Strategy to Overcome the Limited Therapeutic Efficacy of the Current Cell Therapy for MI

Cytokine-based Cell Therapy for Myocardial Infarction:

MAGIC Cell-5-Combicytokine (EPO & G-CSF) Trial

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Disclosure Statement of Financial Interest

I, (Hyo-Soo Kim) DO NOT have a financial interest/arrangement or affiliation with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of the subject of this presentation.

Contents

- Introduction to MAGIC Cell program
- Current status and future direction
- Combination cytokine therapy with Erythropoietin and G-CSF for acute myocardial infarction

Cytokine-based Cell Therapy ; Advantage

Therapy with the mobilized peripheral blood progenitor cell by G-CSF

☐ Potential advantages

- Simple and noninvasive
- Mobilization, homing, differentiation of stem cells, results in angiogenesis and improvement of cardiac function

Orlic et al. Proc Natl Acad Sci USA 2001;98:10344-9

Adachi et al. Journal of Molecular and Cellular Cardiology 2004

Kawada et al. Blood. 2004;104:3581-3587

- Direct effects on prevention of pathologic remodeling

Ohtsuka et al. FASEB 2004

Sugano et al. Cardiovascular Research (2005) 446- 456

- Direct effects on survival of cardiomyocytes and endothelial cells

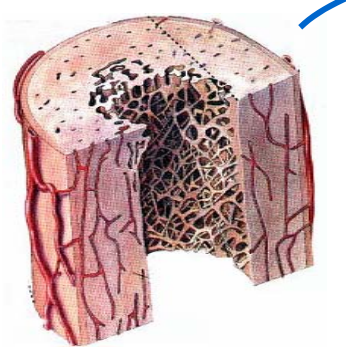
Harada et al. Nature medicine 2005

Two Strategies in Cytokine-based Cell Therapy

G-CSF injection

Mobilization alone

Bone marrow



Direct Mobilization and homing of stem cells

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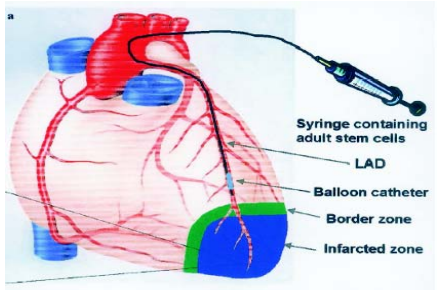
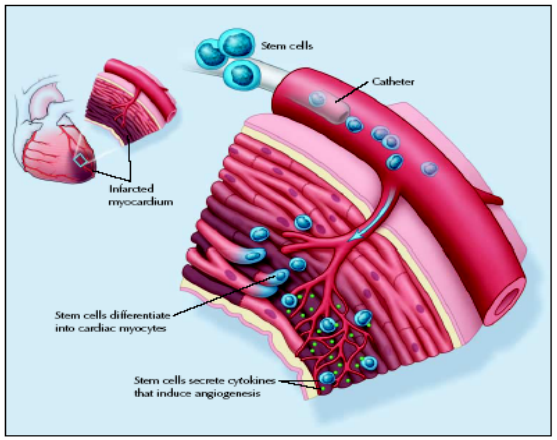




Apheresis

Intracoronary infusion of stem cells

G-CSF injection

Additional IC Infusion

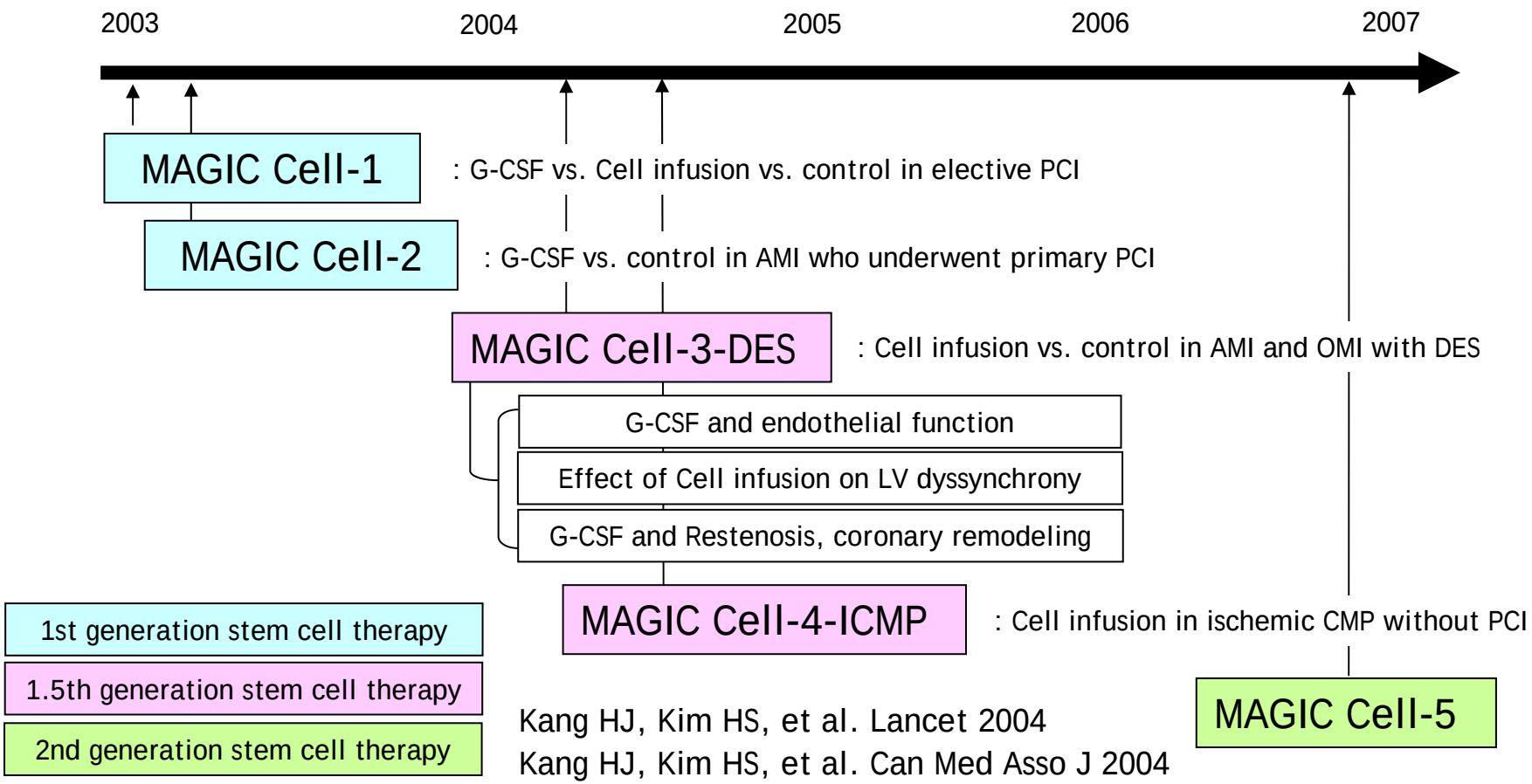
Stem cell therapy trials using G-CSF for ischemic heart disease

	Number of patients	Underlying disease	Results
G-CSF mobilization only			
Hill et al	N = 16	Refractory angina	No improvement
FIRSTLINE-AMI	N = 50	AMI after primary PCI	Improvement of LVEF
Valgimigli et al	N = 20	AMI after PCI	No improvement
STEMMI	N = 78	AMI after PCI	
REVIVAL-2	N=114	AMI after PCI	No improvement
MAGIC Cell -1, 2	N= 42	G-CSF alone or Intra-coronary infusion after PCI after/before PCI (AMI+OMI)	No improvement in G-CSF alone group
G-CSF with cell infusion			
Erbs et al	N= 26	Chronic total occlusion after PCI	Improvement of LVEF
MAGIC Cell-3-DES	N = 96	Intra-coronary infusion after PCI (AMI+OMI)	Improvement of LVEF in AMI

Seoul National University Hospital

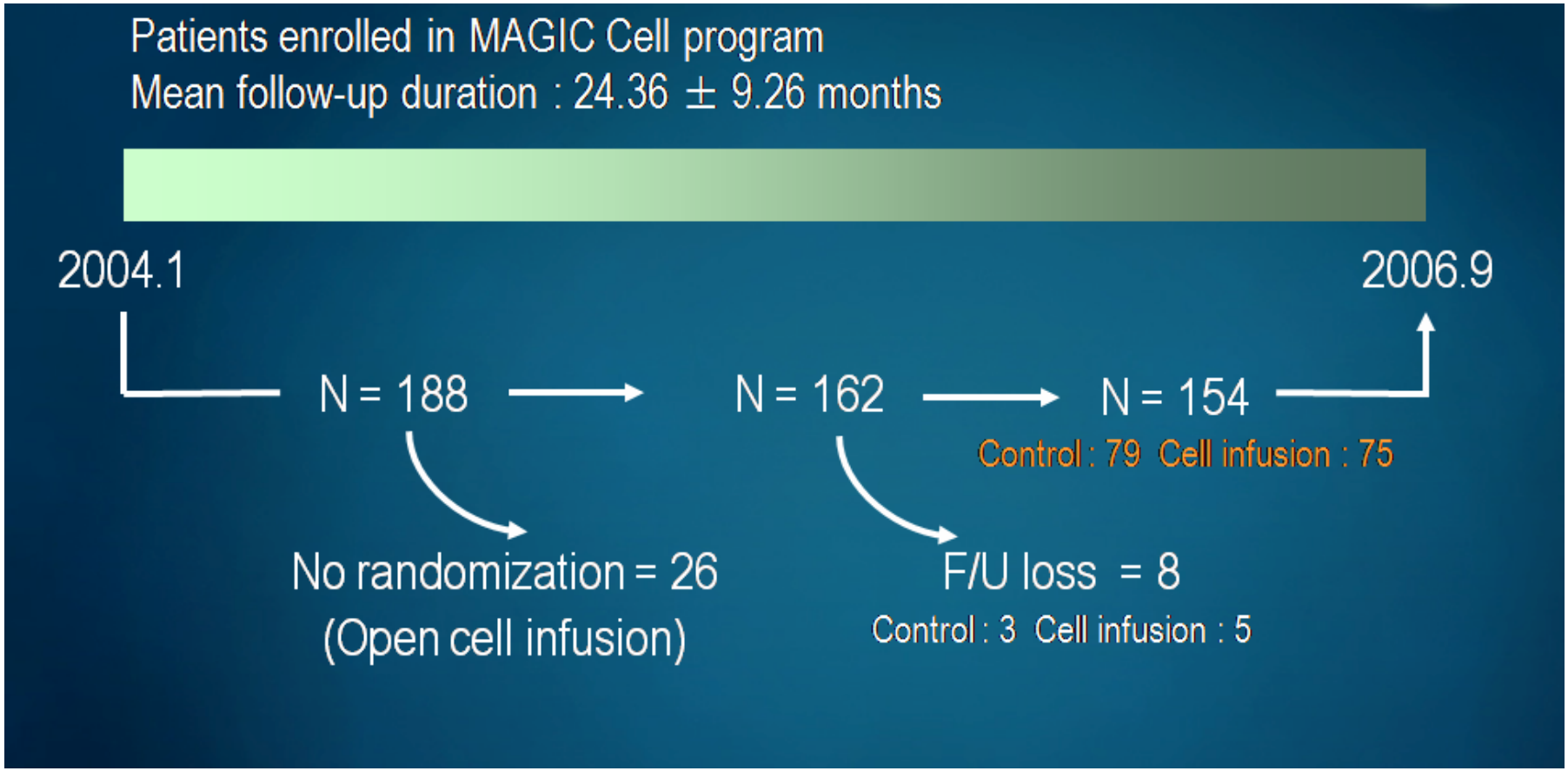
MAGIC Cell program in SNUH

(Myocardial Regeneration and Angiogenesis in Myocardial Infarction with G-CSF and Intra-Coronary Stem Cell Infusion)

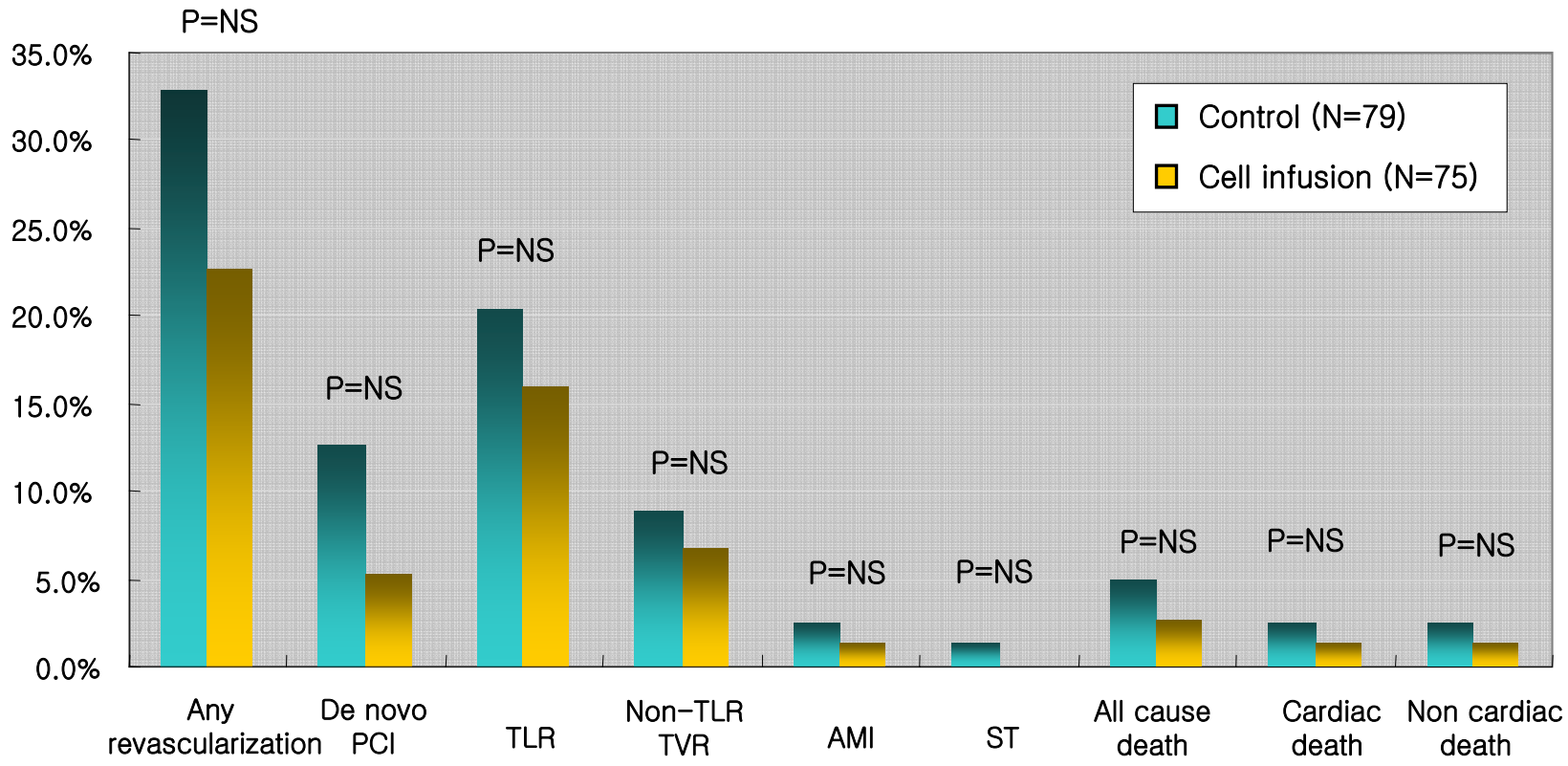


Kang HJ, Kim HS, et al. Lancet 2004
Kang HJ, Kim HS, et al. Can Med Asso J 2004
Kang HJ,, Kim HS. Circulation 2006
Kang HJ,, Kim HS. Am Heart J 2007
Kang HJ,, Kim HS. Heart 2007
Chang SA, Kang HJ,, Kim HS. Heart 2008

Clinical outcomes in MAGIC Cell program



Clinical outcomes at 24 month



* ST: 351 days after index PCI

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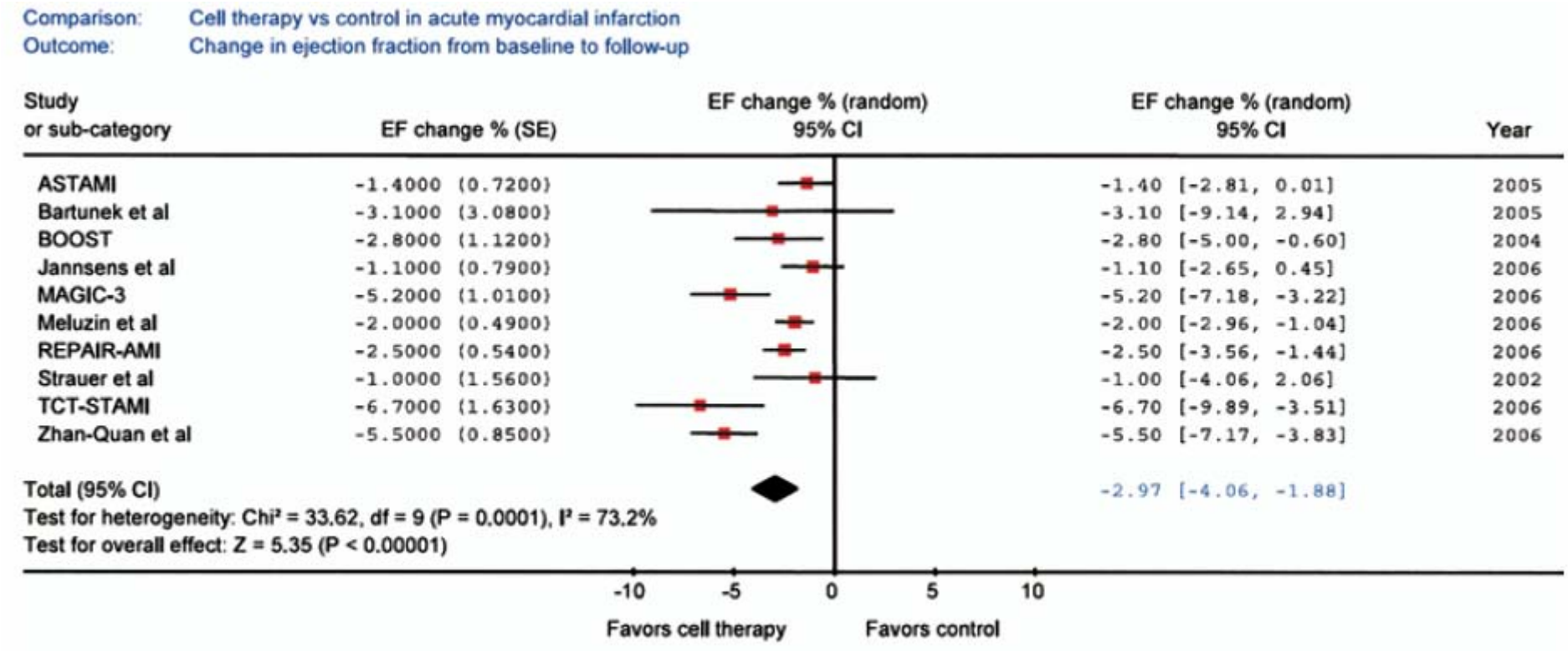
Effects of stem cell therapy on LV systolic function



Lipinski, et al. JACC 2007

Hurdles to the efficacy of cell therapy

quality of stem cells & environment of infarct

V
S

Weak stem cell in diseased old patients

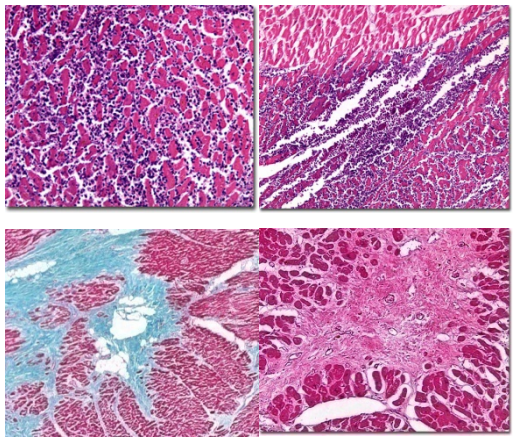
Enhance potency of stem cells

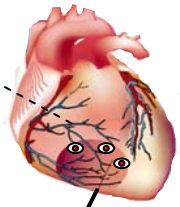
&

Improve micro-environment

AMI

OMI





Harsh Millieu of Infarcted Myocardium

Strategy to improve efficacy of stem cell therapy in acute myocardial infarction

- Adjunctive measures to stem cell therapy
  - Enhancing effects on stem cell
  - Protective effects on infarcted heart

- *In-vivo* priming strategy

  for cell and infarcted heart

  with another cytokine

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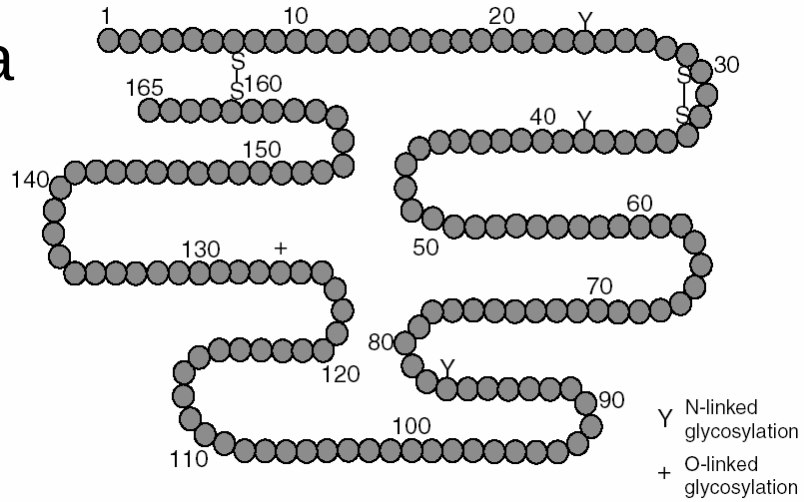
Erythropoietin

- 165-amino acid, 30.4 kD Glycoprotein hormone
- Primary regulator of erythropoiesis
- Stimulating growth, preventing apoptosis, and inducing differentiation of RBC precursors
- rHuEPO: treatment of anemia due to renal failure, cancer



Pleiotropic effects of erythropoietin

- Angiogenesis
  - late long term benefit >> acute benefit
- Mobilization of stem cells or EPCs
- Cytoprotective effect in myocardial infarction
  - preserve the ventricular function (Cai et al., 2003; Joyeux-Faure et al., 2006)
  - reduce lethal arrhythmia (Hirata et al., 2005)
  - reduce apoptosis (Cai et al., 2003; Calvillo et al., 200; Moon C, et al.Proc Natl Acad Sci U S A 2003; Parsa CJ,et al. J Clin Invest 2003; Fiordaliso F, et al.. Proc Natl Acad Sci U S A 2005)
  - reduce necrosis (Lipsic et al., 2004; Bullard et al., 2005)

Heeschen C, et al. Blood 2003

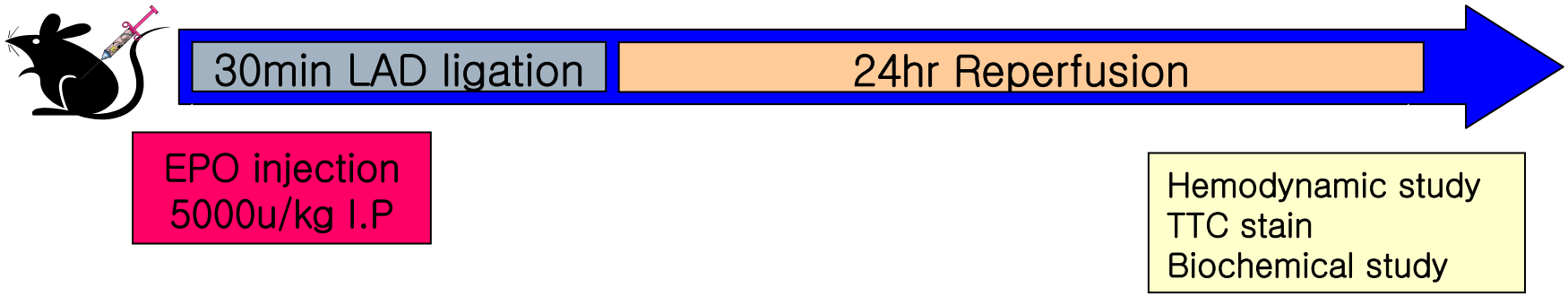
- Anti-inflammatory effect in myocardial infarction

Rui T, et al. Cardiovasc Res 2005

Animal Study [Seoul National University Hospital]

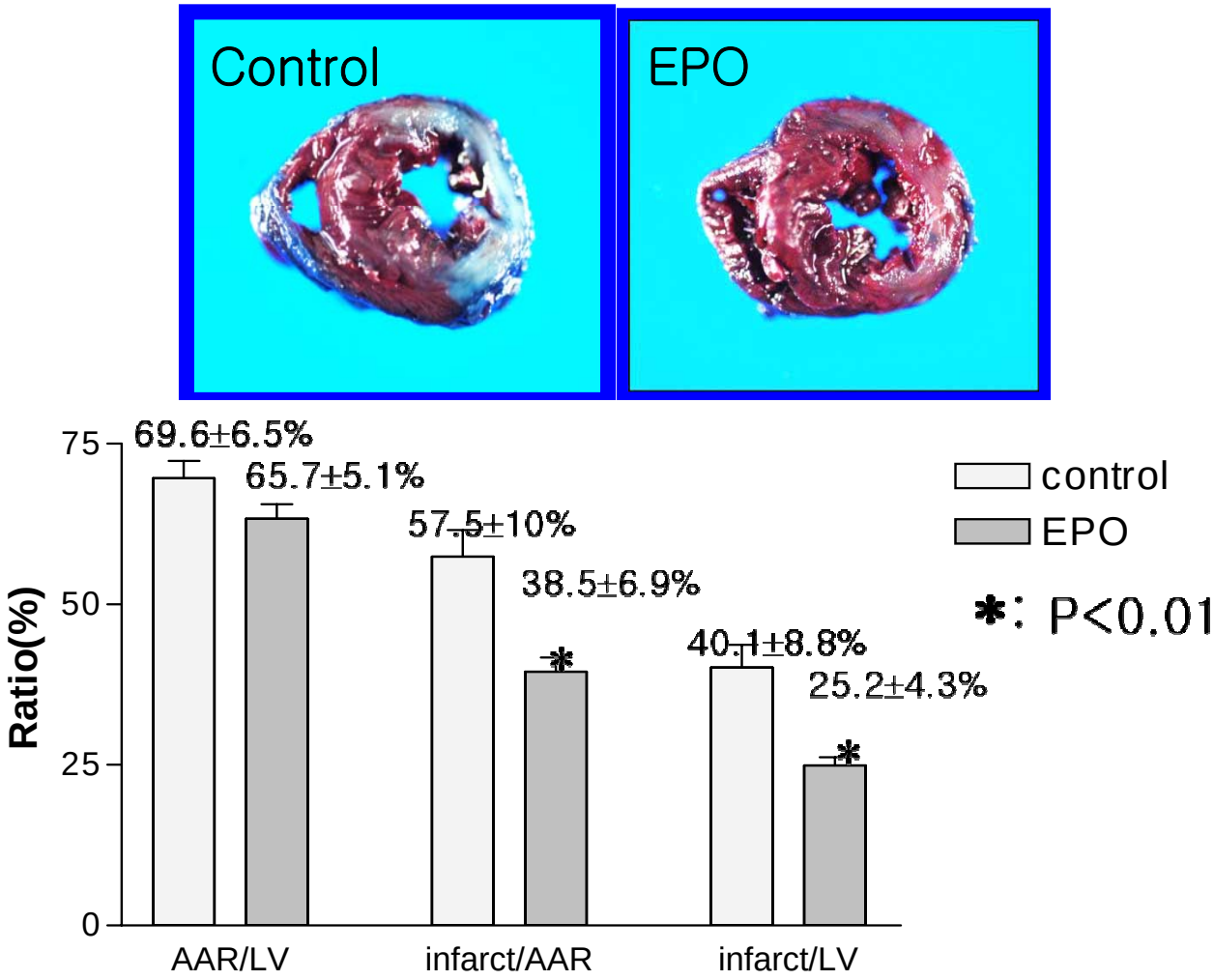
Animals

- 200-250g, male, Sprague-Dawley rats
- Two groups
  - EPO group: EPO 5,000U/kg IP prior to LAD ligation
  - Control group: Saline 0.1cc IP prior to LAD ligation



Choi DJ, et al (unpublished data)

EPO reduces infarct size in rat AMI model



Choi DJ, et al (unpublished data)

EPO in AMI study [Seoul National University Hospital]

- Design
  - Single center, single blinded, placebo controlled
- Objectives
  - To evaluate safety and effect of EPO on infarct size reduction and remodeling in patient with AMI who undergo successful primary PCI
- Population
  - 1st AMI patient who undergo primary PCI < 12 hr
  - Ant wall STEMI
  - Age < 80 YO

(Seoul National University Bundang Hospital)

EPO in AMI study [Seoul National University Hospital]

- Design
  - Control vs. EPO group (1:2 randomization)
  - Placebo (Saline) vs. EPO 500U/kg iv just before primary PCI
- Endpoints
  - Primary endpoint : LVEF at 6m fu (by Cardiac MRI)
  - Secondary endpoint : LV Volume change at 6m fu

(Seoul National University Bundang Hospital)

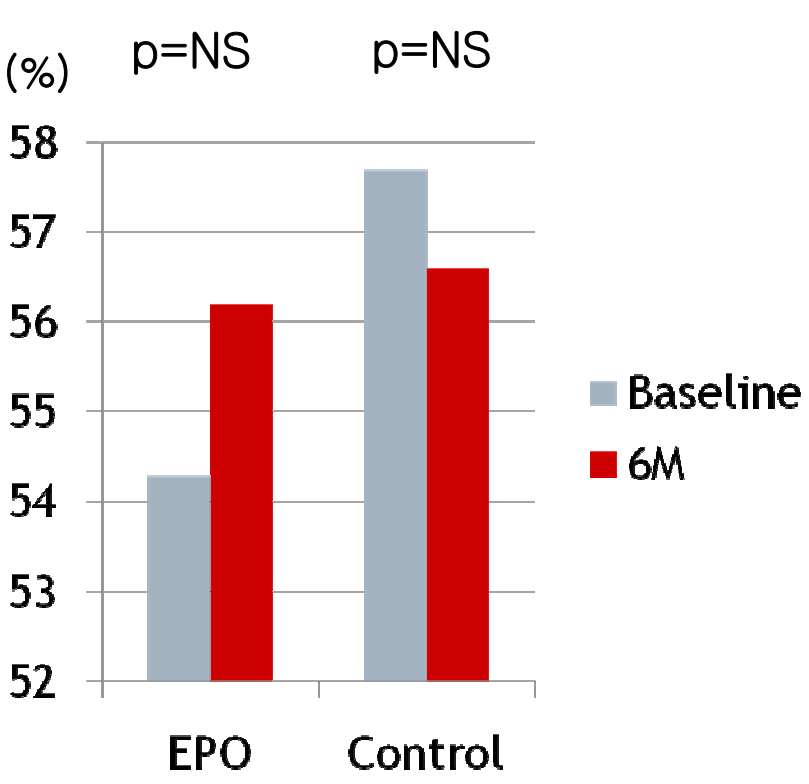
EPO in AMI study [Seoul National University Hospital]

Demographics & Risk Factors

	Control (N=13)	EPO (N=26)	P
Age (years)	59	53	0.200
Sex (Male, %)	69	88	0.194
HT (%)	15	35	0.276
DM (%)	23	31	0.719
Current Smoking (%)	31	50	0.675
Symptom to Balloon time (min.)	222	302	0.095
Erythropoietin level at 24h (mU/mL)	35.6	284.2	<0.001*
Peak CK (IU/L)	3587	3966	0.589
Peak CK-MB (ng/mL)	339	387	0.656

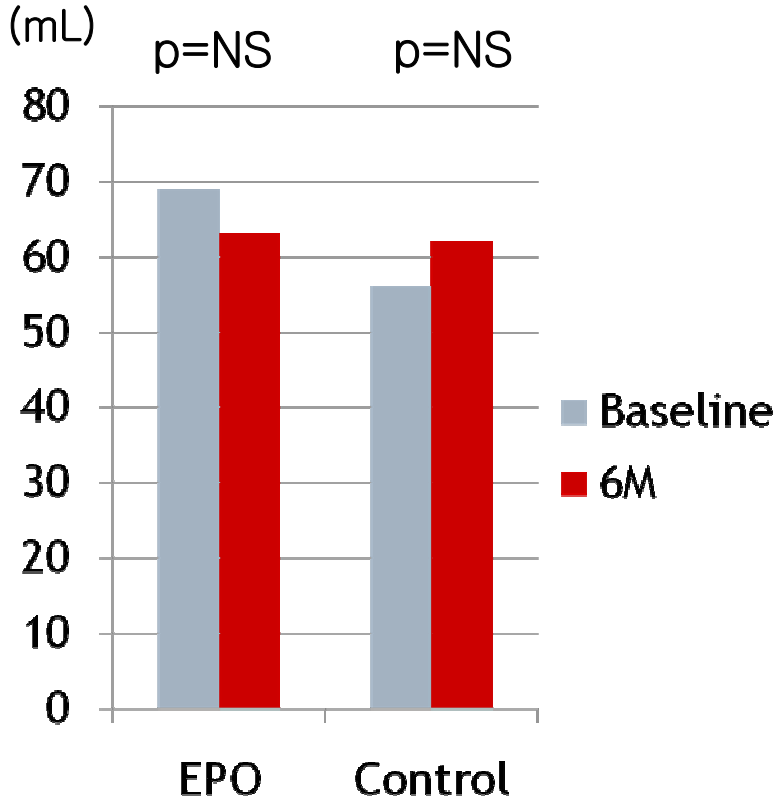
*: by Mann-Whitney Test

EPO in AMI study [Seoul National University Hospital]



EPO: +1.9 % vs. Control: −1.0%, p=NS

LVEF



EPO: −6.2 mL vs. Control: +6.4mL, p=NS

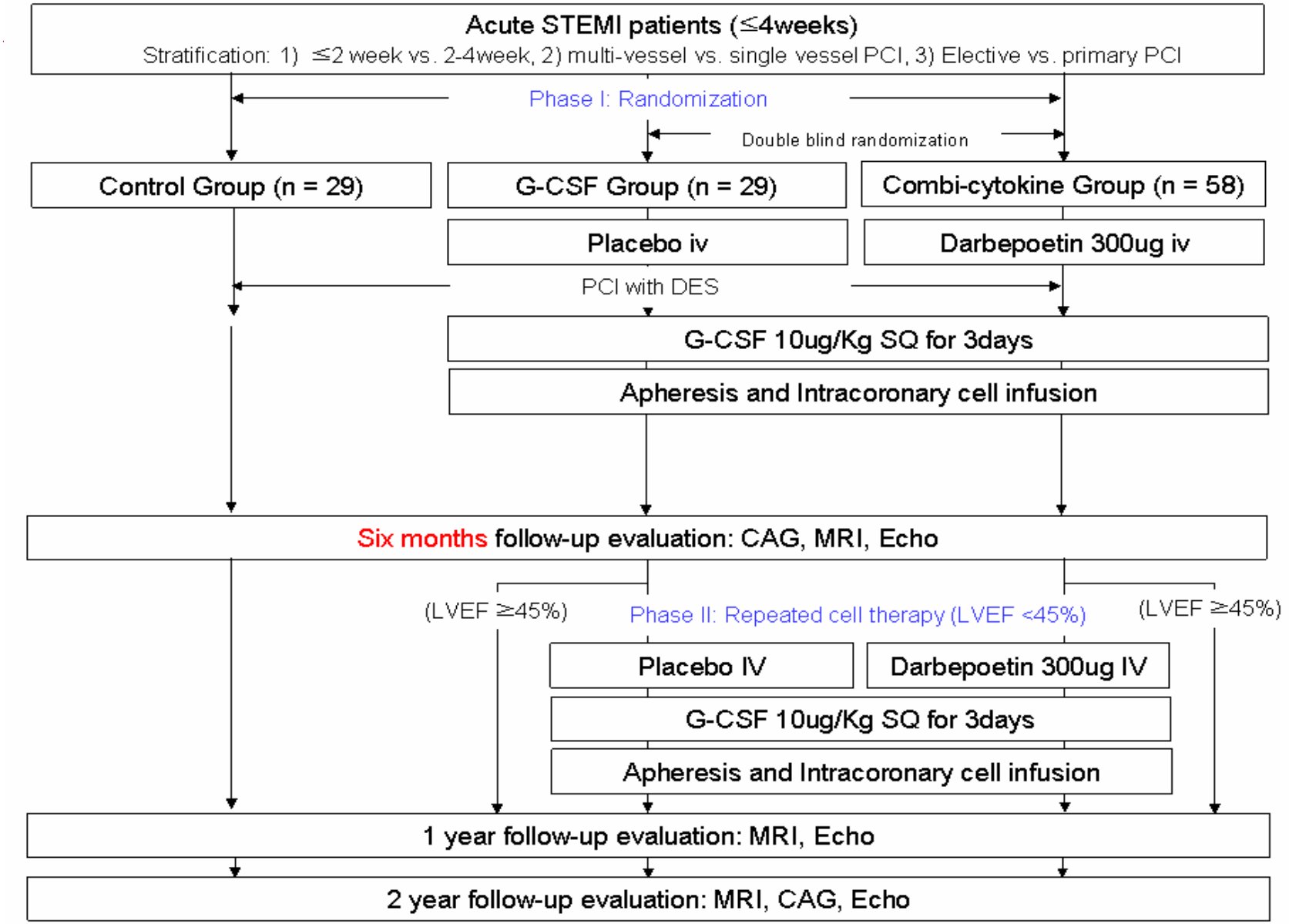
LVESV

MAGIC Cell-5-combicytokine trial

- Design
  - Multi-center, prospective, randomized, partly-double blinded, controlled trial
- Objective
  - Safety and efficacy of Erythropoietein/G-CSF and intracoronary infusion of mobilized PBSC in AMI patients
- Key Issues to be evaluated
  - Combination cytokine treatment
  - Repeated PBSC infusion

MAGIC Cell-5-combicytokine trial

- multicenter, randomized, partially double blinded controlled trial



Cytokine and Cell infusion

- Erythropoietin
  - Darbepoietin 4.5ug/Kg(max: 300ug)/placebo iv infusion during primary PCI (x 1)
- G-CSF
  - G-CSF 5ug/kg twice/day (max:600ug/day) for 3days after successful revascularization and stabilization
- Target Cell number for intracoronary infusion: 2 x 10^9 MNC
- Repeated Cell infusion for patients with LV systolic dysfunction (LVEF<45) at 6m fu will be performed with initially allocated strategy

Current status

- Protocol approval: Feb, 2007
- First patient enroll: Feb, 2007
- Current status: ongoing enrollment
  - 90% of target 120 pts – enrolled
  - half of target 120 pts – 6 month f/u
- Estimated timing for last patient enroll: June, 2009

MAGIC CELL INVESTIGATORS

Seoul National University Hospital, Seoul, Korea

P.I. of MAGIC Cell trial: **Hyo-Soo Kim**

Cardiology, Seoul national university hospital

Young-Bae Park, Byung-Hee Oh, Dae-Won Sohn, Dong-Ju Choi, In-Ho Chae, Bon-Kwon Koo, Woo-Young Chung, Tae-Jin Youn, Young-Seok Cho, Hae-Young Lee, Hyun-Jae Kang

Dept of Radiology

Jae-Hyung Park, Whal Lee

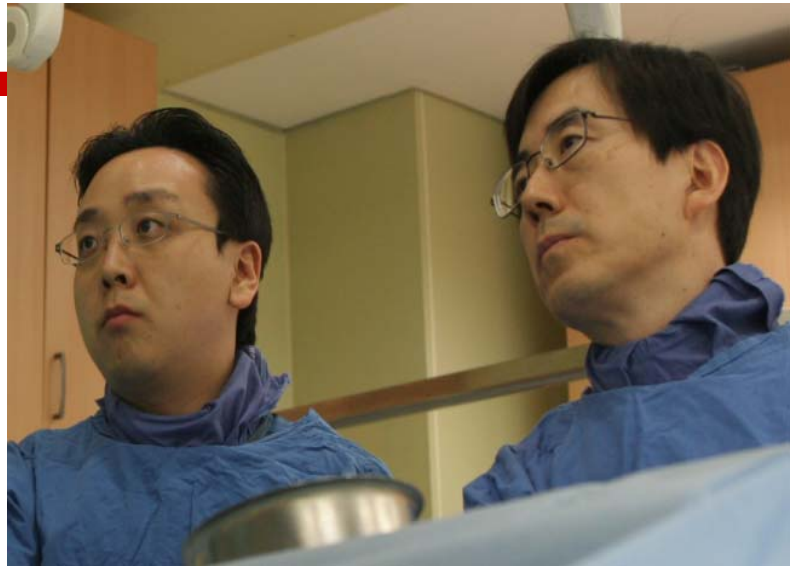
Dept of Clinical Laboratory

Kyu-Sup Hahn

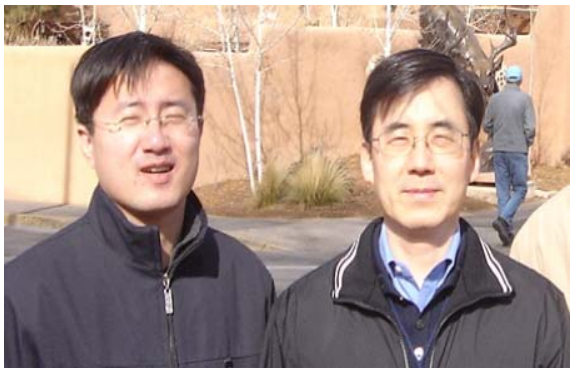
Innovative research institute for cell therapy, SNUH

Stem cell research center

Faculties of National Laboratory for Cardiovascular Stem Cells
Seoul National University Hospital, Seoul, Korea



Dr. KW Park



Dr. HM Yang

Dr. J Hur

Dr. CH Yoon

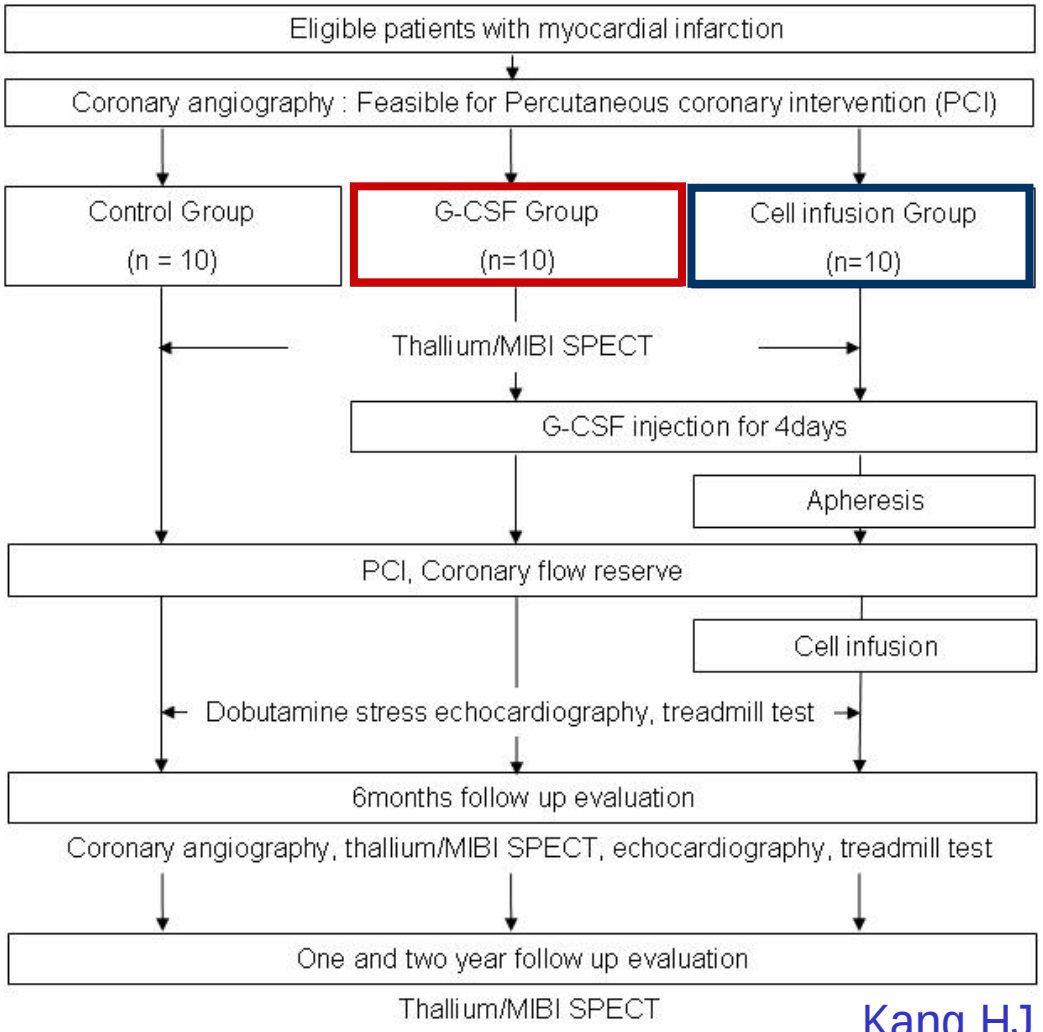
Dr. HJ Cho

EPO derivatives

- Hematopoietic effects of EPO on the bone marrow are mediated by the homodimeric EPOR. Desialylated EPO, which has the same EPOR affinity but with a very short plasma half-life, reducing the hematopoietic response, remains neuroprotective (Erbayraktar et al., 2003).
- carbamylated EPO (CEPO), another EPO analog (with all lysines transformed to homocitrulline by carbamylation), which does not bind to the homodimeric EPOR and lacks erythropoietic activity, confers neuroprotection as well as cardioprotection against various cellular injuries similar to rhEPO (Fiordaliso et al., 2005; Moon et al., 2006). With these compounds, it is now possible to trigger EPO-mediated cytoprotective pathways without cross-talk with hematopoietic system.

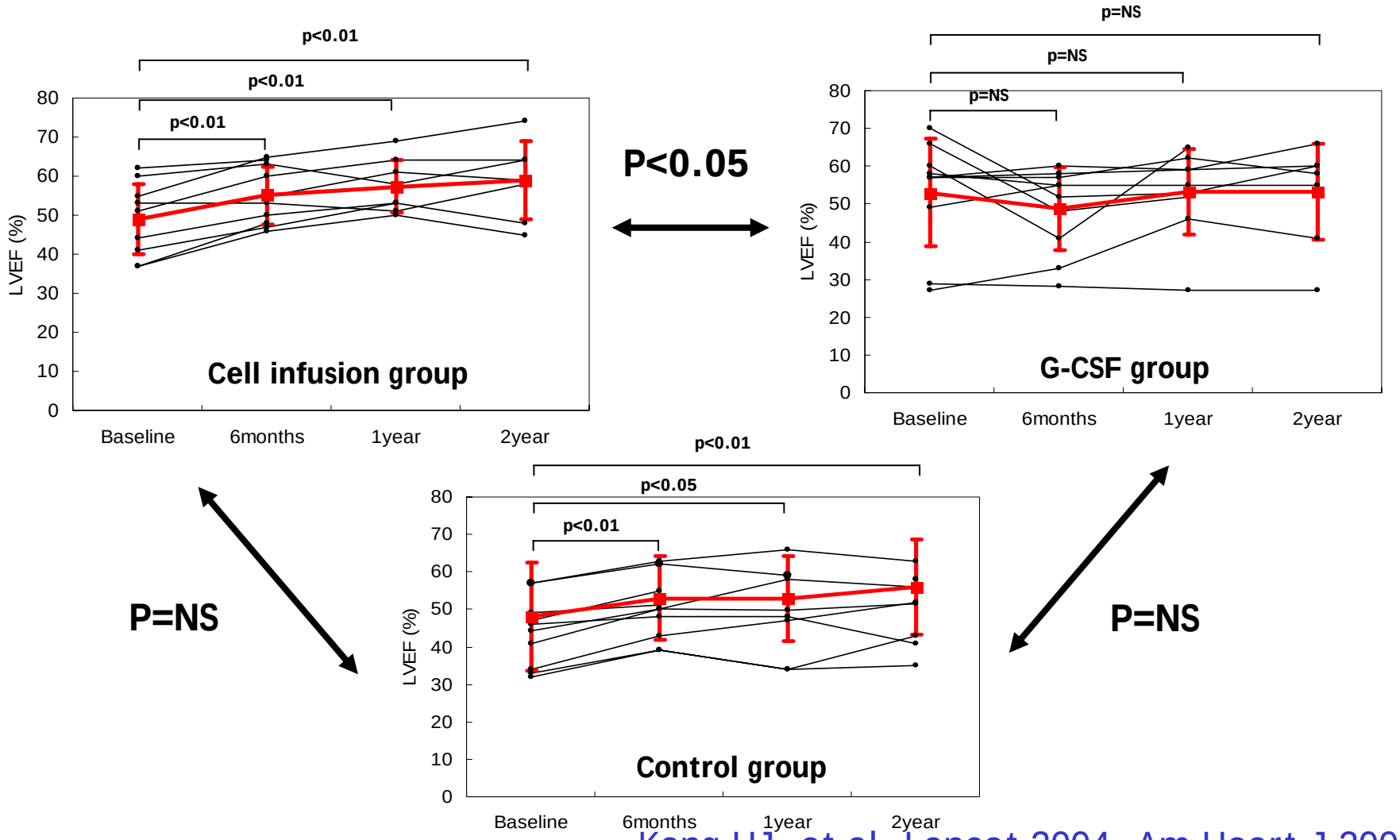
Profiles of MAGIC Cell-1 trial

(Myocardial Regeneration and Angiogenesis in Myocardial Infarction with G-CSF and Intra-Coronary Stem Cell Infusion)



Kang HJ, et al. Lancet 2004

Intracoronary cell infusion is better than G-CSF alone for improvement of LV systolic function

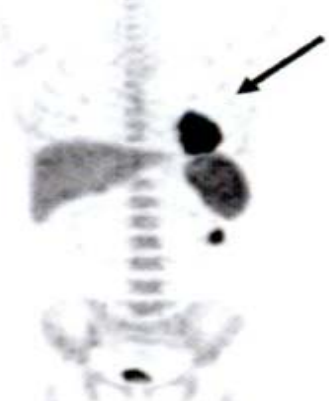


Kang HJ, et al. Lancet 2004, Am Heart J 2007

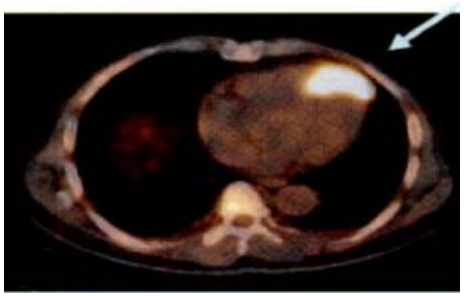
Intracoronary infusion is better than G-CSF only

- To deliver cells efficiently and improve cardiac function, local delivery of concentrated peripheral blood stem/progenitor cell is required .

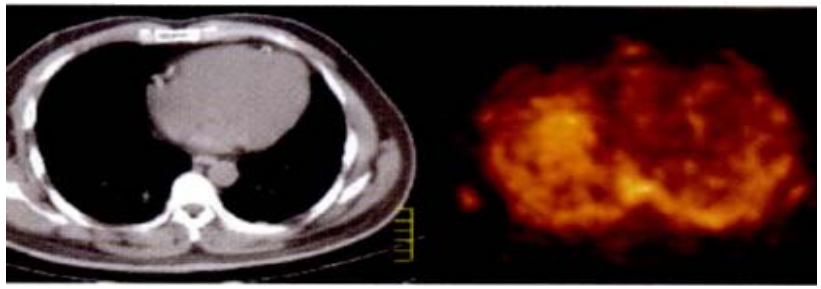
Infusion of FDG-labeled PBSCs in ant wall MI (PET-CT)



Intra-coronary infusion

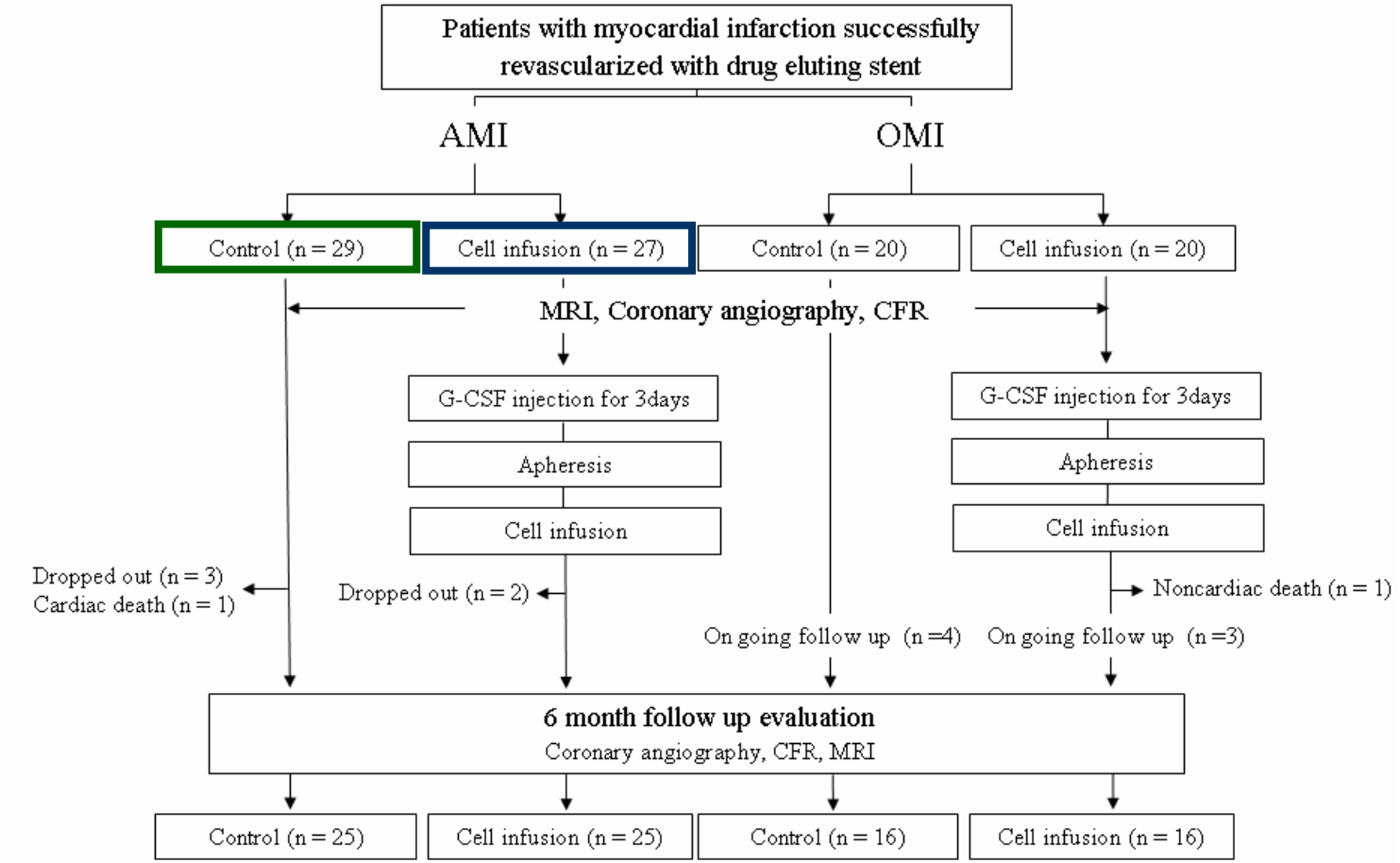


Intra-venous infusion



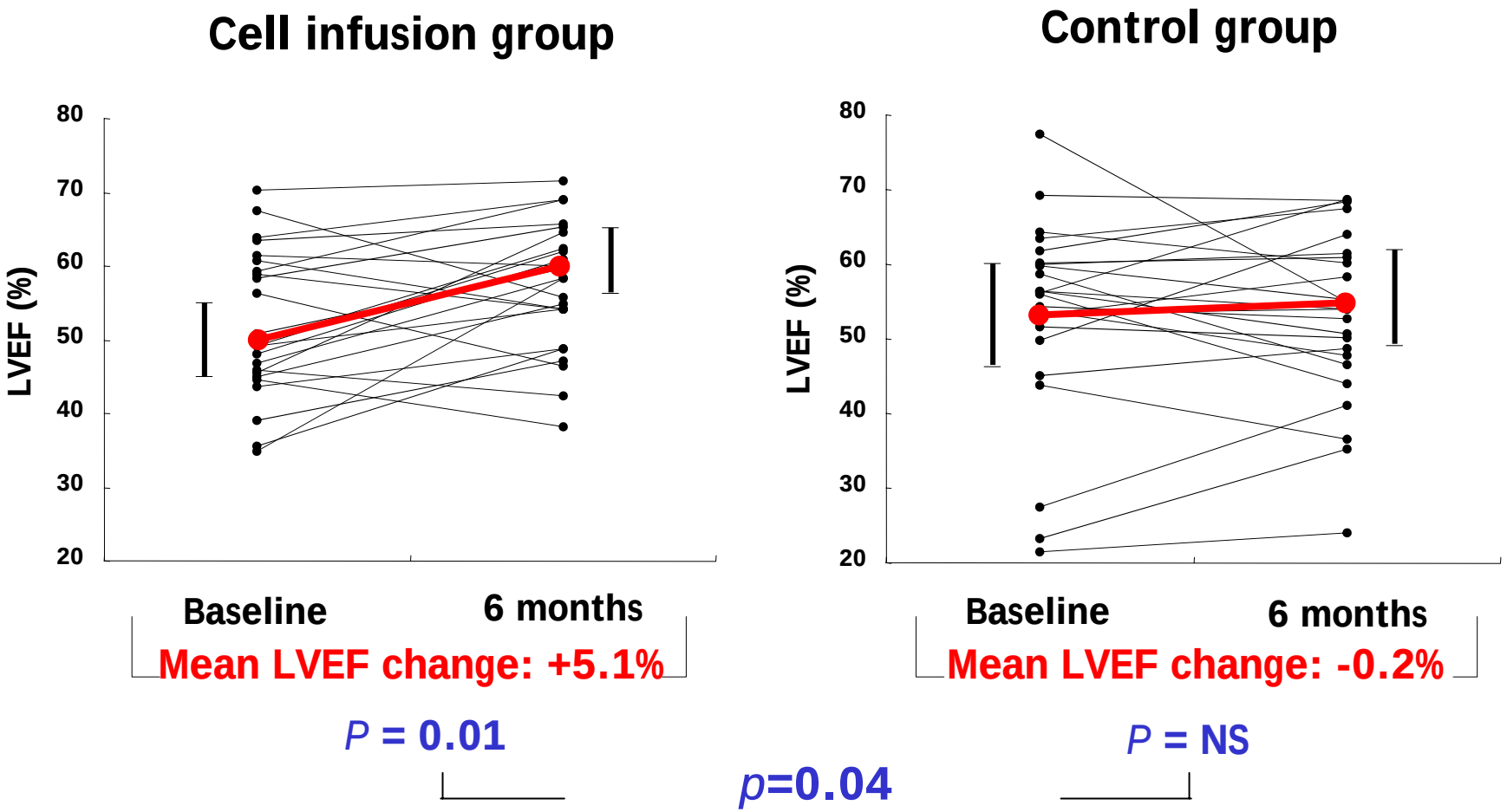
Kang WJ. et al. J Nud Med 2006

Profiles of MAGIC Cell-3-DES trial



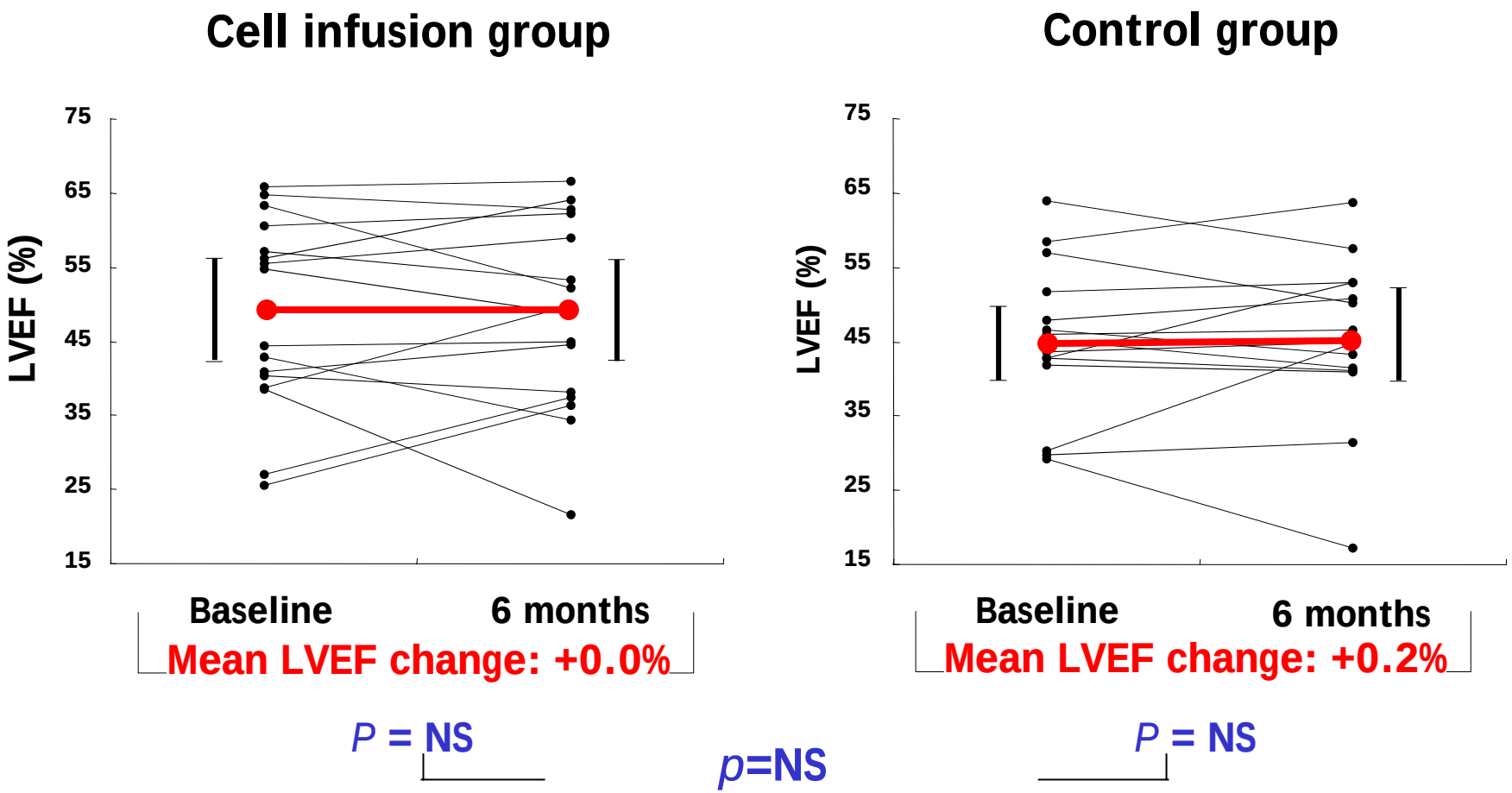
Kang HJ, Lee HY, et al. Circulation 2006

Cell infusion improves cardiac function in AMI



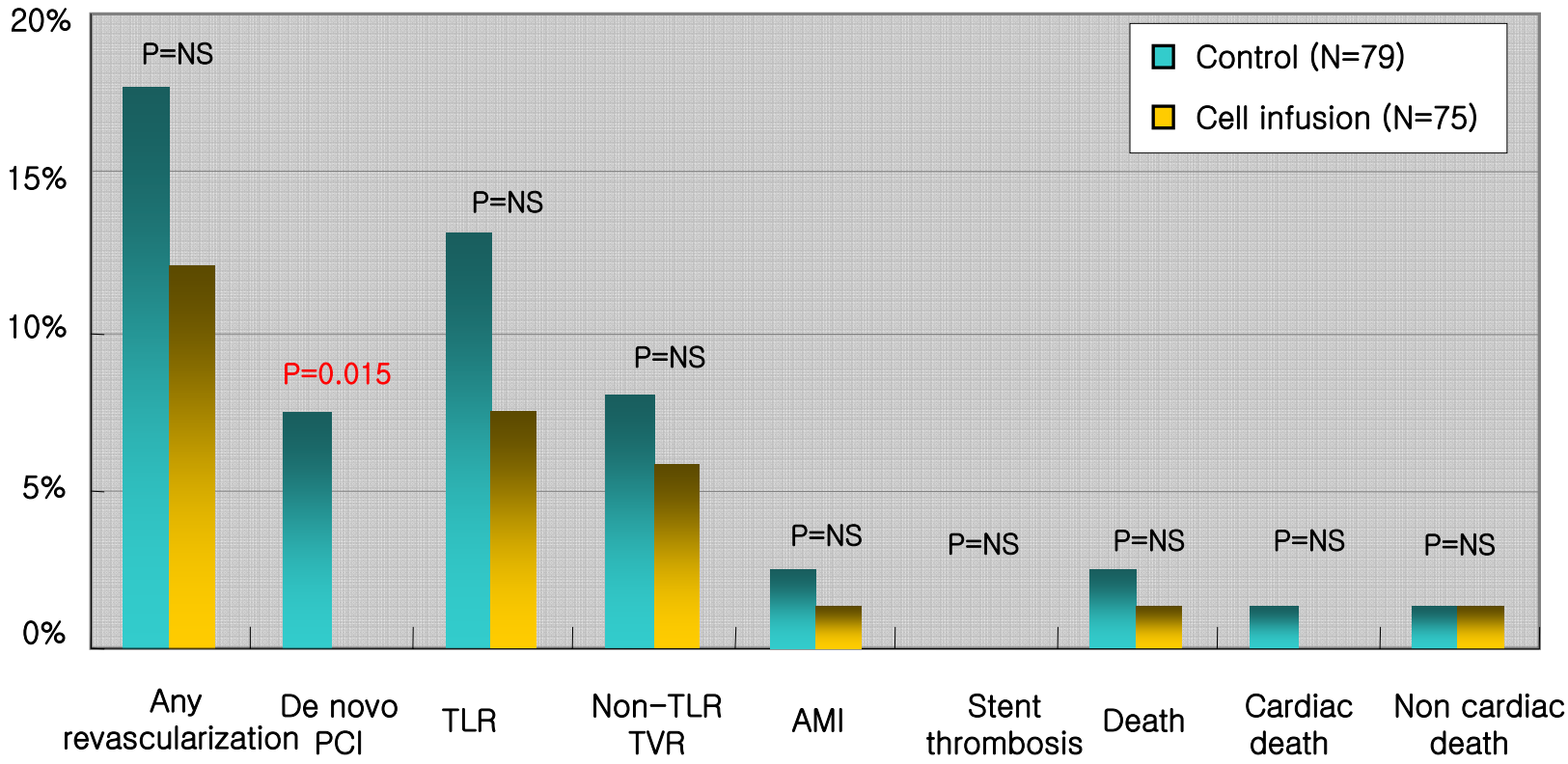
Kang HJ, Lee HY, et al. Circulation 2006

Cell infusion does not improve cardiac function in OMI

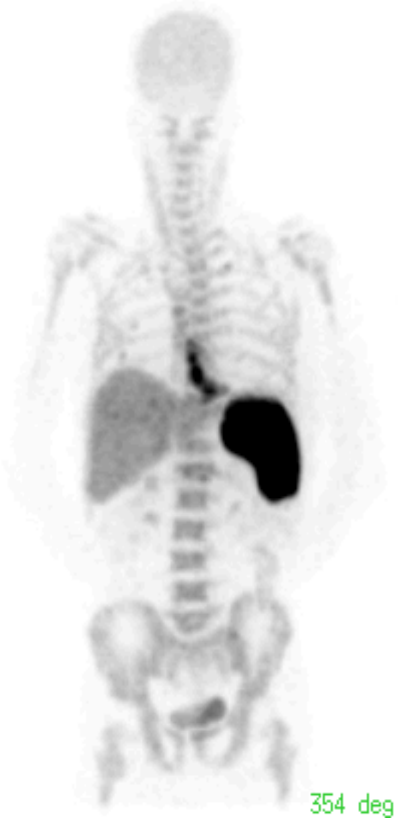


Kang HJ, Lee HY, et al. Circulation 2006

Clinical outcomes at 6 month



Low efficiency of homing and retention to infarct



Distribution of Intracoronary Injected Stem Cells Measured at 2 and 20 Hours

Organ	Activity of injected stem cells (%)	
	2 h	20 h
Myocardium	3.27	1.49
Liver	17.2	16.6
Spleen	6.98	2.4

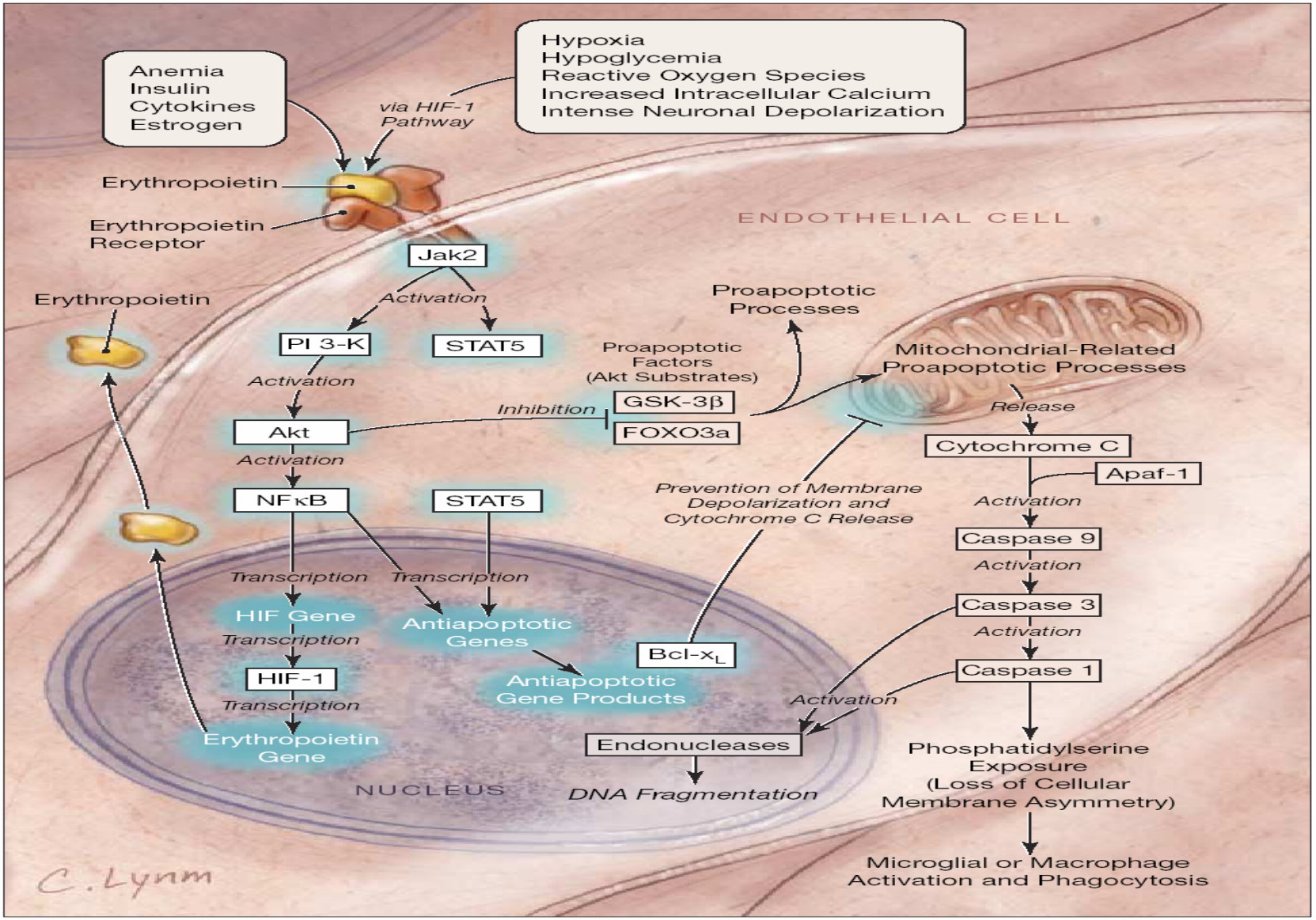
only < 5% of infused cell are

remained in infarcted myocardium

Kang et al, J Nuc Med 2006

Anti-apoptotic & Pro-angiogenic effects of Erythropoietin

Figure. Potential Mechanism of Erythropoietin Cytoprotection

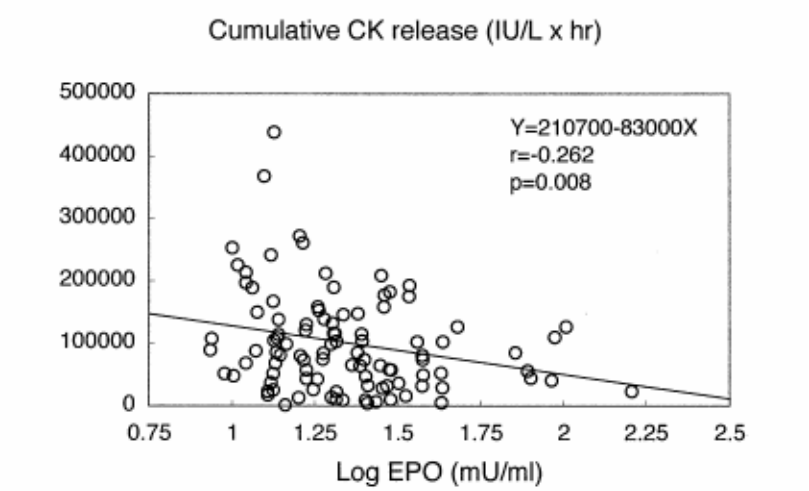


Maiese, et al. JAMA 2005

Clinical Significance of EPO in AMI

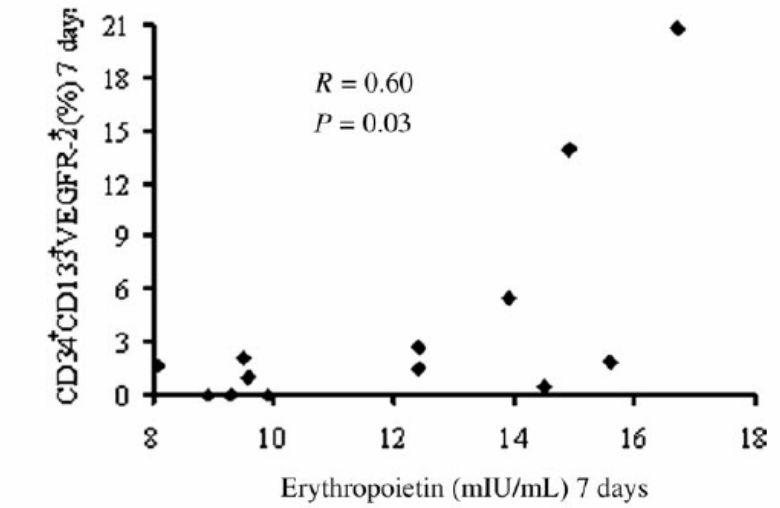
- High EPO level is associated with smaller infarct size in 101 AMI patients undergoing primary PCI

  Namiuchi et al, J Am Coll Cardiol. 2005



- EPO level is associated with number of PB-EPCs (CD34+ CD133+ VEFGR2+) in 50 AMI patient undergoing primary PCI

  Ferrario et al, Eur Heart J. 2007



Therapeutic Efficacy of EPO in AMI Patients

- 20 STEMI patients (Control: n=10 vs. EPO: n=10): pain onset<6h, feasible for primary PCI without anemia
- Single bolus (300 ug) of darbepoetin before PCI in EPO group
  - Increase in circulating EPCs at 72hours post injection
  - Trends to reduce infarct size at 4m,
  - LVEF of EPO: 52 vs. Control: 48%, p=NS

Lipsic et al, Cardiovasc Drugs Ther. 2006

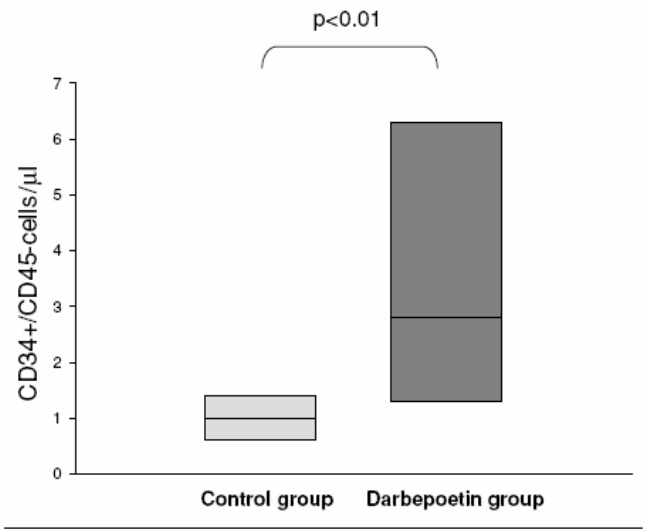


Fig. 2. Number of CD34 + /CD45 – cells, 72 h after MI. Box plot show the median with 25–75% range.

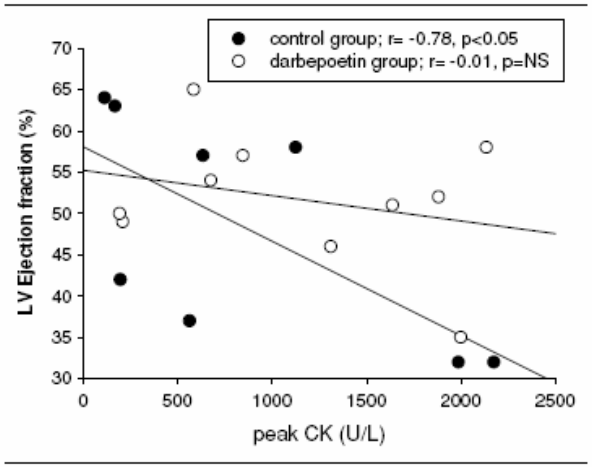


Fig. 3. Correlations between peak CK levels after MI and LV-ejection fraction measured 4-months later.

Safety Issues of EPO

- Conflicting data from previous reports
  - Chronic Epo treatment decreases bleeding time and increases in vitro measures of platelet reactivity

    Moia M, et al. Lancet 1987; van Geet C, et al. Thromb Haemost 1989; Cases A, et al. Kidney Int 1992
  - Epo treatment did not interfere antiplatelet effects of dual antiplatelet treatment in volunteers

    Yi-Da Tang, et al. Am Heart J 2007

- Short term EPO treatment in our study
  - No change in Hb and platelet count
  - No serious thrombotic complications

Acute cardioprotection with EPO in experiments

Table 1. Experimental Studies Showing Acute Cardioprotection With Erythropoietin (EPO)

	Animal Model	Dosage of EPO	Outcome	Reference(s)
Ex vivo I/R	Isolated rat heart, 30 min/2 h I/R	5,000 IU/kg 24 h before I/R	Increased functional recovery, apoptosis inhibition	(11,24)
	Isolated rabbit heart, 30 min/35 min I/R	0.5–10 IU/ml, 15 min before I/R	Increased recovery through activation of MAPK and potassium channels	(25)
	Isolated rat heart, 40 min/2 h I/R	10 IU/ml throughout the protocol	Improved recovery of LV pressure, coronary flow, reduction of cellular damage	(19)
	Isolated rat heart, 20 min/25 min I/R	10 IU/ml before I/R	Improved postischemic recovery of LVDP, preservation of ATP levels in ischemic myocardium	(18)
	Isolated rat heart, 20 min/40 min I/R	10 IU/ml at various time points during I/R	PI3K/Akt-dependent postischemic EPO cardioprotection	(26)
In vivo I/R	Rat, 30 min/7 days I/R	5,000 IU/kg for 7 consecutive days	Reduction in cardiomyocyte loss by ≈50%, normalization of hemodynamic function	(12)
	Rabbit, 30 min/3 days I/R	5,000 IU/kg at the time of reperfusion	Decreased infarct size, enhancement of LV function, mitigation of myocardial cells apoptosis	(14,31)
	Rat, 40 min/24 h I/R	5,000 IU/kg 30 min after ischemia	Reduced infarct size, dependent on MAPK, PI3K/Akt activation	(27)
	Rat, 40 min/24 h I/R	5,000 IU/kg at different time points during I/R	Reduction in infarct size and apoptosis even when EPO administered after the onset of reperfusion	(13)
	Dog, 90 min/6 h I/R	100–1,000 IU/kg just before reperfusion	Reduction in infarct size with low dose, via PI3K/Akt-dependent pathway	(32)
In vivo permanent occlusion	Rat, 60 min	5,000 IU/kg after coronary artery ligation	Reduction in apoptosis	(17)
	Rabbit, 3-day follow-up	5,000 IU/kg at the time of coronary artery ligation	Improvement in inotropic reserve, inhibition of apoptosis	(14)
	Rat, 8-week follow-up	3,000 IU/kg after coronary occlusion	Smaller infarct size, prevention of LV dilation, improved LV ejection fraction	(34)
	Rat, 9-weeks follow-up	40 μg/kg darbepoetin after coronary occlusion	Reduction in infarct size, improved LV function, increased capillary density	(42)

ATP = adenosine triphosphate; I/R = ischemia/reperfusion; IU = international units; LVDP = left ventricular diastolic pressure; LV = left ventricular; MAPK = mitogen-activated protein kinase; PI3K/Akt = phosphatidylinositol 3-kinase/Akt kinase.

Lipsic et al, JACC. 2006

Target patients of MAGIC-CELL-5-COMBICYTO

- Initial enrollment
  - AMI (< 4weeks from pain onset)
  - Successful Revascularization of culprit lesion
  - Age < 80YO
- Repeated PBSC infusion at 6months follow up
  - LVEF at 6m fu < 45%
  - Patients who were previously allocated to cell infusion group
  - No significant major epicardial coronary artery stenosis (can be included after additional PCI)

End points of MAGIC-CELL-5-COMBICYTO

- Primary end point
  - Change of LVEF (Cardiac MRI at 6m/12m/24m)
  - Safety measure including MACE