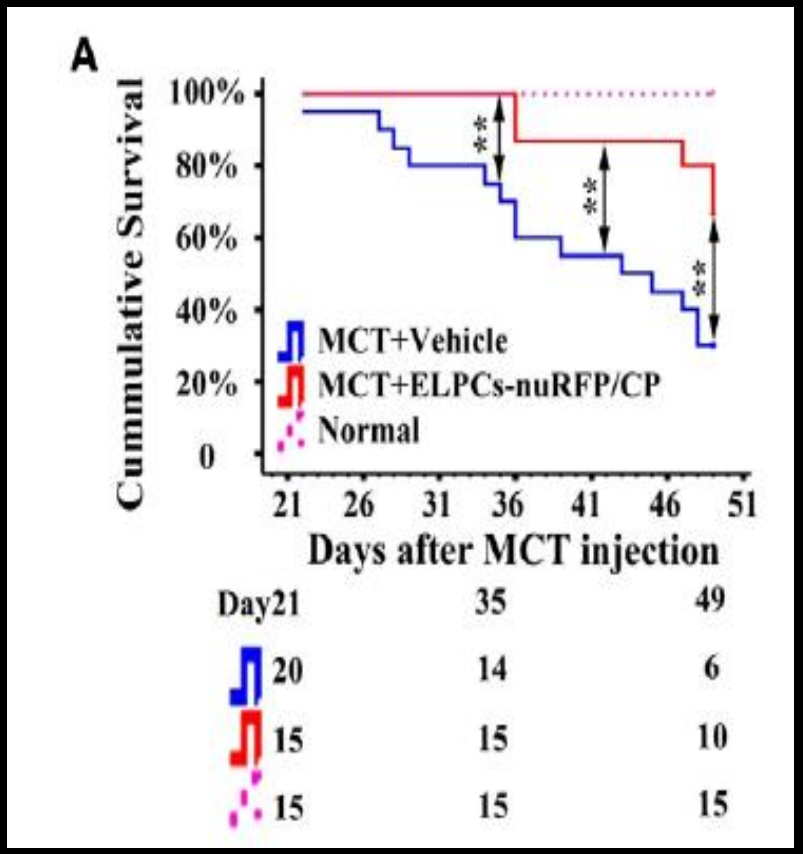
-A single jugular vein injection offered survival benefits for at least 4 weeks and may provide a promising option for PAH patients.

Zhou, et al. Circulation. 2013;128:982-994.

Endothelial progenitor cells



Engineered ELPCs attenuated monocrotaline (MCT)-induced pulmonary vessel wall thickening



Both engineered ELPCs and control ELPCs provided a survival benefit that was accompanied by a decrease in right ventricular hypertrophy.

Zhou, et al. Circulation. 2013;128:982-994.

WHAT IS AN EPC?

What is an EPC?

There is as yet no DEFINITIVE description of what is an EPC.
Different putative EPC assays reflect different cell populations.

↓

EPCs cannot be reliably quantified or enriched by any agreed standard

↓

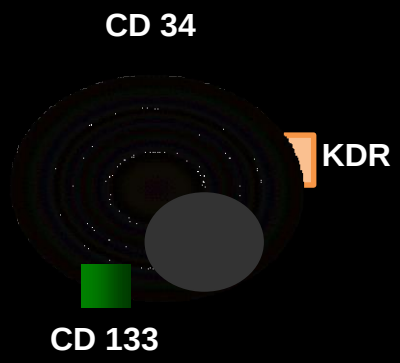
This controversy has HINDERED the development of effective cellular therapies for vascular regeneration

EPCs CAN BE defined by 3 methods:

1

1997
Asahara *et al*, Science

Phenotypic analysis by Flow Cytometry
(Original description)



2

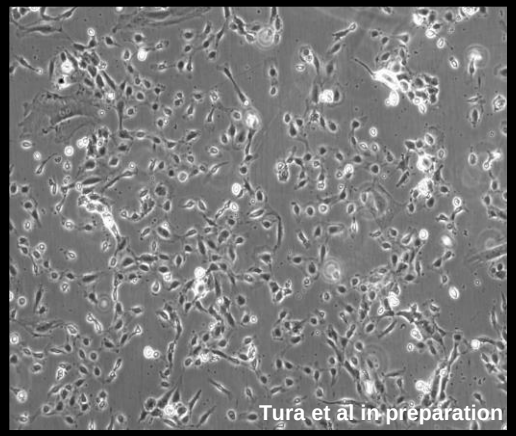
2003
Hill *et al*, N Eng J Med

Functional analysis by colony forming assay
(CFU-EPC)

3

2007
Yoder *et al*, Blood

Functional analysis by colony forming assay
(Late outgrowth endothelial cells, EOC)



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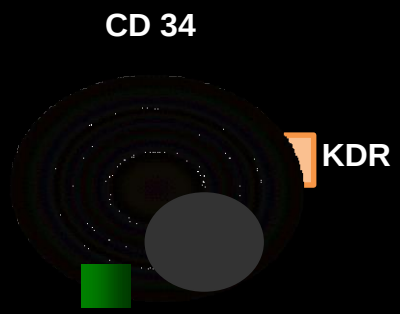

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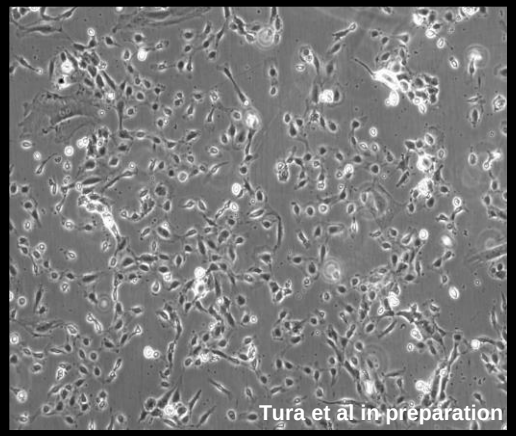
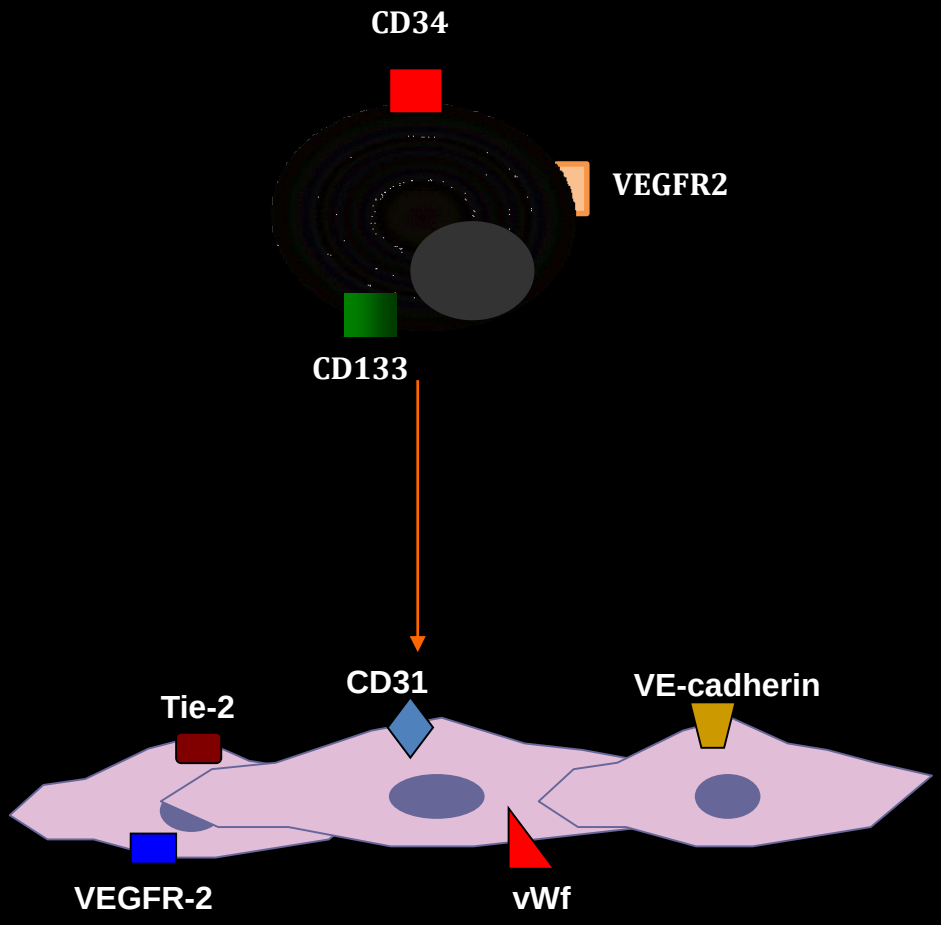


1 EPC defined by phenotypic analysis (original description, Asahara et al,.)

Endothelial Progenitor cell

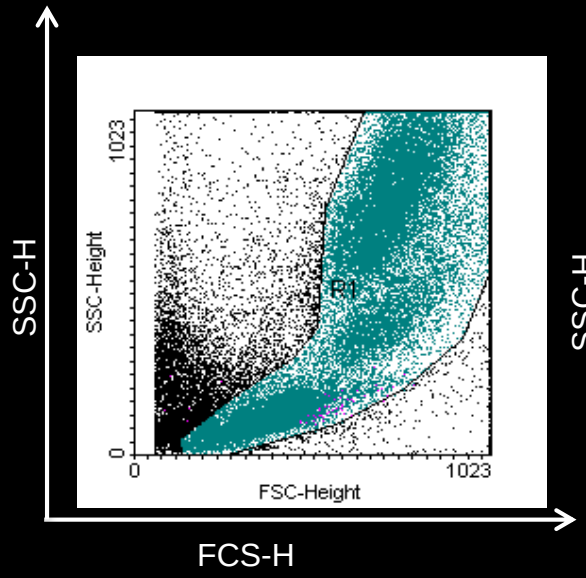


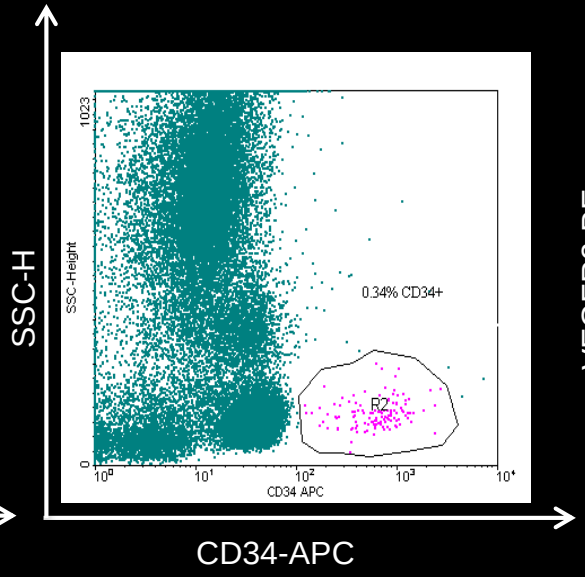
Mature endothelial cells

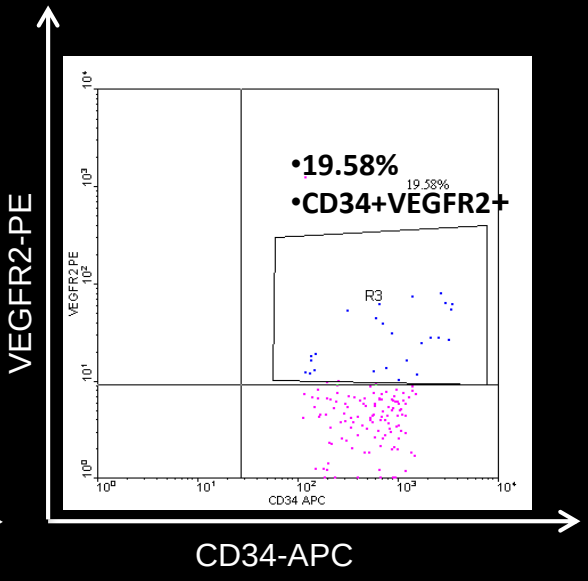
- Early studies characterised EPCs based on the expression of:
- CD34 and/or CD133 (haematopoietic stem cell markers)
- VEGFR2 (Vascular endothelium growth factor receptor 2)

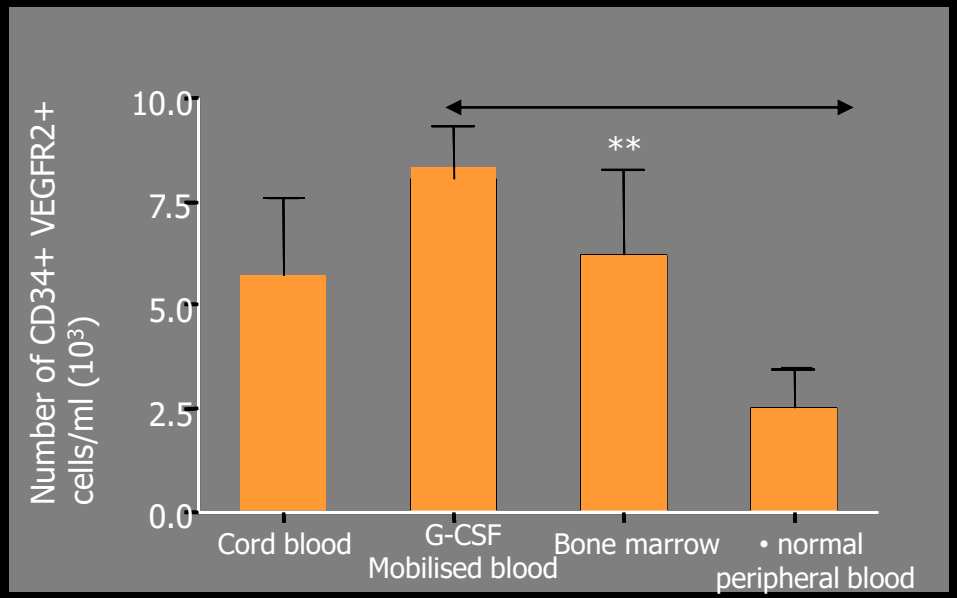
- In the original description authors described a population of adult human circulating $CD34^{+}VEGFR2^{+}$ cells that could differentiate into cells with endothelial-like characteristics *in vitro* **(Asahara *et al*., 1997 Science; 275:964-7).**

1 EPC defined by phenotypic analysis (original description, Asahara et al,.)









Sangre periférica movilizada es la fuente más rica de CPE tal como se define por las células que expresan número de células CD34 + en combinación con VEGFR2.

Tura O, et al, Journal of Translational Medicine 2007.

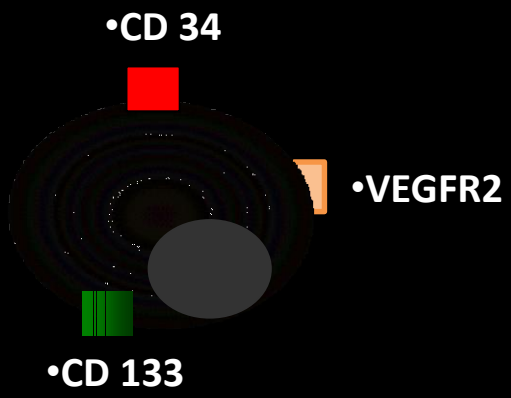
EPC CAN BE DEFINED BY 3 METHODS:

1 **1997**
Asahara *et al.*,
Asahara *et al,* Science; 275:964-7

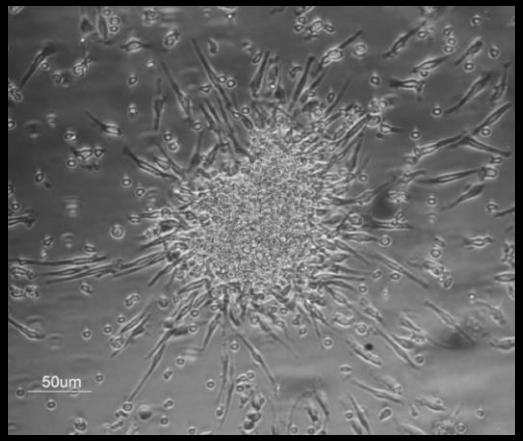
2 **2003**
Hill *et al.*,
Hill *et al*.,2003 N Engl J Med;348:593-600

3 **2007**
Yoder *et al.*,
Yoder et al.,2007 Blood;109:1801-9

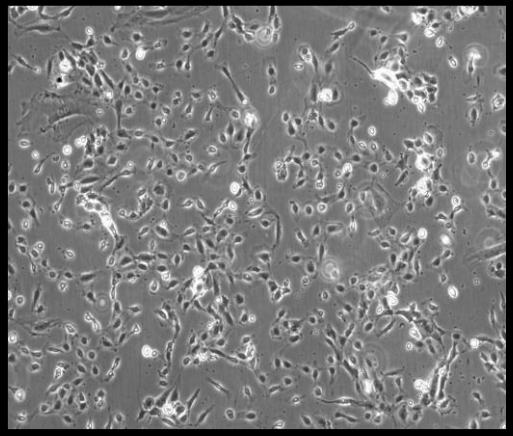
- Phenotypic analysis by
- Flow Cytometry
- (Original description)



Colony assay (CFU-EPC)

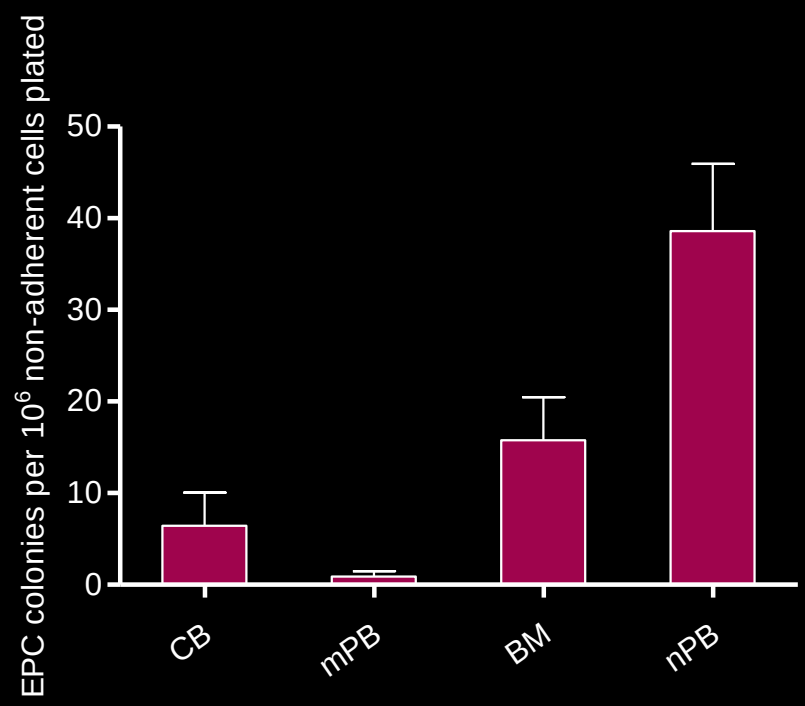


Late outgrowth endothelial cells (EOC)



2 ENDOTHELIAL COLONY ASSAY (CFU-EPC) (early EPC) Hill et al

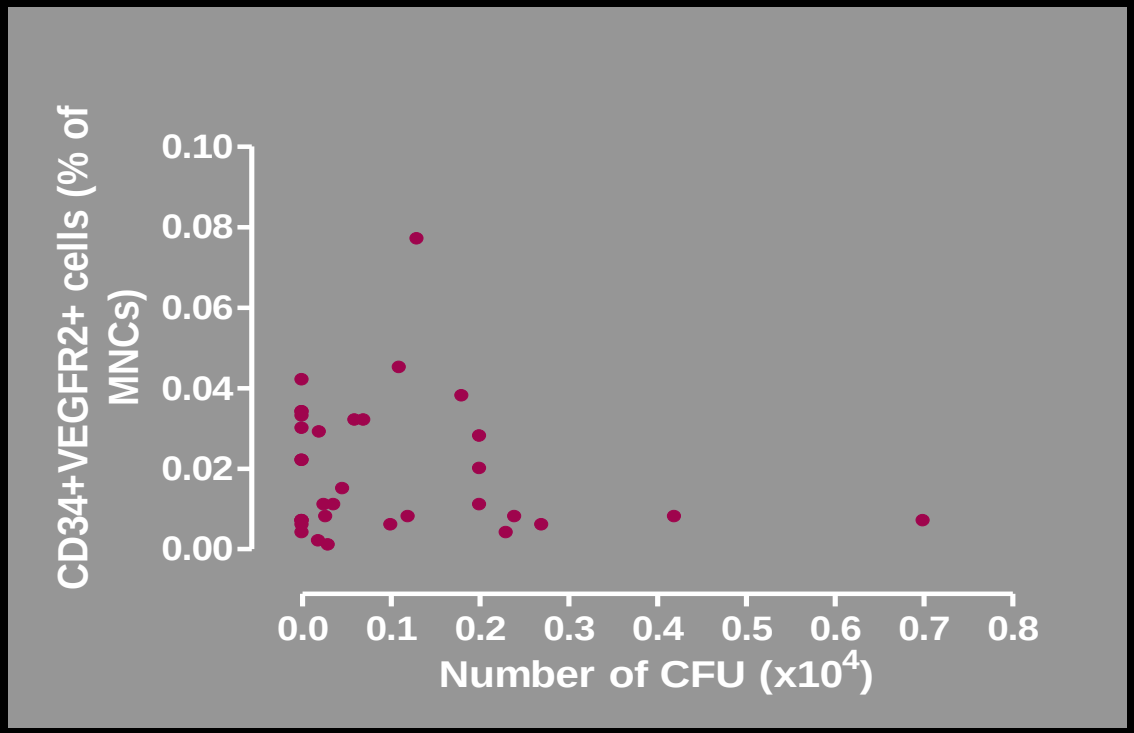
2 ENDOTHELIAL COLONY ASSAY (CFU-EPC) (early EPC) Hill et al



Normal peripheral blood (nPB) had the greatest number of CFU-EPC (CFU-Hill), while mobilised PB (mPB) which contained the most CD34+VEGFR2+ are virtually devoid of CFU-EPC.

Tura O, et al, Journal of Translational Medicine 2007.

2 ENDOTHELIAL COLONY ASSAY (CFU-EPC) (early EPC) Hill et al

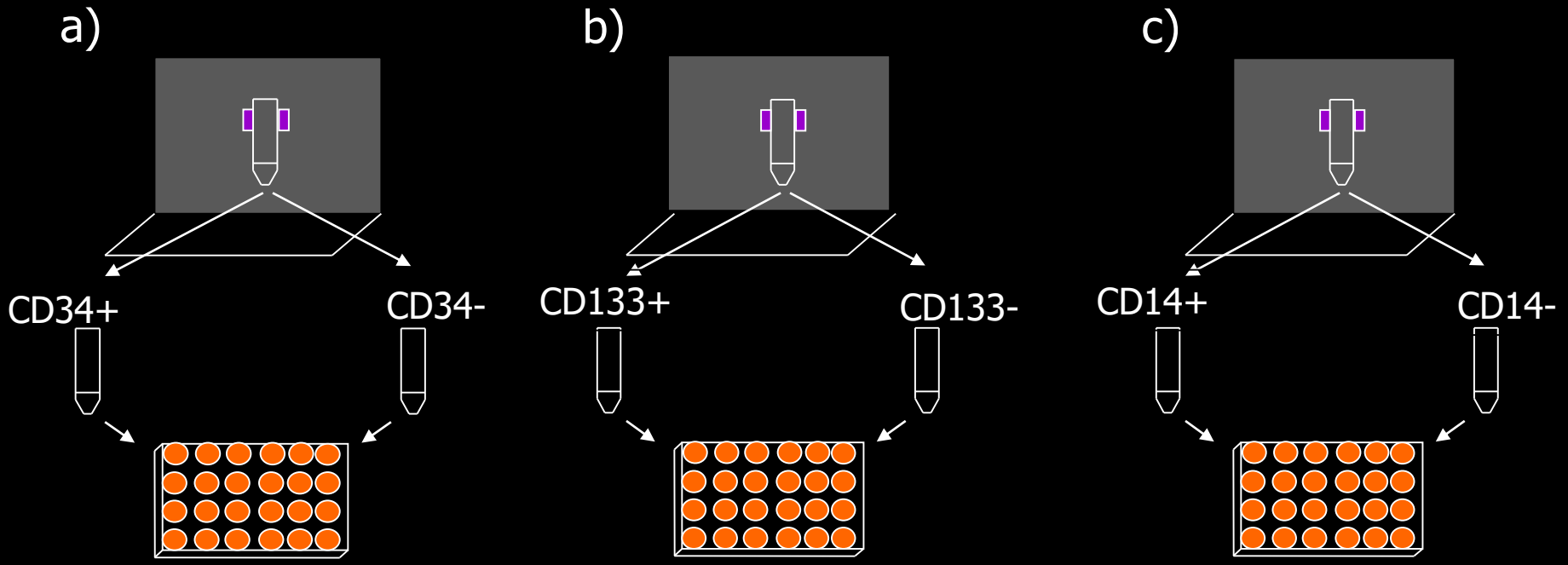


There is no correlation between the CFU-EPC 'functional' definition and the phenotypic definition of EPCs.

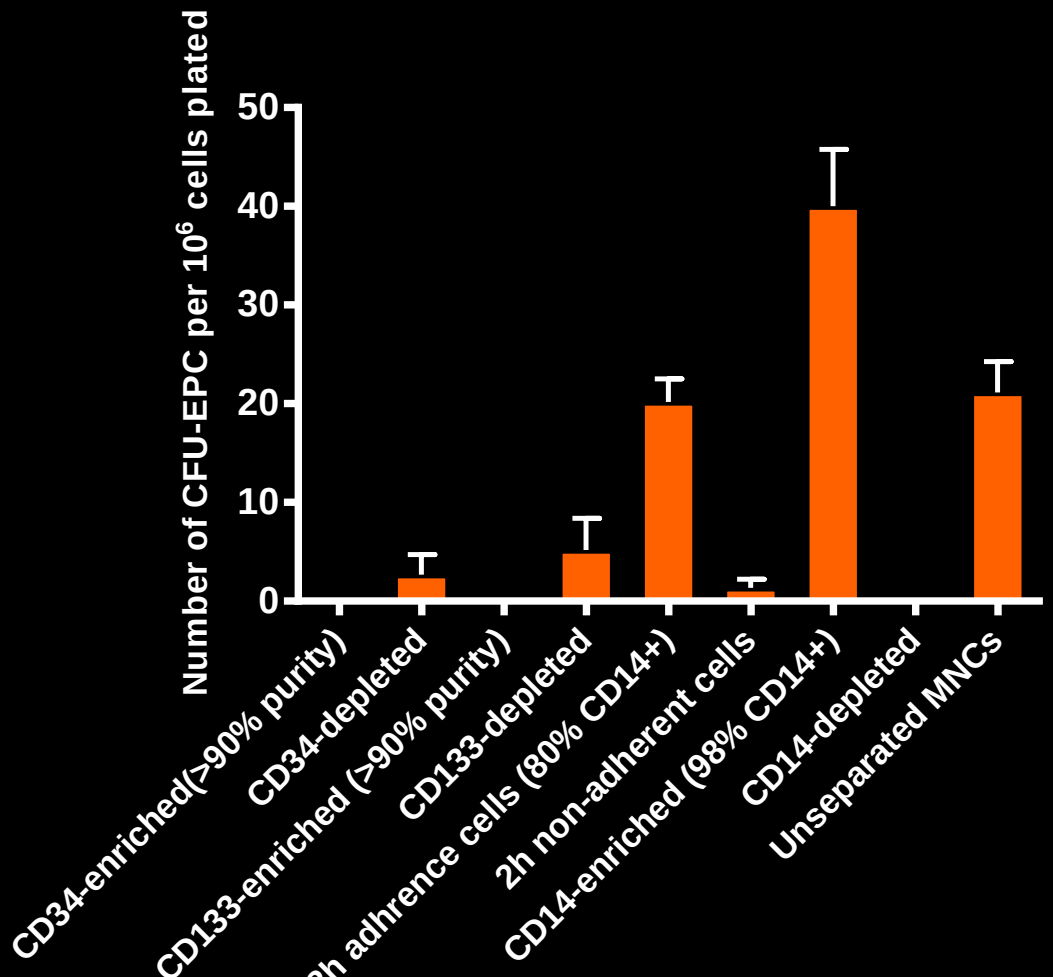
Cells identified using current phenotype EPC definitions (CD34+ and/or CD133+ jointly expressing VEGFR2) do not appear to be the population of cells defined in the CFU-EPC assay.

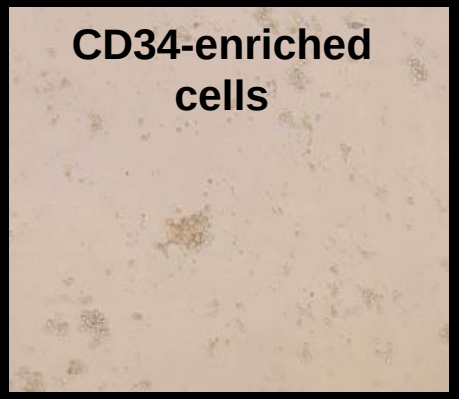
Tura O, et al, Journal of Translational Medicine 2007.
Mills and Tura et al , *Heart. 2009;95(24):2003-8.*

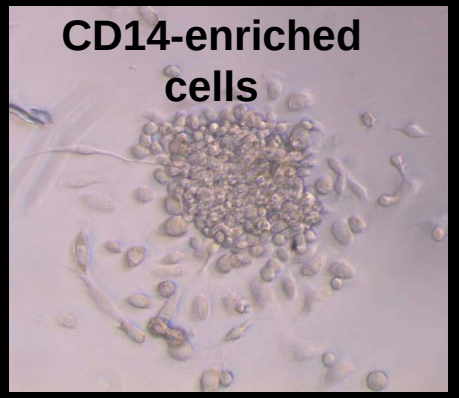
Selective enrichment of different cell populations and test their EPC colony capacity



Selective enrichment of different cell populations and test their EPC colony capacity







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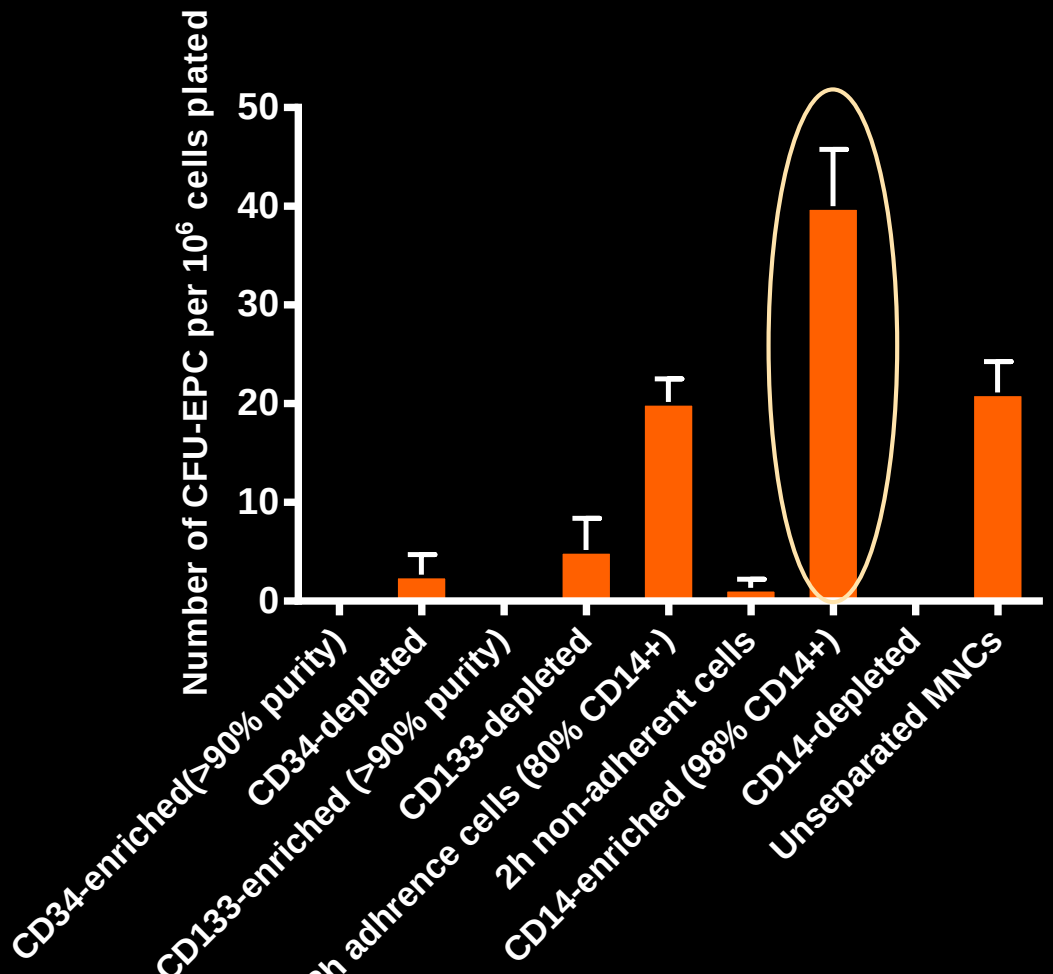

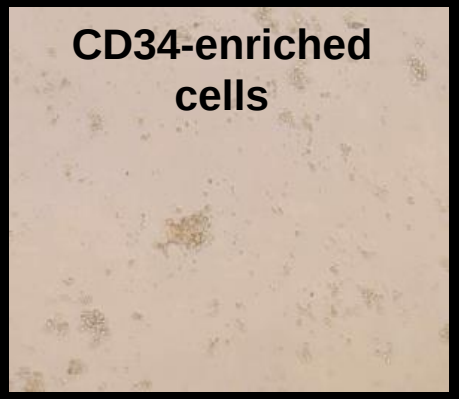

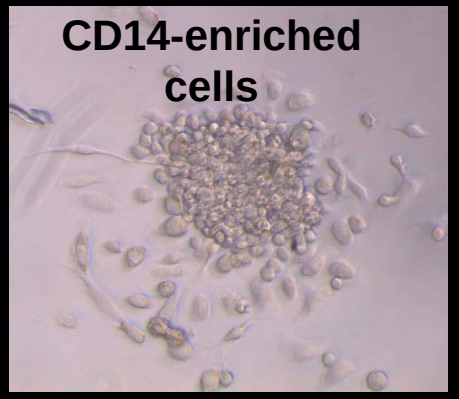

EPCs CAN BE defined by 3 methods:

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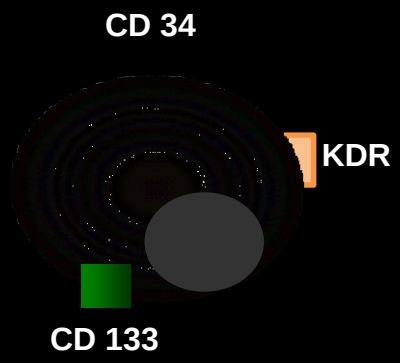

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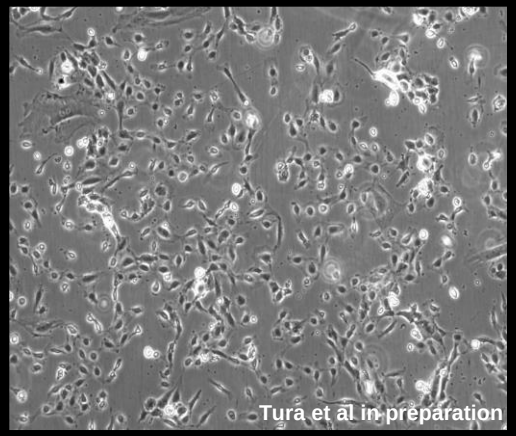
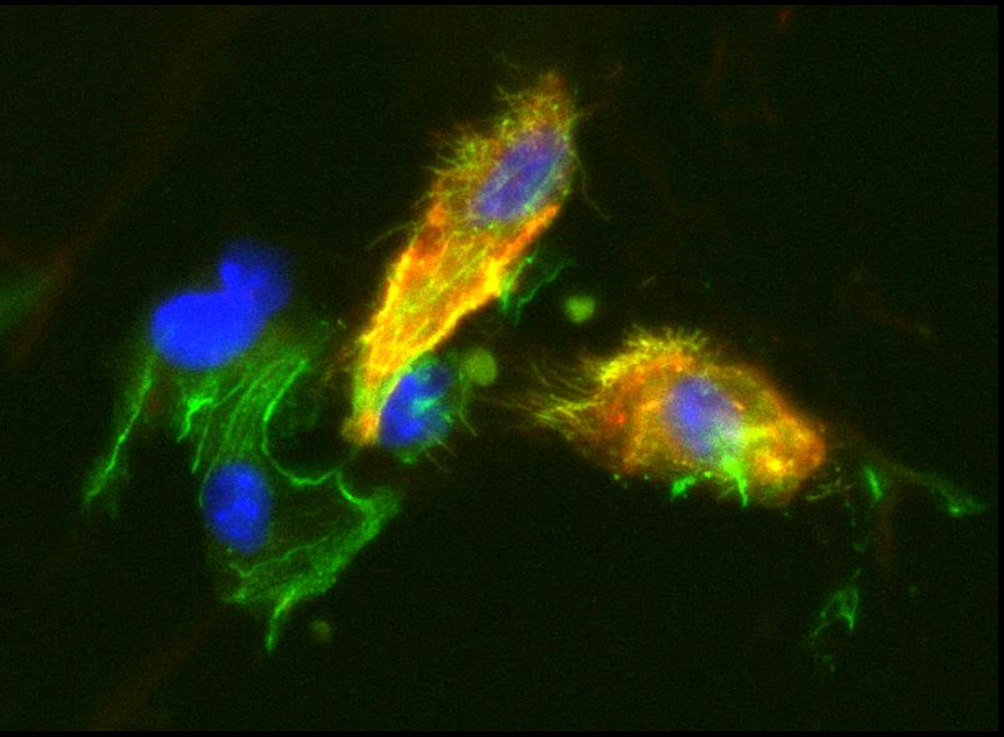
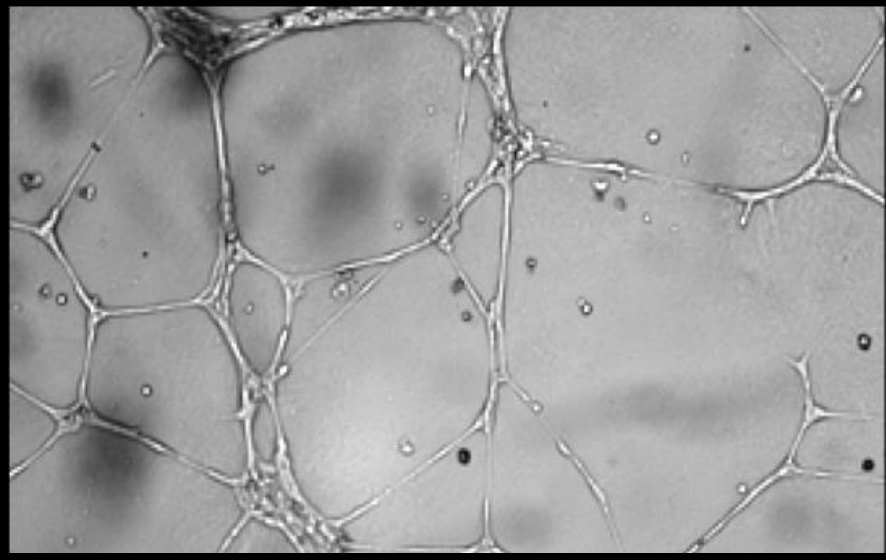


EPC defined by late outgrowth Endothelial cells (EOC) or (ECFU)

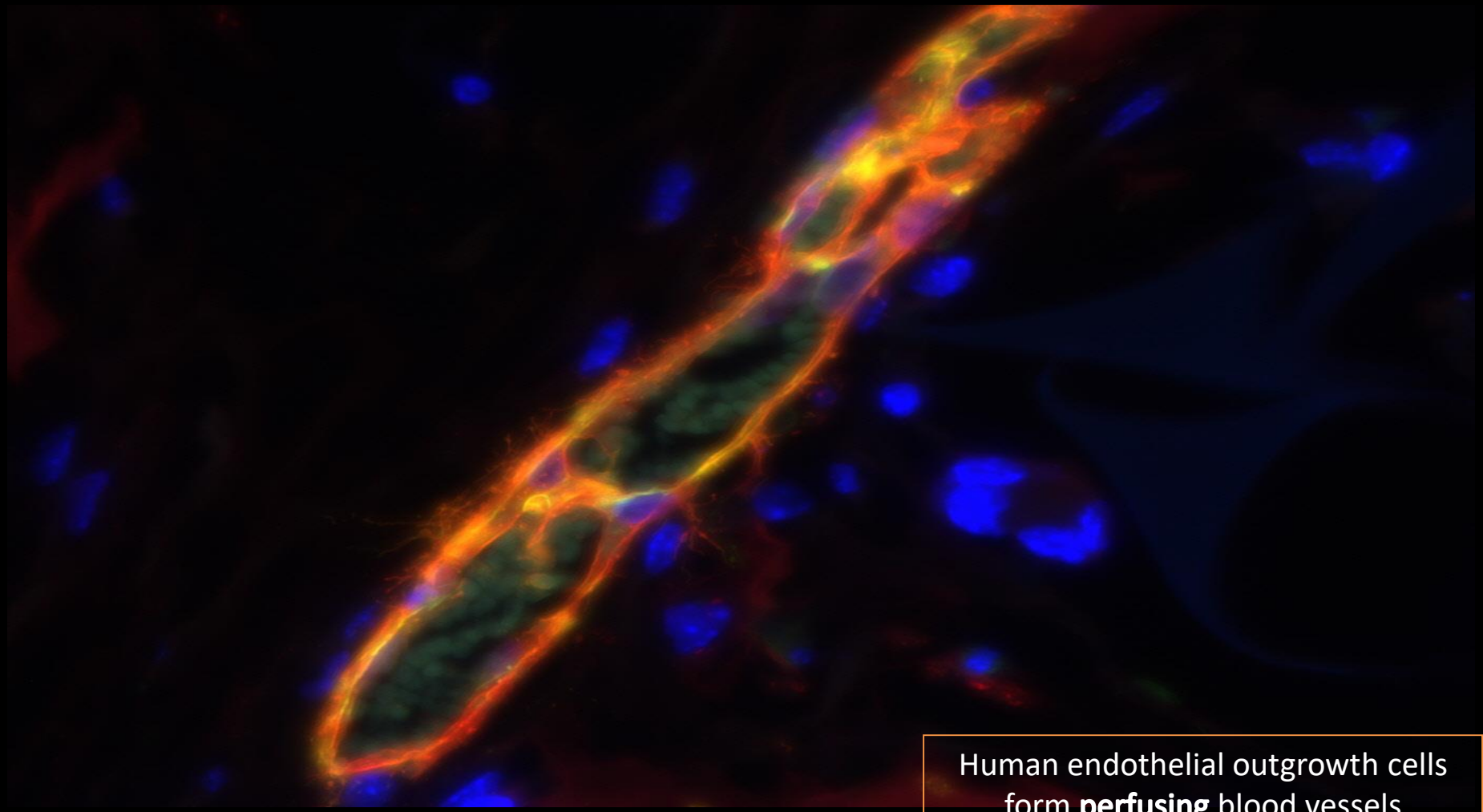




These cells resemble mature endothelial cells, and express the same surface marker/receptor profiles by immunophenotyping (CD31 (red), CD146 (green)).

They form tube-like structures in Matrigel

EPC defined by late outgrowth Endothelial cells (EOC) or (ECFU)



Human endothelial outgrowth cells form **perfusing** blood vessels

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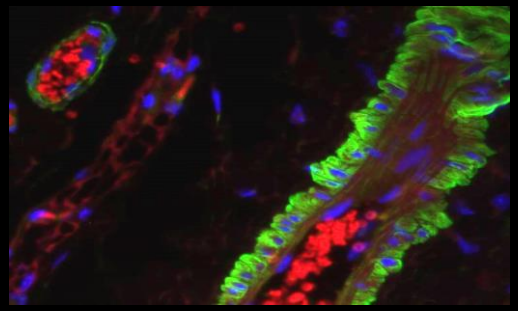

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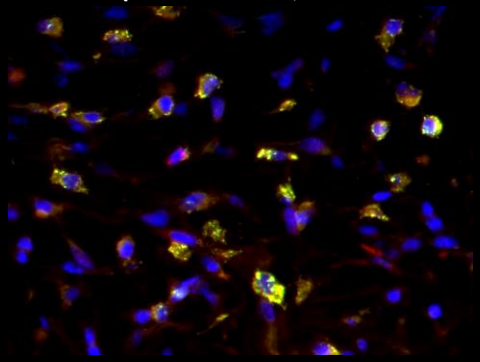

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Yoder *et al*, Blood

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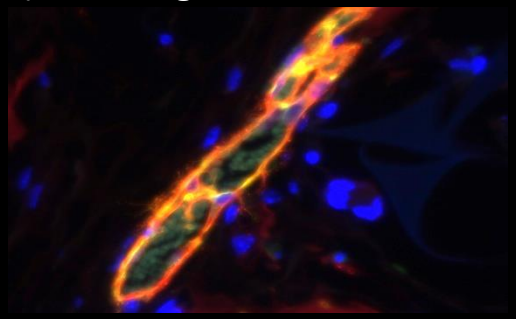
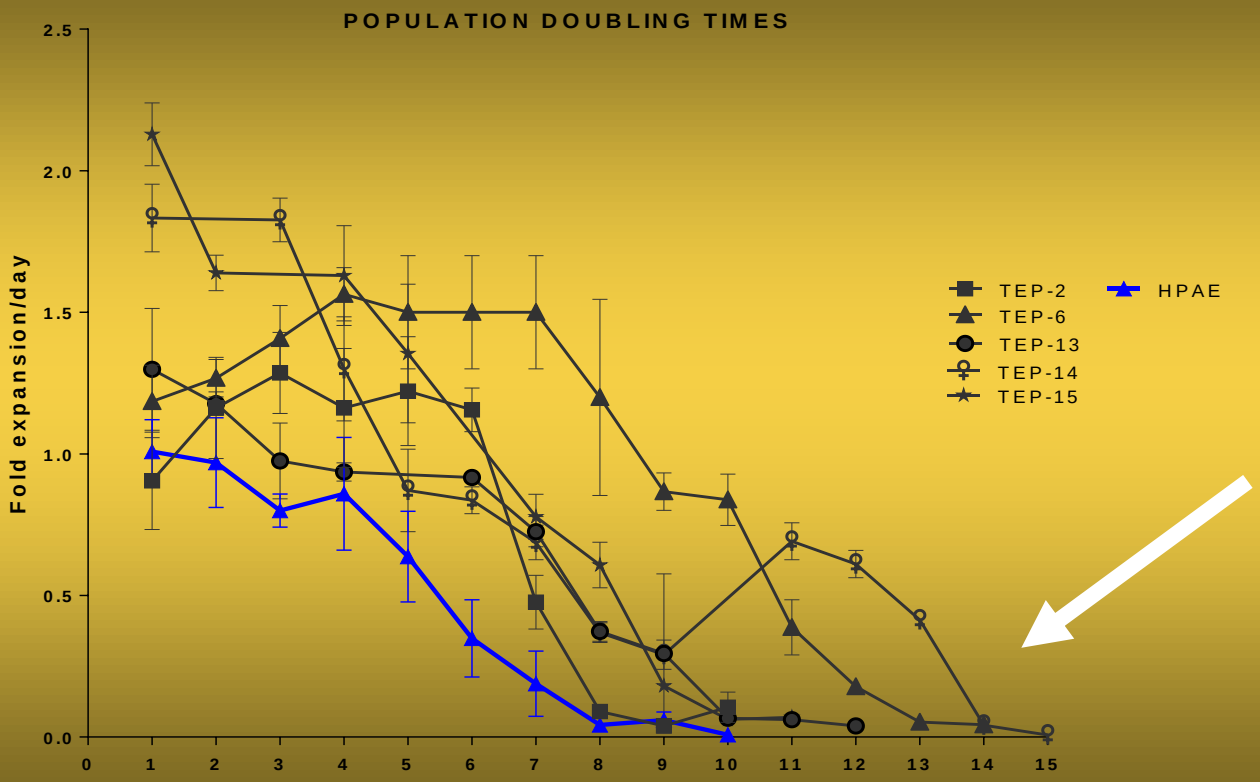


Results-Endothelial cell proliferative capacity

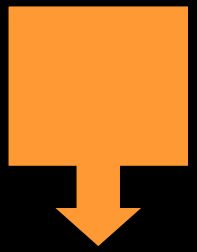


They showed an hyperproliferative phenotype when compared with control endothelial cell lines (HPAE), Fold expansion/days of culture ($p<0.002$ at P4)

Fold expansion/day= (Nº of final cells/Nº of seeded cells)/days of culture.

WHY?

WHY?

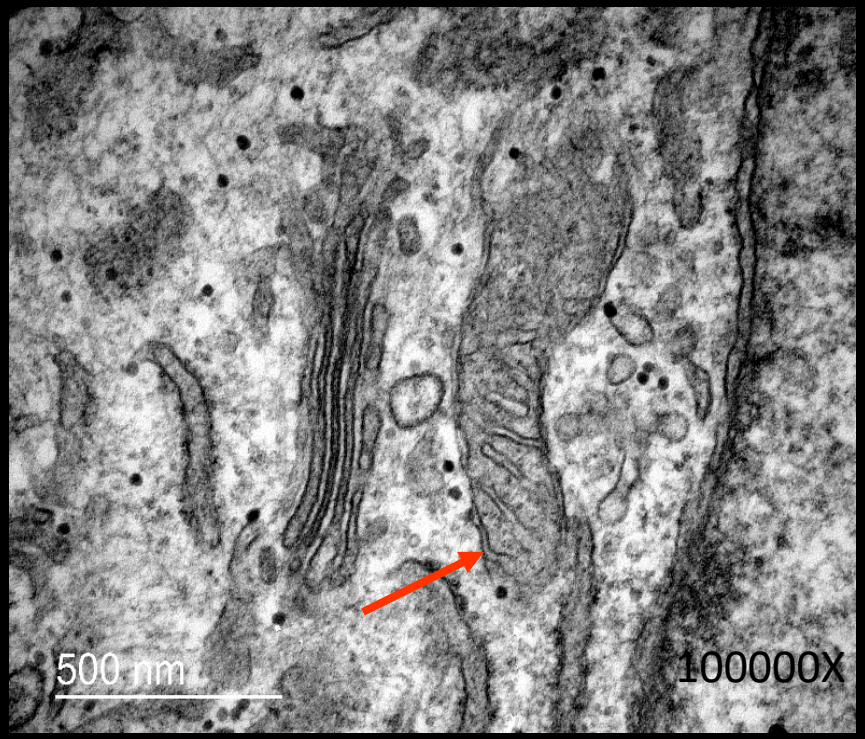


Endothelial dysfunctionality

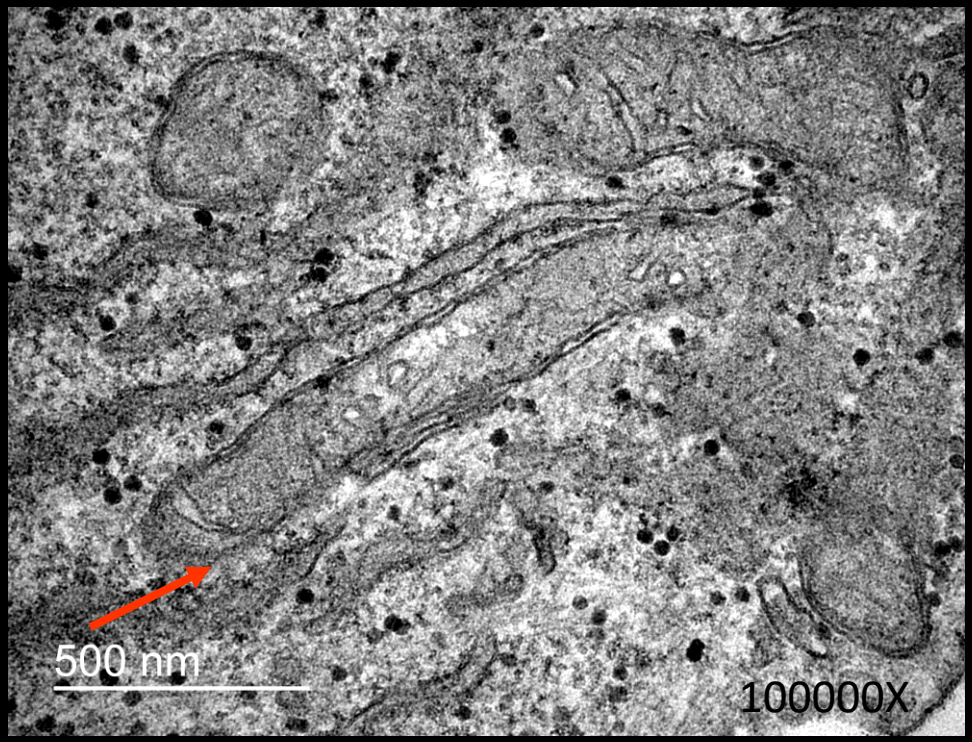
Endothelial metabolism?

Endothelial metabolism

HPAE

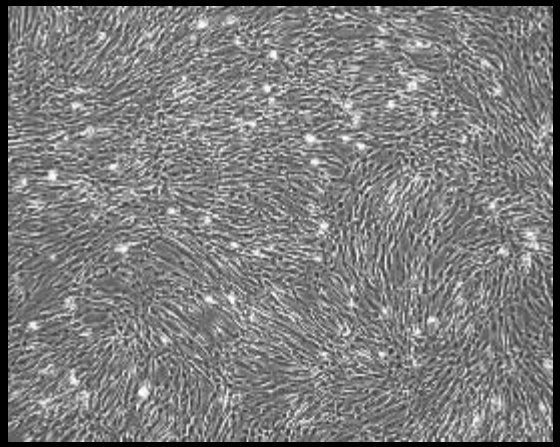


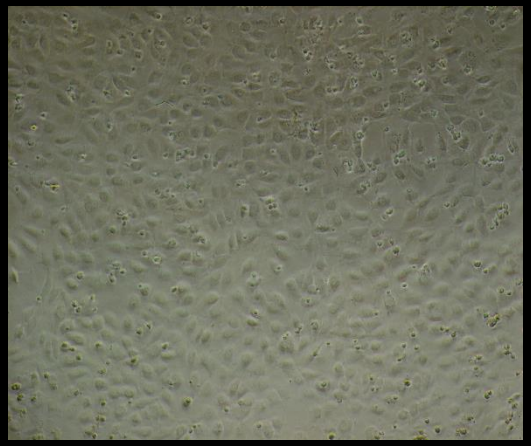
CPTEH-EC



TEM pictures show that in CTEPH mitochondrial inner membranes and crestae are strange. CTEPH-EC cells have more multivesicular bodies than HPAE and an inflamed and disorganised ER.

MSCs have beneficial effects on EPC





-MSCs have beneficial effects on endothelial progenitor cells their co-injection could allow the formation of a more developed vascular network than with either MSC or EPC alone.

-As a result it might be intersting to administrate MSC in combination with EPC in a PAH to achive greater benefits.

Different types of stem cells

- Mononuclear stem cells (MNCs)
- Mesenchymal stromal cells (MSCs)
- Endothelial progenitor cells (EPCs)
- Adipose-derived stem cells (ADSc)
- iPS derived cells (iPS)
- Cardiac progenitor cells (CPCs)

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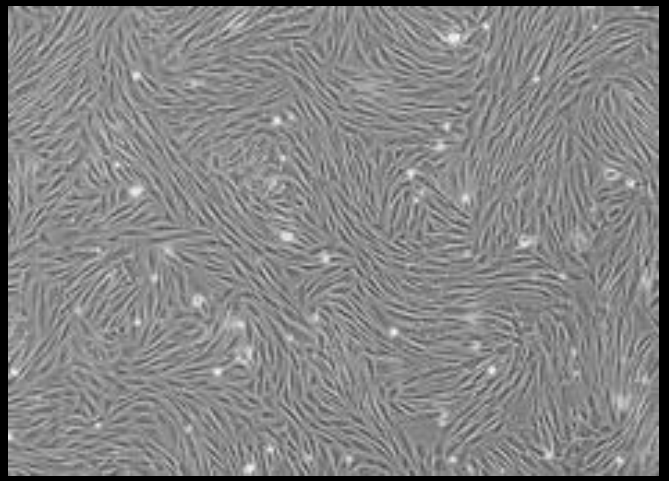
Adipose-derived stem cells (ADSc)

- Can be readely isolated from white adipose tissue by liposuction
- They are suitable for autotransplantation
- Pro-angiogenic and anti-apoptotic
- Multipotential
- plentiful
- Proven to be safe and feasible (phase I trial in ischemic cardiomyopathy (Precise t
- Appear to be a good choice of stem cells to treat PAH

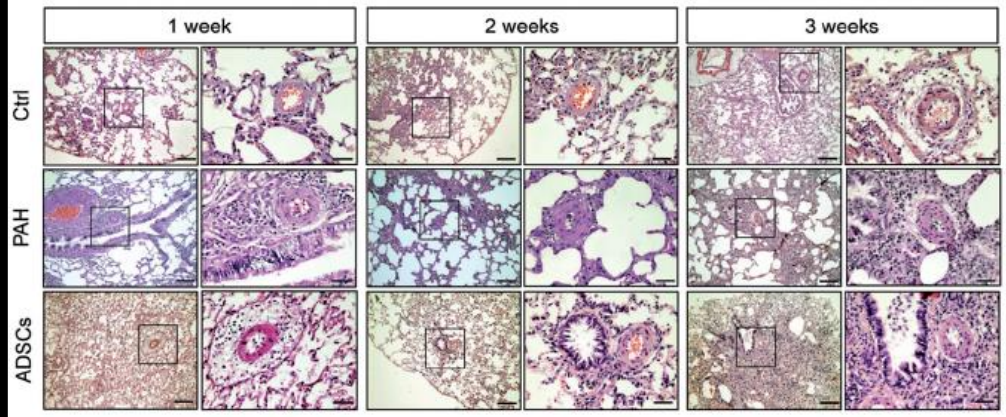


Adipose-derived stem cells (ADSc)

Clinical and Experimental HYPERTENSION
http://informahealthcare.com/ceh
ISSN: 1064-1963 (print), 1525-6006 (electronic)
Clin Exp Hypertens, 2015; 37(3): 241–248
 DOI: 10.3109/10641963.2014.954710
informa healthcare

Adipose-derived stem cells attenuate pulmonary arterial hypertension and ameliorate pulmonary arterial remodeling in monocrotaline-induced pulmonary hypertensive rats

Li Luo, Taijie Lin, Suli Zheng, Zhenguo Xie, Ming Chen, Guili Lian, Changsheng Xu, Huajun Wang, and Liangdi Xie



ADSC therapeutic effects on pulmonary small artery remodeling in MCT-induced pulmonary hypertension in rats (Mean ± SD)

		1w	2w	3w
Ctrl group	WA%	50.27±4.92	51.61±3.29	52.02±5.87
	WT%	32.46±5.19	32.52±3.19	31.42±4.53
PAH group	WA%	68.70±3.43*	80.48±6.19*	84.01±2.76*
	WT%	46.09±4.70*	57.26±4.32*	64.64±3.86*
ADSCs group	WA%	58.23±4.08#	62.97±6.58#	65.27±5.45#
	WT%	38.06±4.15#	40.91±5.24#	41.91±5.16#

Note: $^{*}P < 0.05$ vs Ctr group at the same time points; $^{\#}P < 0.05$ vs PAH group at the same time points.

Table 1. Effects of ADSCs on mean pulmonary artery pressure (mPAP, mmHg) (mean ± SD).

	1w	2w	3w
Ctrl group	14.81 ± 1.95	15.08 ± 2.35	15.65 ± 2.27
PAH group	24.53 ± 2.90*	33.18 ± 2.30*	36.38 ± 3.28*
ADSCs group	18.63 ± 2.15#	23.07 ± 2.84#	22.98 ± 2.34#

* $p < 0.05$ versus Ctrl group at the same time points.
# $p < 0.05$ versus PAH group at the same time points.

Table 2 The effect of ADSCs treatment on right ventricular hypertrophy index (RVHI) in rats (mean ± SD).

	1w	2w	3w
Ctrl group	28.25 ± 2.15	28.71 ± 2.08	28.54 ± 2.19
PAH group	41.01 ± 1.29*	48.75 ± 2.13*	50.52 ± 1.49*
ADSCs group	36.21 ± 4.27#	39.47 ± 4.02#	41.02 ± 0.9 #

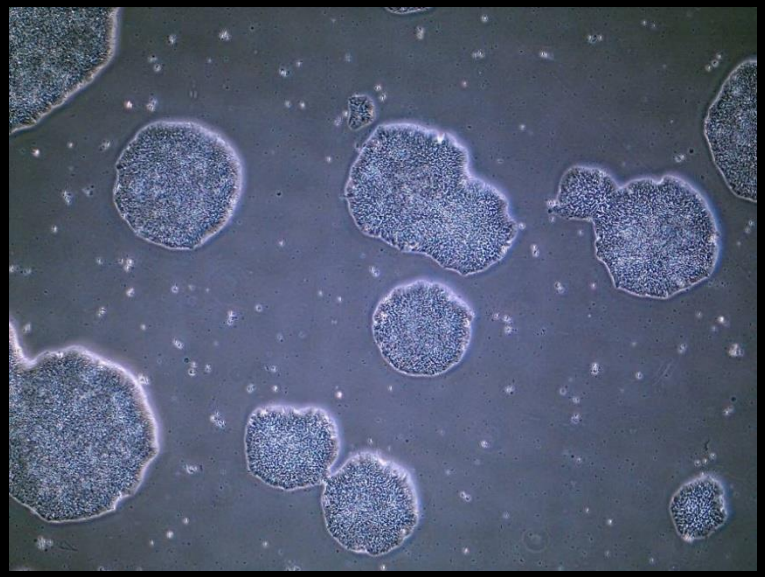
*$p < 0.05$ versus Ctrl group at the same time points.

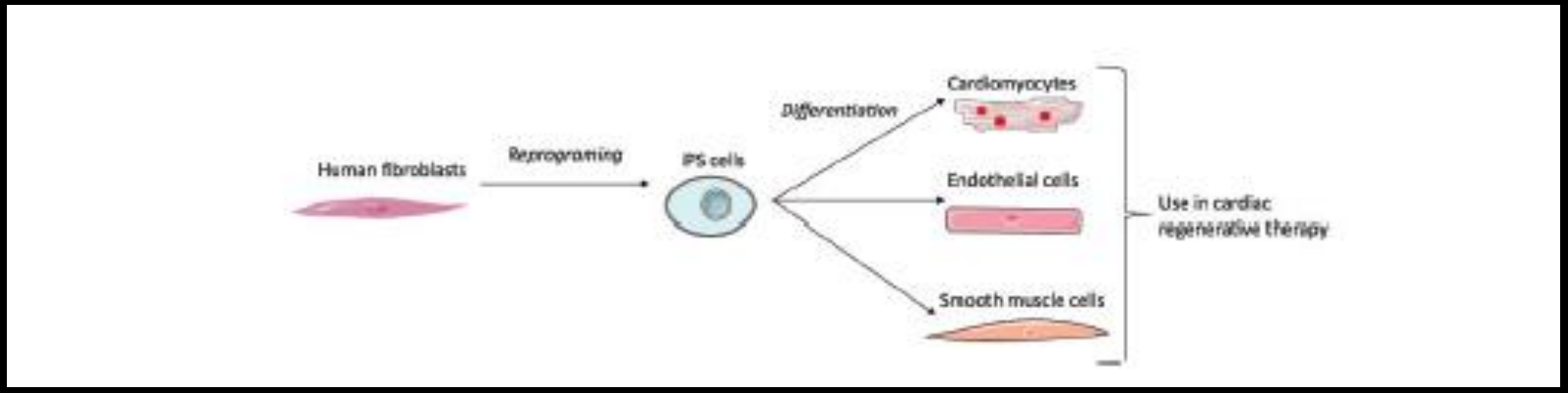
ADSC colonize the pulmonary arteries, attenuate pulmonary arterial hypertension and ameliorate pulmonary arterial remodeling.

Luo, et al. Clin Exp Hyperten. 2015;37 (3):241-248.

➢iPS derived cells (iPS)

- iPS might provide a useful source of patient specific cells,as they can be isolated from their own dermal fibroblasts.
- Big numbers
- Tumorigenic

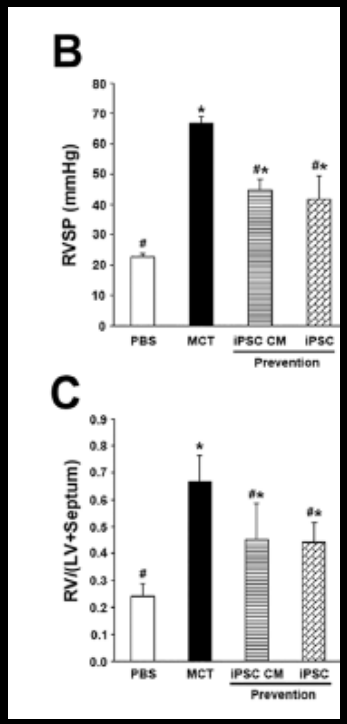


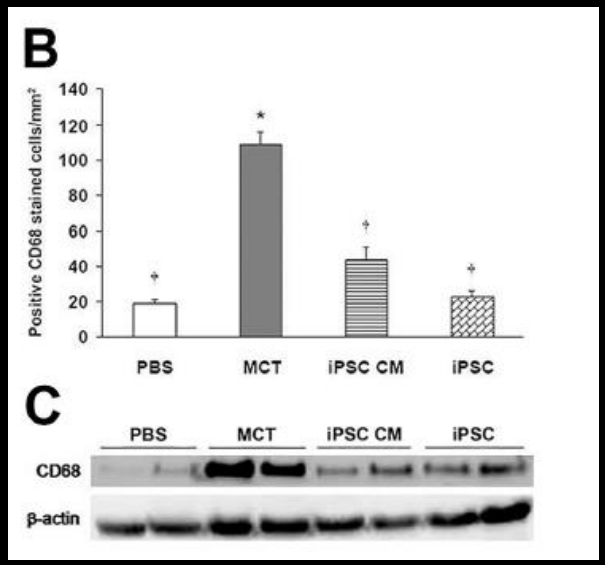


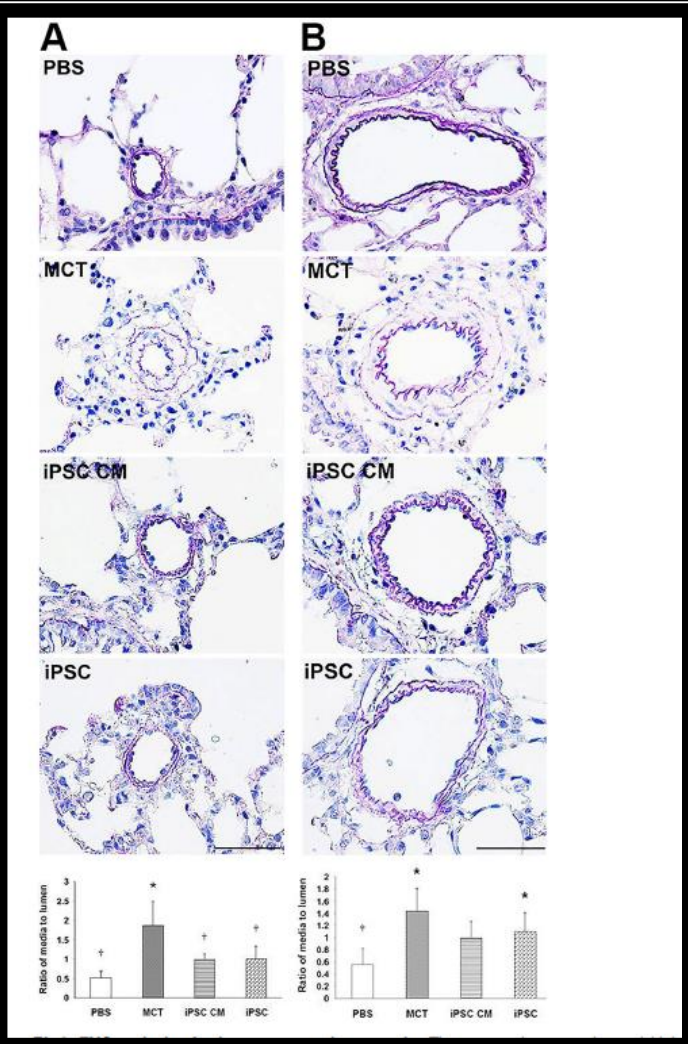
➢iPS derived cells (iPS)

Therapeutic Benefits of Induced Pluripotent Stem Cells in Monocrotaline-Induced Pulmonary Arterial Hypertension

Wei-Chun Huang[1,2,3], Meng-Wei Ke[1], Chin-Chang Cheng[1,2,3], Shih-Hwa Chiou[4,5], Shue-Ren Wann[7]*, Chih-Wen Shu[6], Kuan-Rau Chiou[1,2], Ching-Jiunn Tseng[6], Hung-Wei Pan[6], Guang-Yuan Mar[1], Chun-Peng Liu[1,2]*

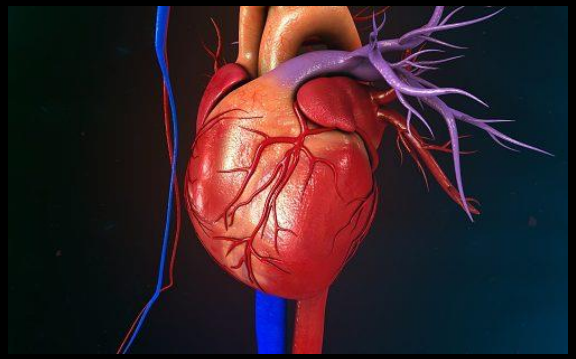






iPSC-based therapy could resotre the hemodynamic function of the RV with benefits for preveniting the ongoing inflammation in the lungs of MCT-induced PAH rats.

PLOS ONE | DOI:10.1371/journal.pone.0142476 February 3, 2016



- Right ventricular function is the main prognostic risk factor in PH.

- Stem cell therapies targeting the left ventricle (LV) are proving to be promising therapies in animal models.

- In contrast to left heart disease, stem cell therapy applied to the RV has not been studied much, despite indications that it may be a viable therapeutic option.

- Future new therapeutics in the next decade that specifically target the RV are required.
  - -Improvements in the RV are they due to direct effect of stem cell therapy or due to indirect reduction of pulmonary resistance?

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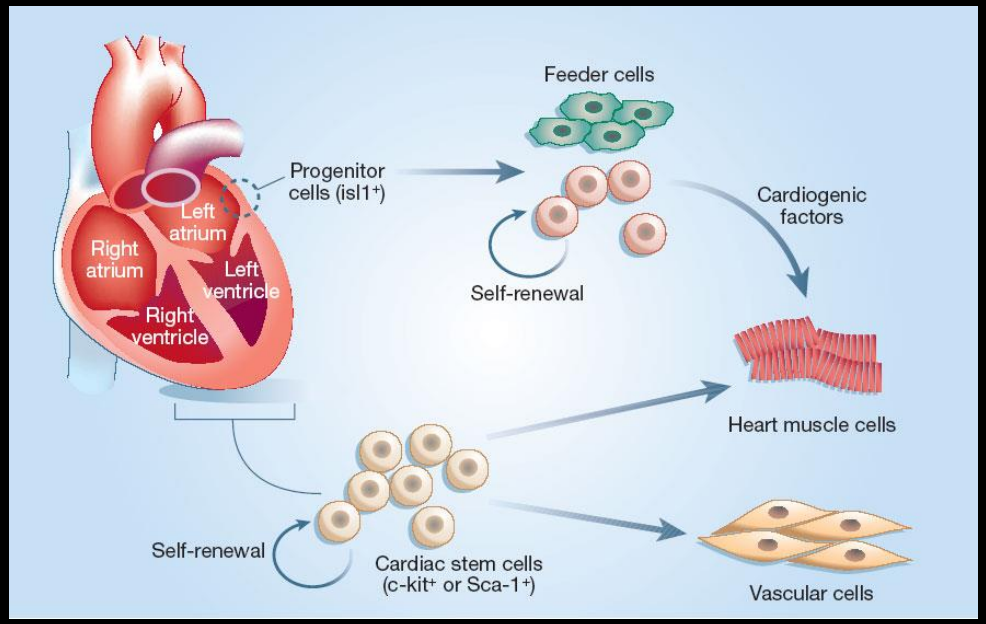
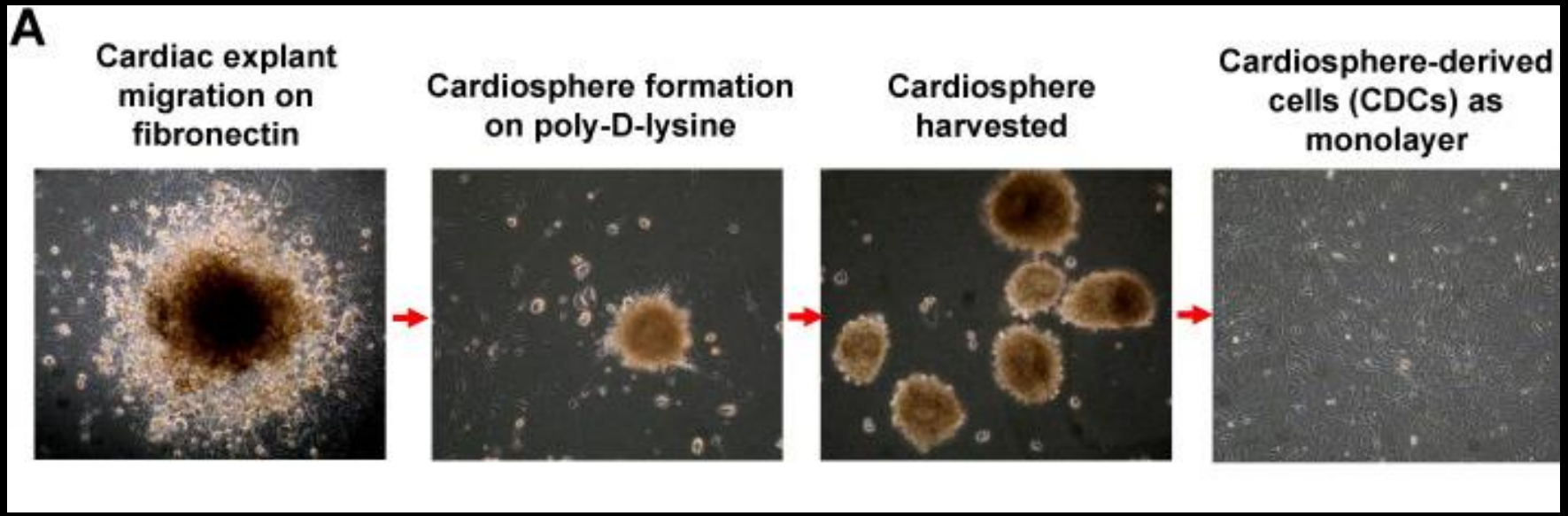


➢Cardiac progenitor cells (CPCs)

- Heterogenous group of cells in the adult heart (c-kit+, Sca-1+..) similar problem than EPC.
- Clear identification and characterization of the cells used are crucial to discuss the results of studies.
- The therapeutic potential of CPCs has been documented for myocardial infarction.
- Thought to stimulate cardiomyocytes and vascular cell trandifferentiation as well secreting paracrine factors that promote neovasularizarion and activation of endogenous CPCs.
- These properties could be beneficial for the RV in PAH by causing an increase in capillary density and by activating the production of efficient new cardiomyocytes.
- Is an invasive procedure isolated from the heart tissue

➢Cardiosphere derived cells (CDC)

- Can be obtain from human biopsies.
- CDC were tested clinically ina CADUCEUS trial (cardiosphere-derived autologous stem cells to reverse ventricular dysfunction), which examined safety and efficacy of intracoronary autologous administration of CDC in patients with LV dysfunction after MI.



➢Conclusions

- Stem cell therapies in pulmonay hypertension seem very promising
- Clear identification and characterization of the cells used are crucial
- Ethical, embryo-derived cells (CPC), immunological issues
- Reprogramming may induce genetic changes –tumor formation
- For practical reasons, cells should be readely available at the right clinical dose at the time of injection. Isolated cells previously criopreserved.
- Autologous--- PAH-EPC numbers are reduced compared to controls.
- Route of administration, scaffolds..

Choose the best cells and the best way to administer them

Thank you