

# **Inflammatory Reactions after Myocardial Infarction in a Rat LAD Ligation Model**

and their Suppression by Endogenous Cell derived Mechanisms

**Diplomarbeit**  
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ausgeführt an der  
Universitätsklinik für Chirurgie  
Klinische Abteilung für Herz-Thorax-Chirurgie

unter der Anleitung von  
Univ.-Doz. Dr. Hendrik Jan Ankersmit

7. 1.2010

# Background

## Cell-based Therapies for Myocardial Infarction A Meta-Analysis

THE NEW ENGLAND JOURNAL OF MEDICINE

ORIGINAL ARTICLE

### Intracoronary Bone Marrow-Derived Progenitor Cells in Acute Myocardial Infarction

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

## THE LANCET

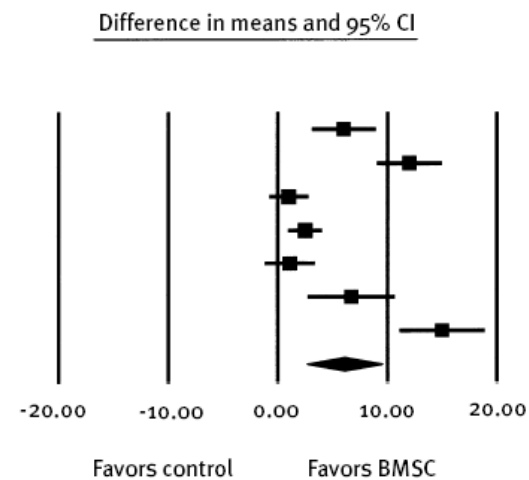
### Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial

Karl C. Wollert, Gerd P. Maier, Joachim Lotz, Stefania Ringler, Lutz Lehmann, Peter J. Spoell, Christiane Brachmann, Stephanie Eichinger, Thomas Kretz, Burkhard Hammig, Diethelm Messinger, Lubomir Arsenau, Bernd Hartmann, Arnold Garner, Helmut Drexler  
Lancet 2004, 364, 520-28  
See Comment page 521  
Departments of Cardiology and

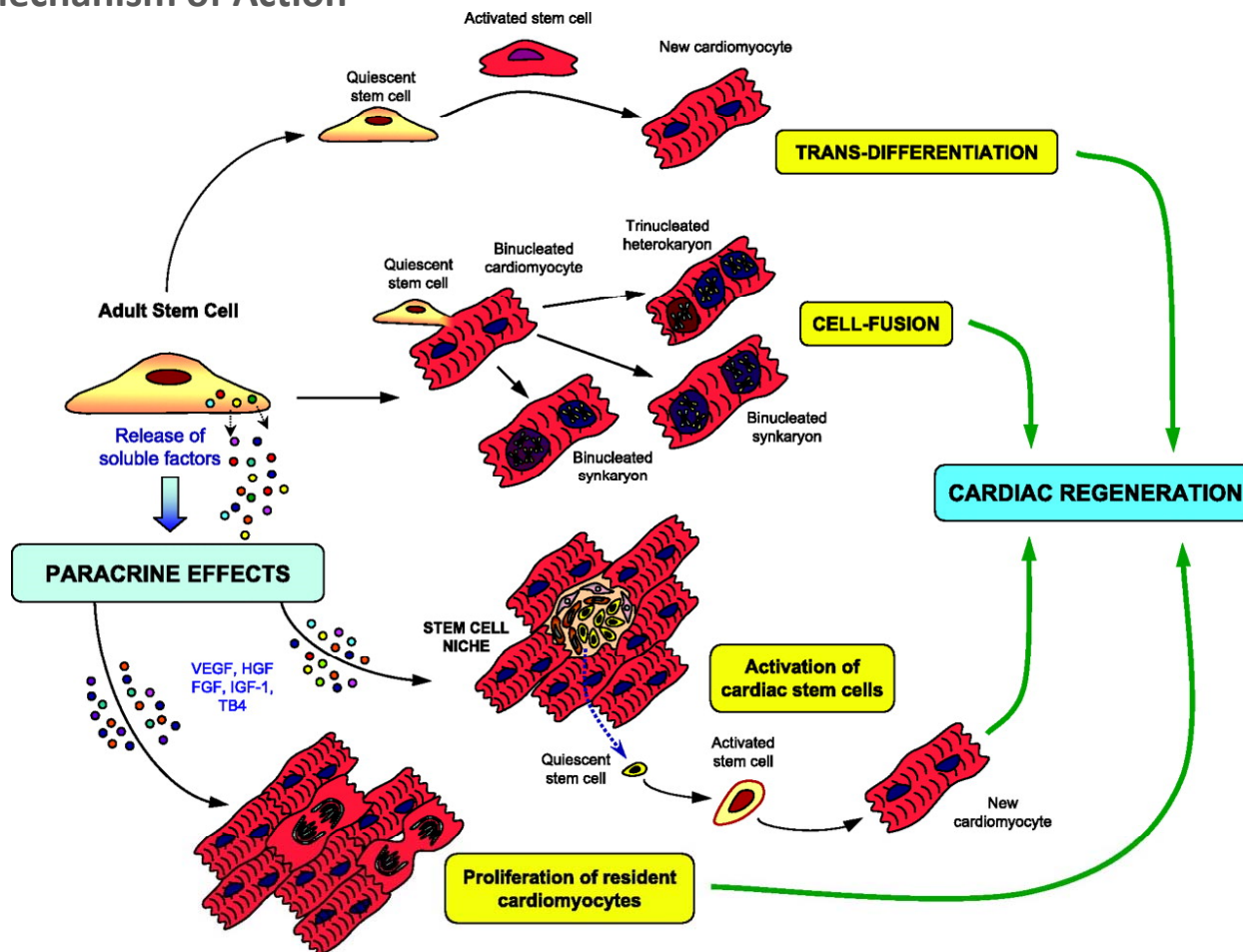


Autologous stem cell transplantation in acute myocardial infarction: The ASTAMI randomized controlled trial. Intracoronary transplantation of autologous mononuclear bone marrow cells, study design and safety aspects  
Ketil Lunde, Svein Solheim, Svend Aakhus, Harald Arnesen, Michael Abdelnoor, Kolbjørn Forfang and on behalf of the ASTAMI investigators  
Scandinavian Cardiovascular Journal, 2005, Vol. 39, No. 3, Pages 150-158, DOI 10.1080/14017430510009131.  
Summary | Full Text | PDF (99 KB) | PDF Plus (100 KB)

Model	Study name	Statistics for each study				
		Difference in means	Standard error	Lower limit	Upper limit	p-Value
	Wollert et al	6.000	1.460	3.139	8.861	0.000
	Chen et al	12.000	1.487	9.085	14.915	0.000
	Lunde et al	1.000	0.895	-0.754	2.754	0.264
	Schächinger et al	2.500	0.755	1.019	3.981	0.001
	Janssen et al	1.100	1.141	-1.136	3.336	0.335
	Ge et al	6.700	1.999	2.782	10.618	0.001
	Suarez de Lezo et al	15.000	1.965	11.149	18.851	0.000
Random		6.108	1.753	2.672	9.543	0.000

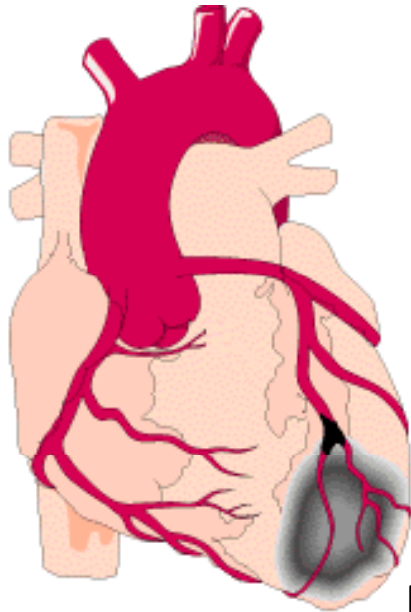


## Cell-based Therapies for Myocardial Infarction Mechanism of Action



# Background

## Myocardial Infarction



**Necrosis**

Attraction of immune cells

Secretion of pro-inflammatory cytokines

IL-1 IL-6 TNF- $\alpha$

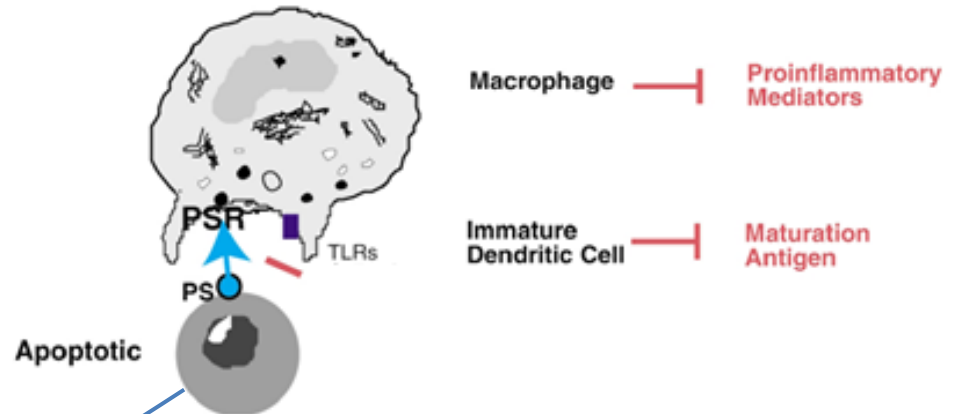
Amplification of inflammation

## The Dying Stem Cell Hypothesis

by Anker *et al.*

up to 25% of all transplanted cells are in the state of apoptosis

apoptotic cells induce transient immunosuppression



Inhibition of pro-inflammatory Signals

J Am Coll Cardiol. 2005 Nov 15;46(10):1799-802.  
J Clin Invest. 2001 Oct;108(7):957-62.

## ***In vitro* Experiments**

### FACS Analysis

Annexin-V positivity of irradiated PBMC was verified by FACS

### Co-Incubation Assay

PBMC and monocytes stimulated with LPS were co-incubated with autologous apoptotic PBMC

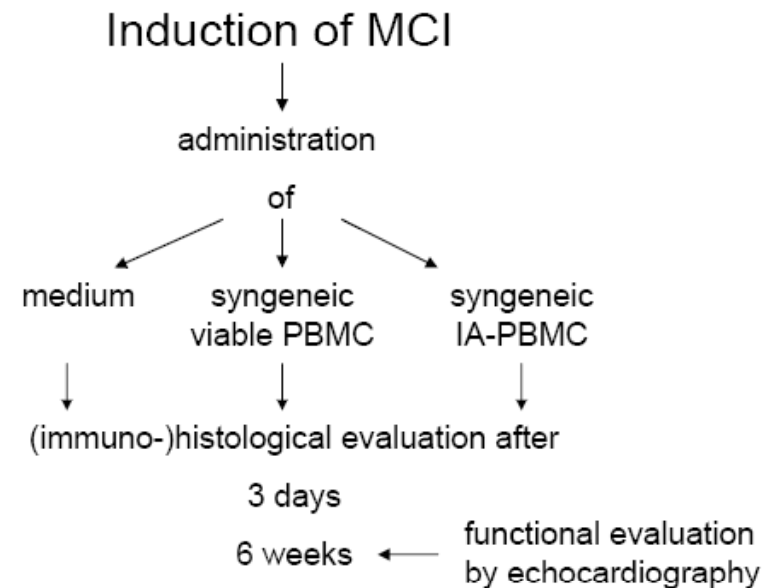
→ ELISA Evaluation for IL-1 $\beta$  and IL-6

### Mixed-Lymphocyte Reaktion (MLR)

RT-PCR for transcripts of pro-angiogenic mediators (VEGF, IL-8 and MMP9) in apoptotic PBMC

RT-PCR for transcripts of pro-angiogenic mediators (VEGF, IL-8 and MMP9) in fibroblasts incubated with cell culture supernatants from apoptotic PBMC

## ***In vivo* Experiments**

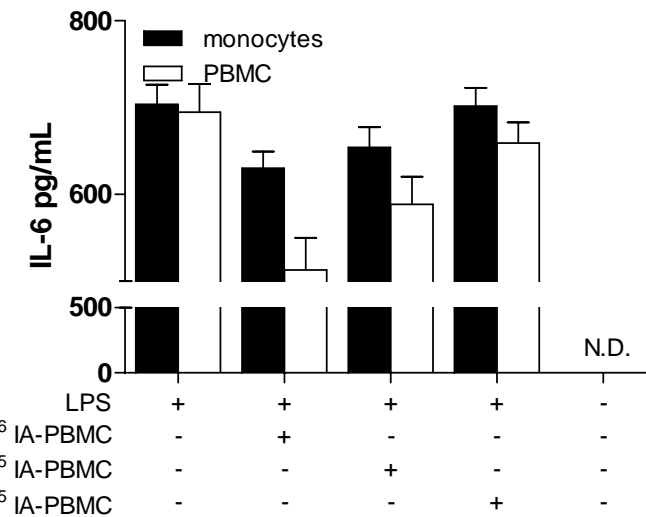
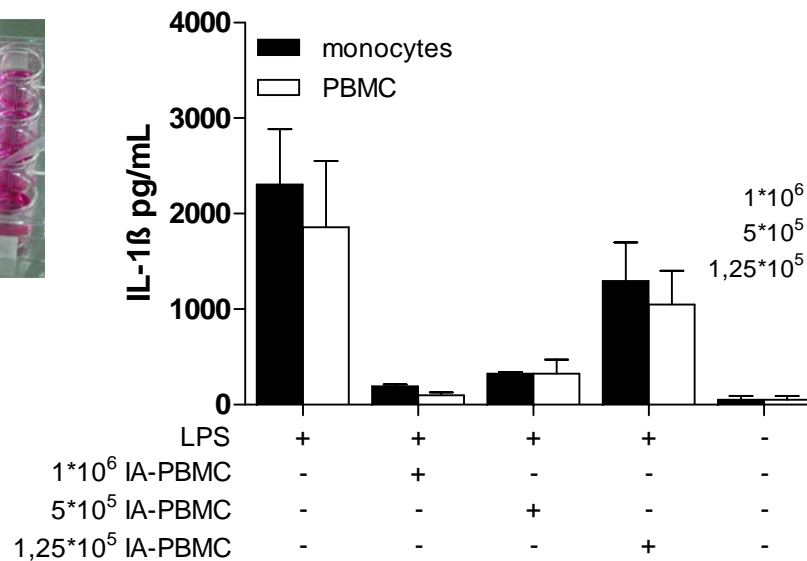
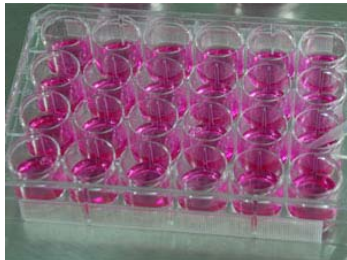


# In vitro Experiments

## Immunesuppressive & Pro-angiogenic Potential of Apoptotic Cells

### Co-Incubation Assay

PBMC and monocytes stimulated with LPS were co-incubated with autologous apoptotic PBMC

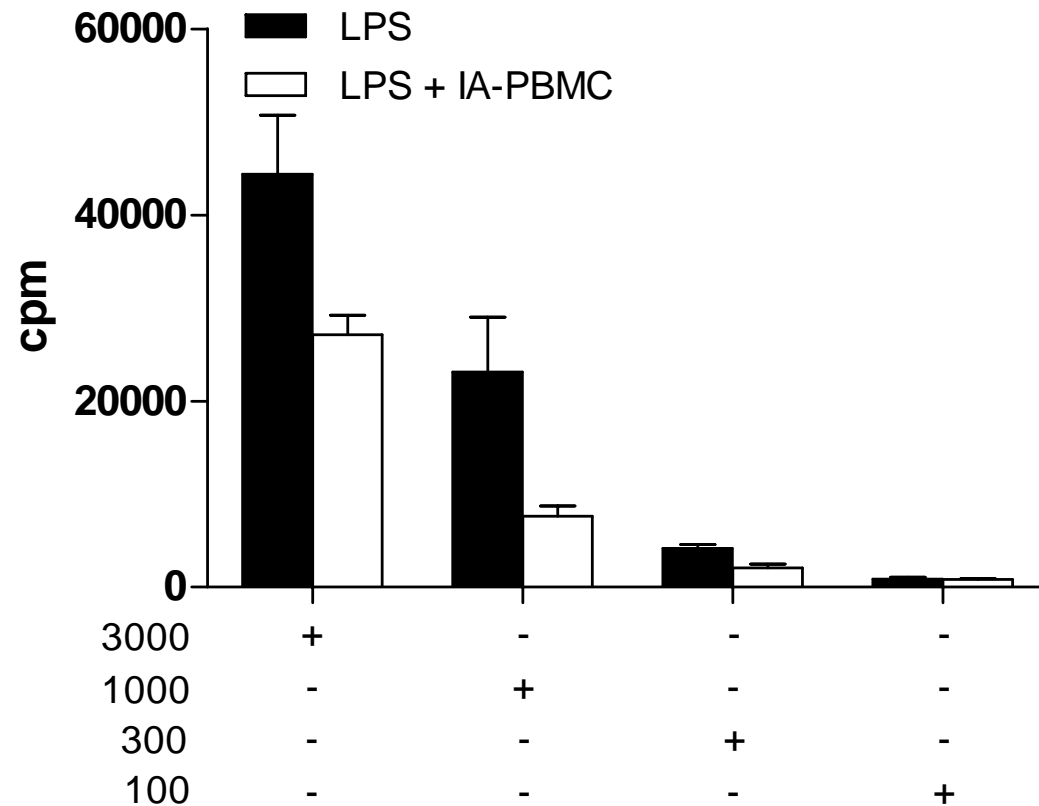


# In vitro Experiments

## Immunesuppressive & Pro-angiogenic Potential of Apoptotic Cells

### Mixed-Lymphocyte Reaction

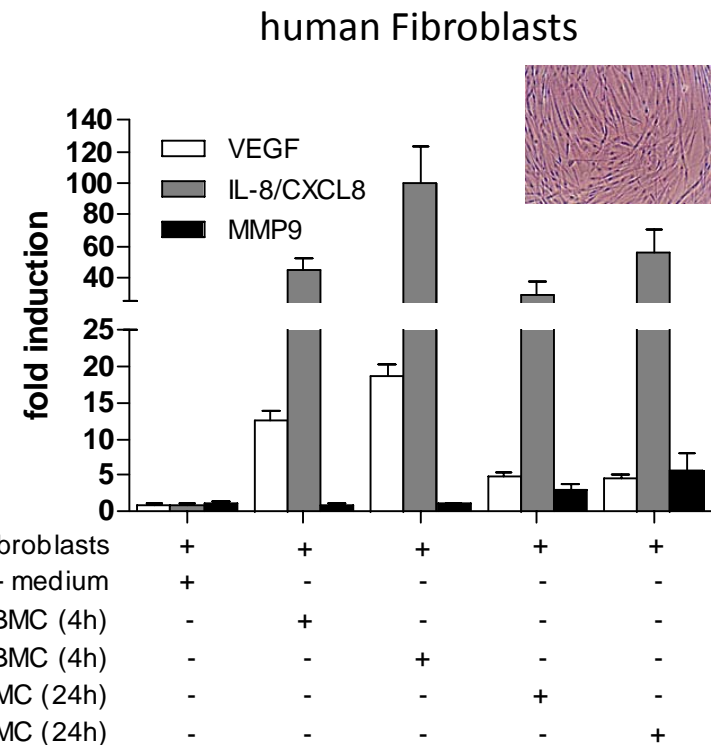
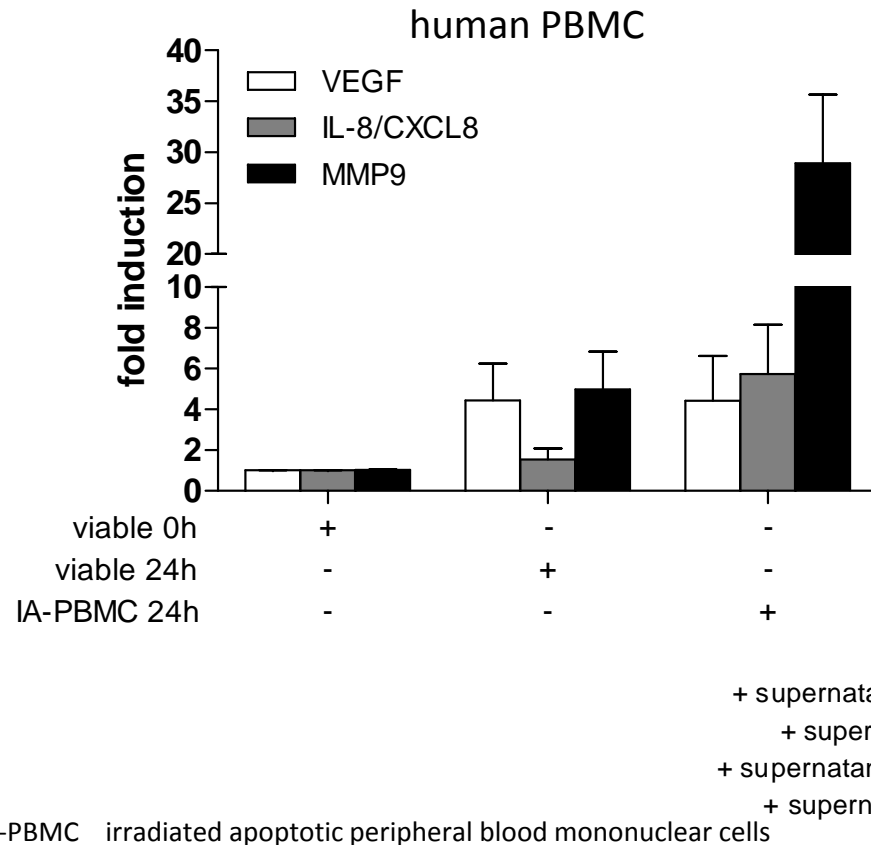
Maturation of monocyte-derived Dendritic Cells was induced by LPS and IA-PBMC were added in a 1:1 ratio. For the mixed leukocyte reaction (MLR), allogenic, T cells ( $1 \times 10^5$ /well) were incubated with graded numbers of stimulated DCs for 6 days. Proliferation of T cells was monitored by measuring [methyl-3H] thymidine.



# In vitro Experiments

## Immunesuppressive & Pro-angiogenic Potential of Apoptotic Cells

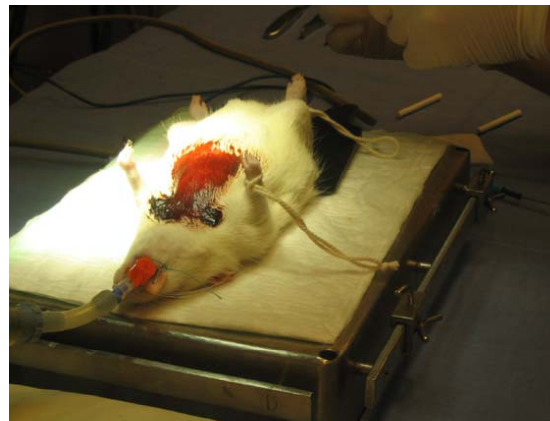
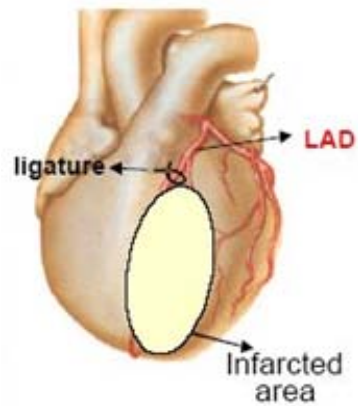
### RT-PCR





# *In vivo* Experiments

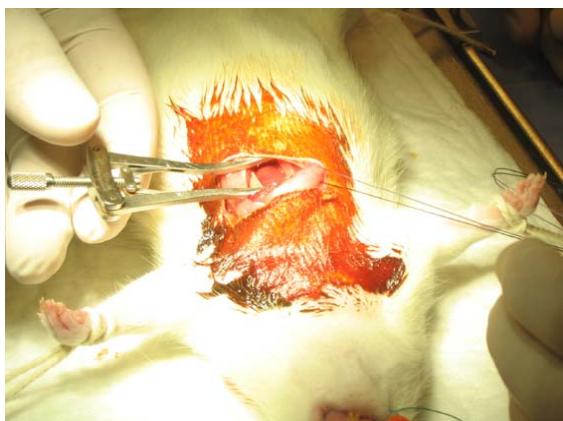
## Induction of myocardial infarction by LAD ligation



anesthetized and mechanically ventilated rat



dermal incision



intercostal thoracotomy



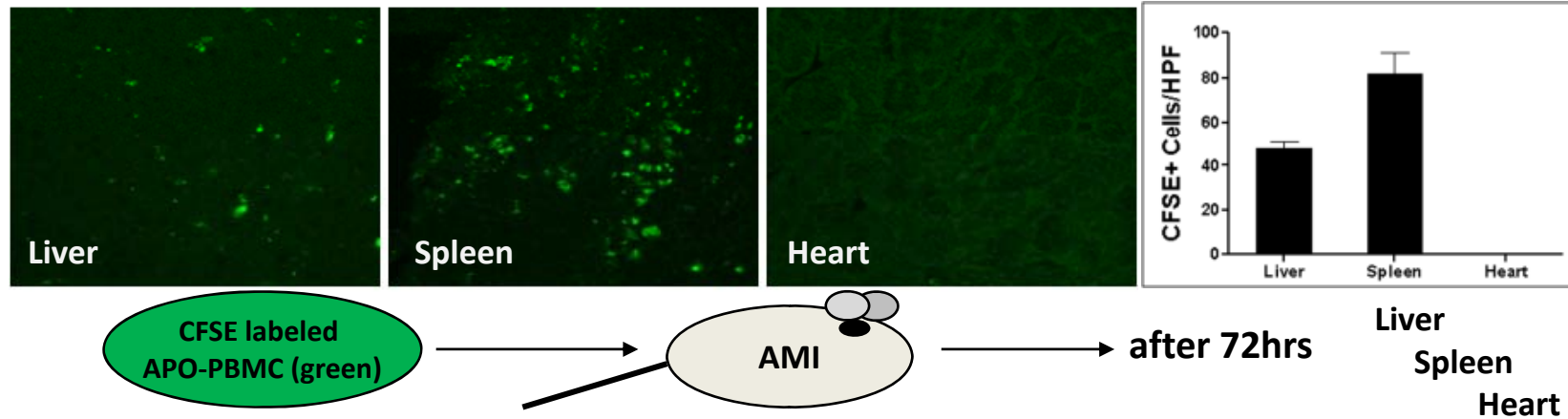
ligation of the coronary artery



dermal suture

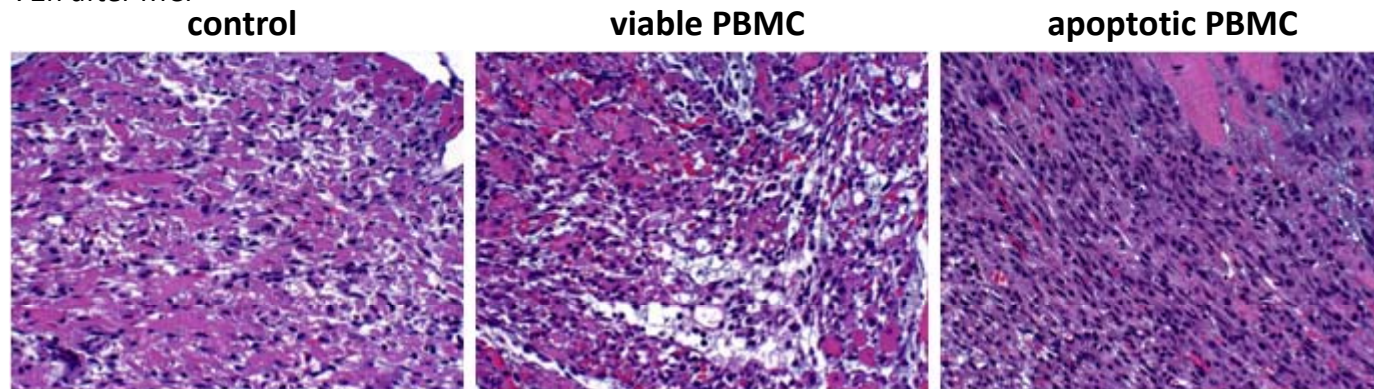
# In vivo Experiments

## Cell Transfer of apoptotic PBMC after MCI



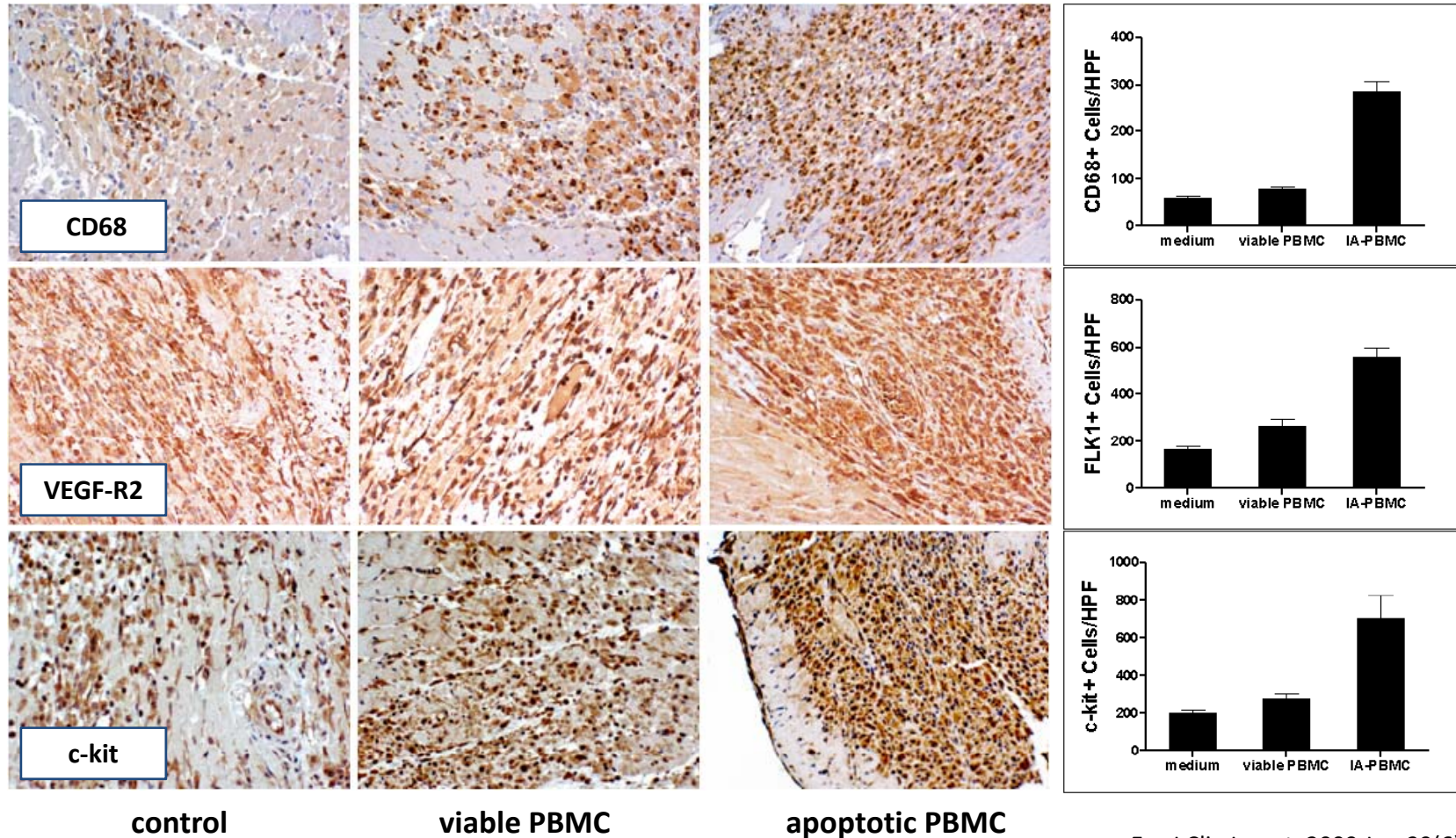
## HE Histology

72h after MCI



# In vivo Experiments

## Immunohistochemistry



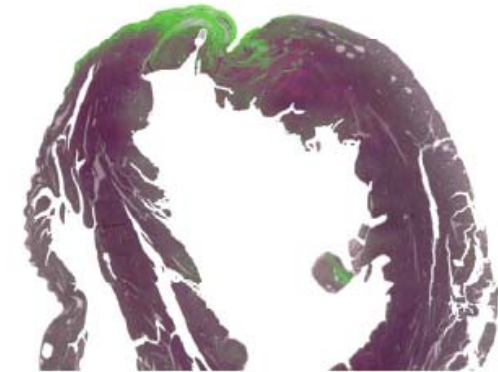
# Results



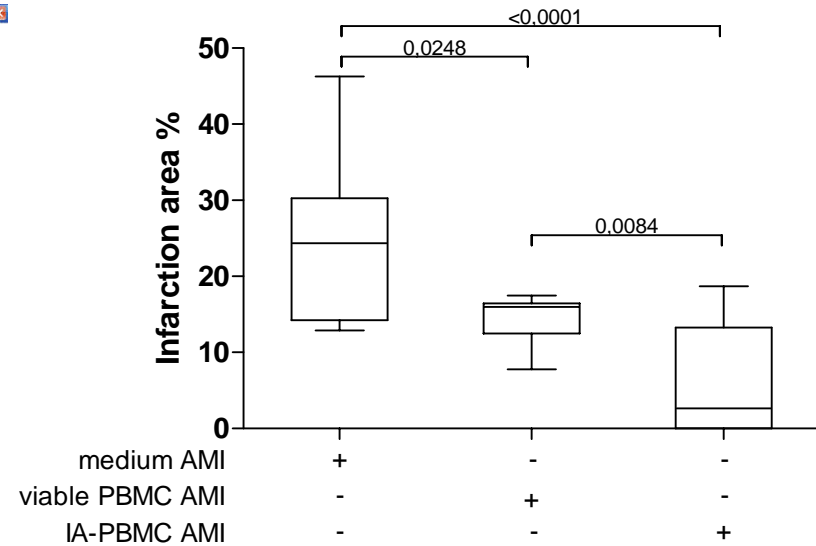
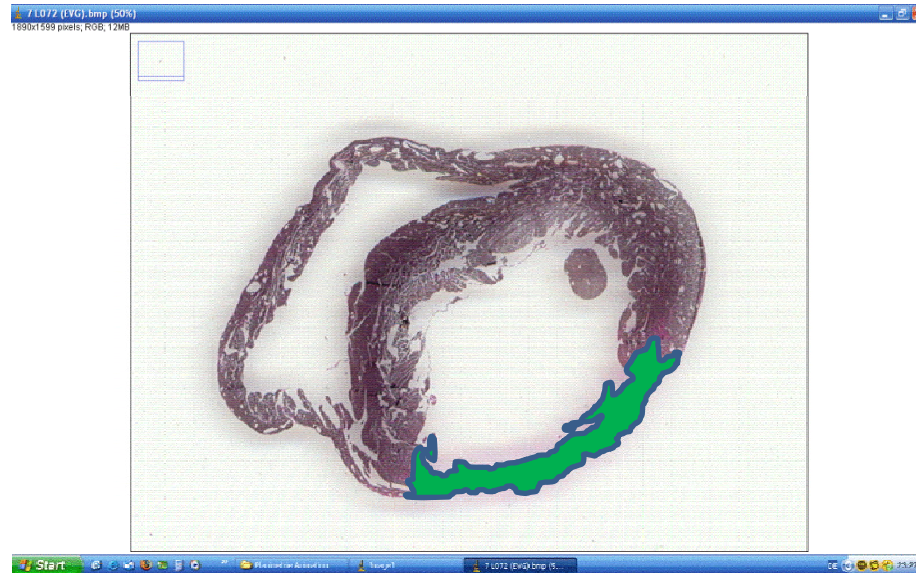
**control**



**viable PBMC**



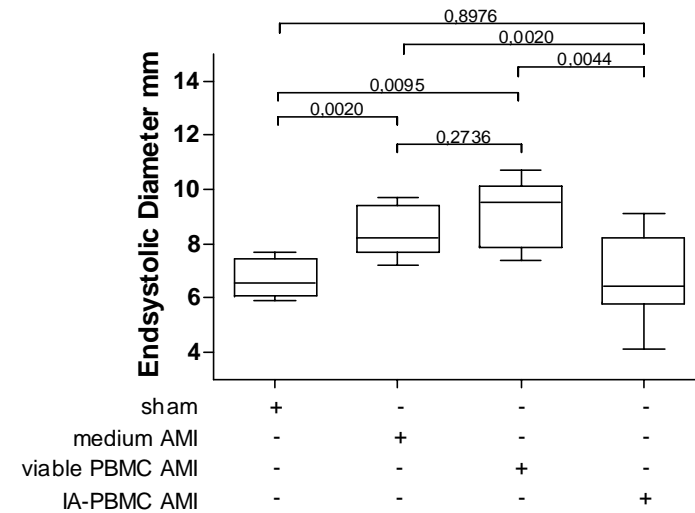
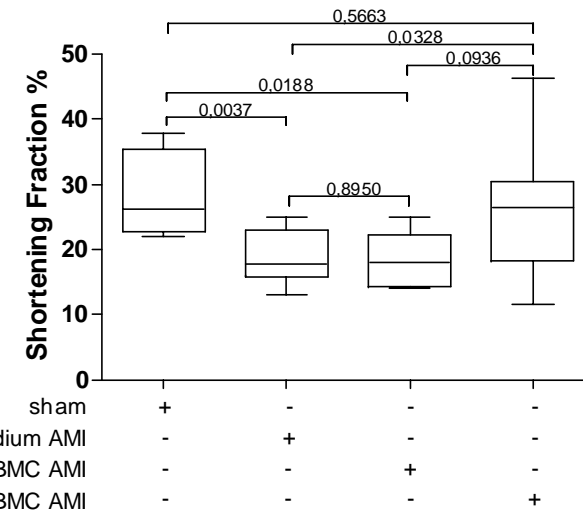
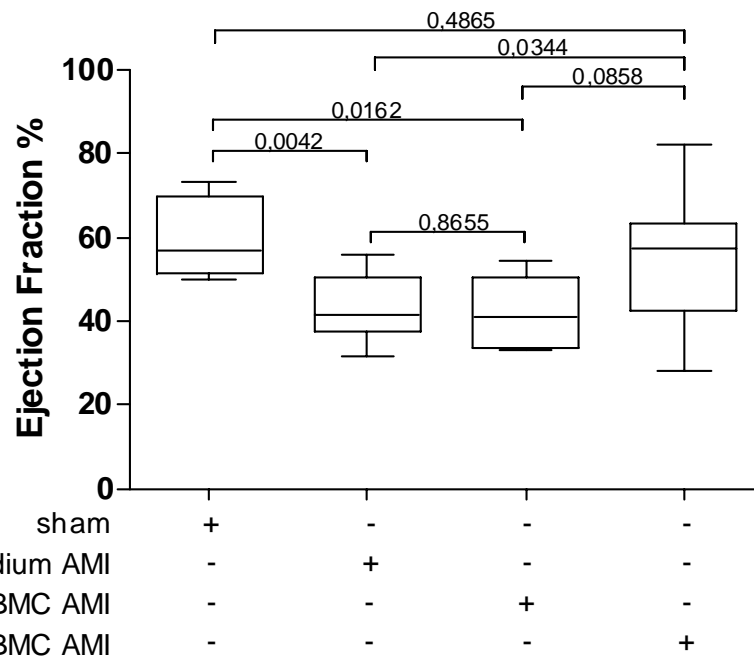
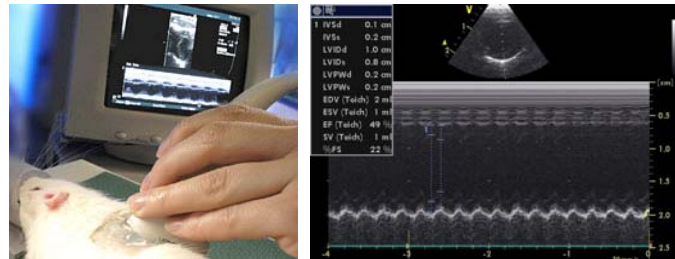
**apoptotic PBMC**



# Results

## Echocardiography

6 weeks after induction of myocardial infarction



## Administration of irradiated apoptotic PBMC after myocardial infarction induces ...

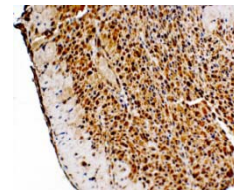
### Reduction of Pro-inflammatory Signals

IL-1 $\beta$  ↓  
IL-6 ↓

### Up-regulation of Pro-angiogenic mediators

Interleukin-8 ↑  
MMP9 ↑

### Increased Homing of c-kit<sup>+</sup> Cells



### Better Recovery of Cardiac Function

Ejection Fraction ↑  
Shortening Fraction ↑  
Dilatation ↓



# Dank an

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**zum Patent angemeldet**

