Stem Cell Therapy for STEMI: When PCI is Not Enough

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Acute MI

CHF and Cardiogenic Shock-Declining but Still Important

- Of 300,000 STEMI patients* surviving to present to hospital annually in US

	Percent	30 day Mortality
Killip II	8-10	15
Killip III	6-8	30
Killip IV	4-5	50

~ 60,000 patients at high risk/yr

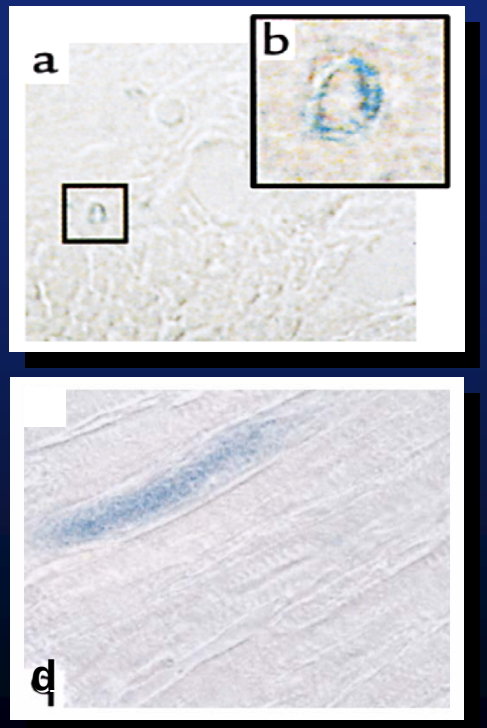
GISSI I, Lancet 2:397 '86
ASSENT 2, Lancet 354:716 '99
AHA Heart Disease and Stroke Statistics, 2005
GUSTO 2B, NEJM 336:1621 '97
SHOCK Reg, JACC 36:1063 '00

* 1,000,000 15 years ago

Cardiac Regeneration

Homing After MI

- Stem cell population (CD 117+ CD 34-) from Rosa 26 mice injected IV into radiated-induced marrow-ablated mice
- LAD occlusion 10 wks later
- Post mortem 3 wks later
- 3% endothelial cell and 0.02% myocytes LacZ ⊕



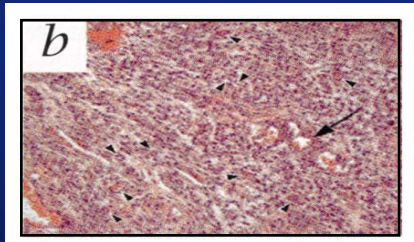
Jackson and Goodell, JCI '01

Stem Cell Therapy and Myogenesis

Homing / BM Cells Injected IV Decrease Infarct Size

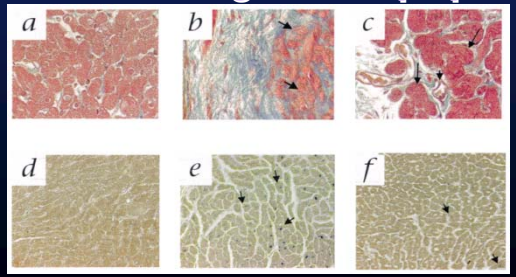
- Athymic nude rat infarct model
- GCSF mobilized human CD34+ cells
- Injection into tail vein 2x 10^6 cells 48 hrs post MI
- Sacrifice at 2 wks

Neoangiogenesis



H and E

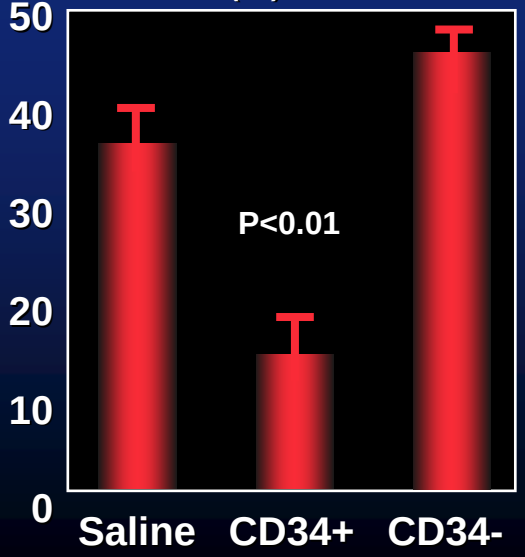
Protection Against Apoptosis



Antidesmin and TUNEL assay

Smaller Infants

Scar Tissue (%) in LV wall



CD45-hematopoietic lineage
CD117-undifferentiated BMSC marker
CD14-monocyte/macrophage marker

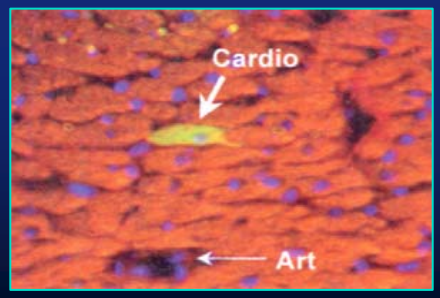
Kocher, Nat Med 7:429,2000

Cellular Cardiac Repair

Doubts about Meaningful Transdifferentiation

Murray, Nature 428: 664 '04

Lin$^-$c-kit$^+$ X-gal or GFP ⊕ cells injected into peri-infarct zone or via bone marrow tx 5 hrs after LAD ligation in a mouse model

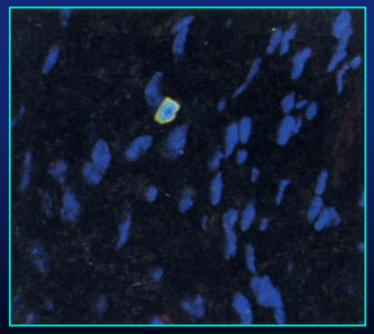


Bone marrow transplant (2-4 cells/heart)

No labeled cardio-myocytes seen after myocardial injection (7-36d)

Balsam, Nature 428: 668 '04

Lin$^-$c-kit$^+$ or Lin$^-$c-kit$^+$ Thy 1.1^{lo} Sca-1$^+$ GFP labeled cells injected into border zone or IV (parabiotic vasculature) 3-5 hrs post MI



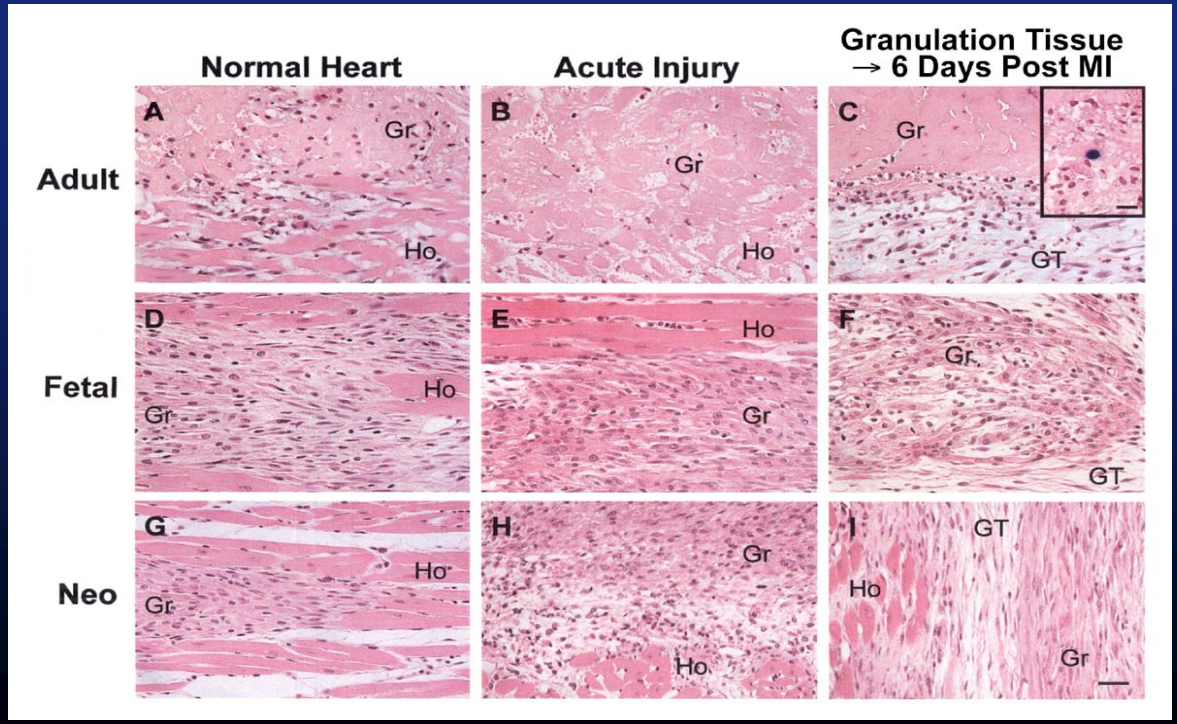
GFP/B220 merge

No GFP ⊕ cardio-myocytes, but GFP CD45, B220 and Gr-1 ⊕ (hema-topoietic) cells

No ↓ MI size or ↑ survival

Cellular Cardiac Repair

Cell Death After Transplantation



- Syngeneic Fischer 344 rats
- 0.5-4x10^6 cells injected into center of cryoinfarct
- Adult cells do not survive
- Only 25% of animals with neonatal cells have host-transplant connection by 8 weeks vs 60% early

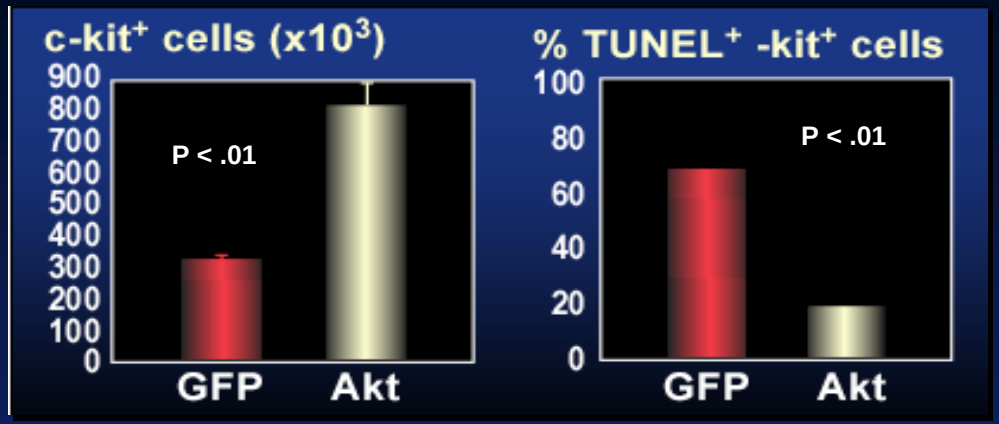
Gr=Grafted cell

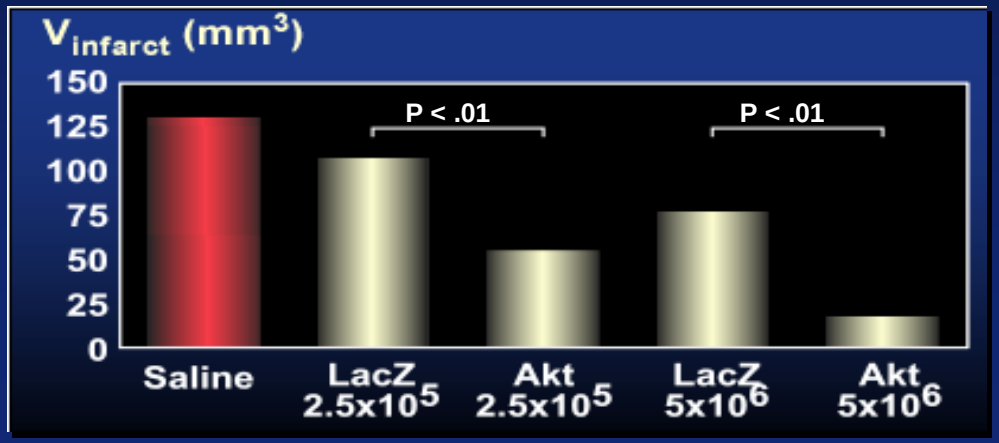
Reinecke Circ. 100:193 '99

Cellular Cardiac Repair

Enhancing Stem Cell Survival

- Rat infarct model (LAO ligation)
- MSC retrovirus-transfected (pMSCV) with either Akt or GFP/LacZ
- MSC injected 1 hour later into border zone

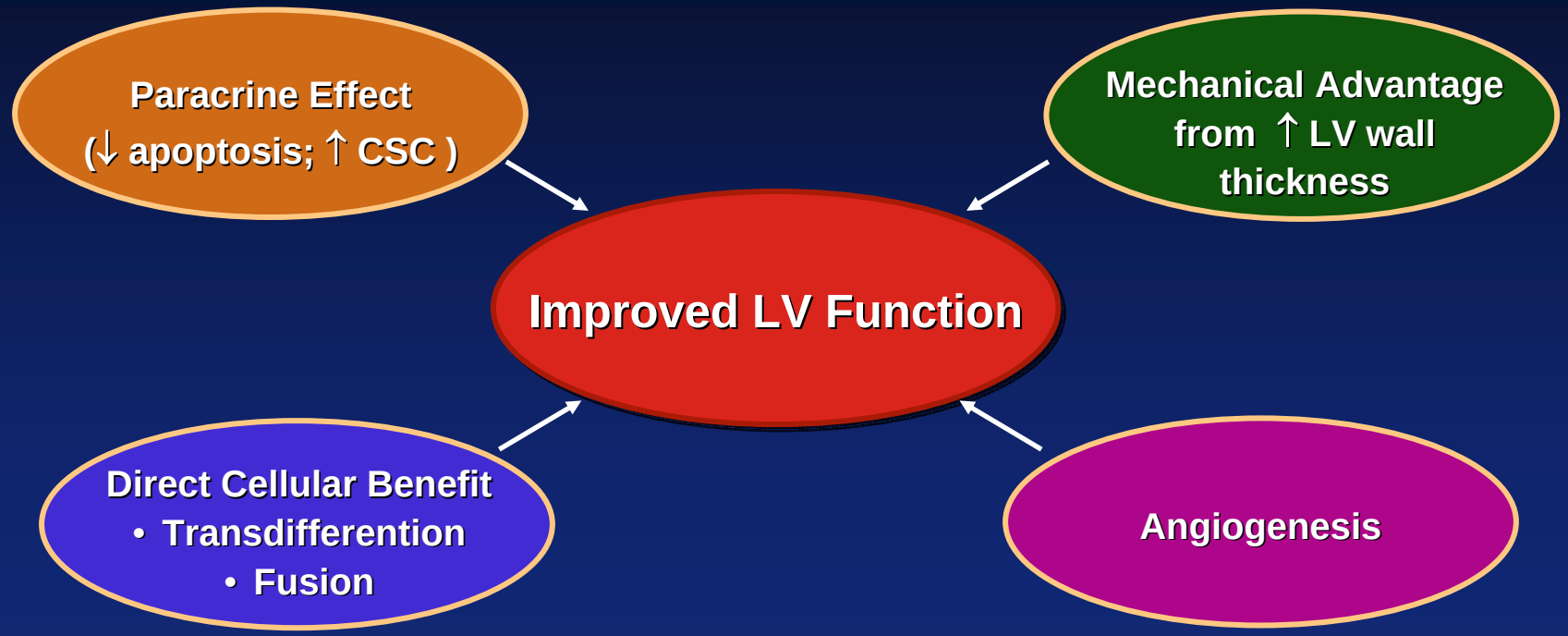




Mangi and Dzau, Nat Med 9:1195, 2003

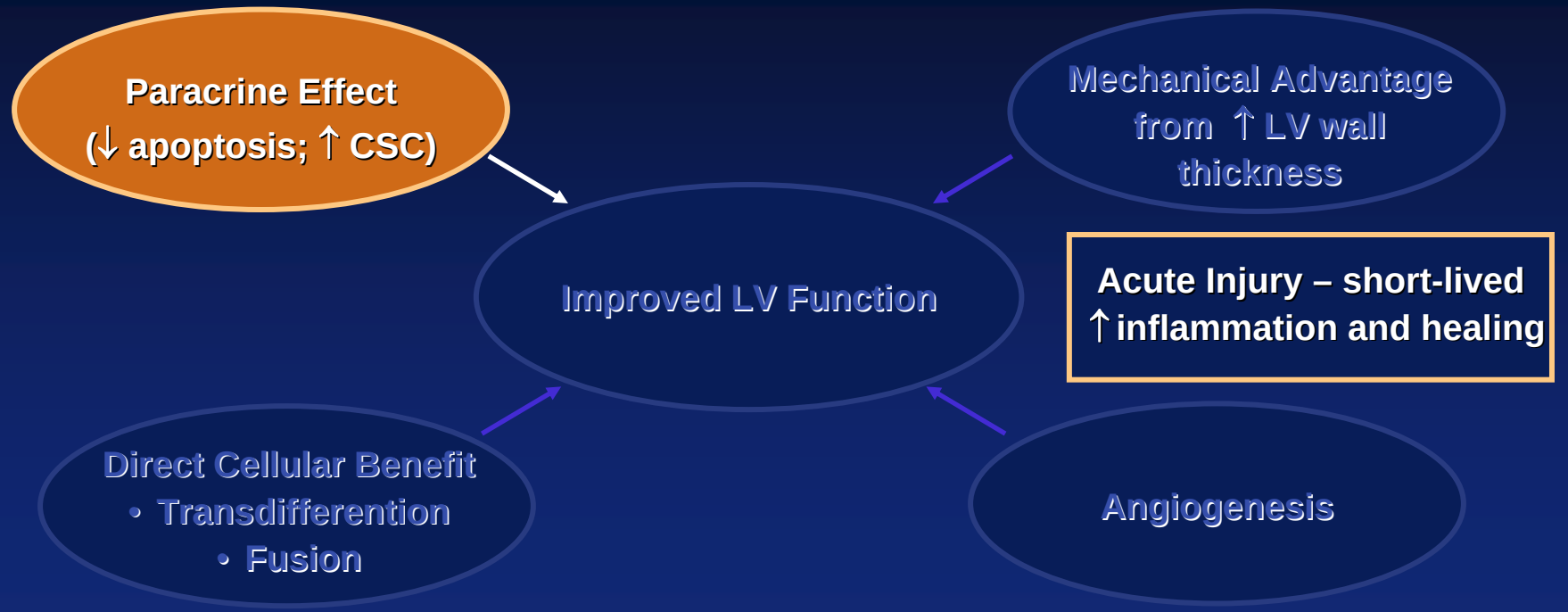
Cellular Cardiac Repair

Possible Mechanisms of Action



Cellular Cardiac Repair

Possible Mechanisms of Action



Stem Cell Rx for AMI or CHF

Delivery Options

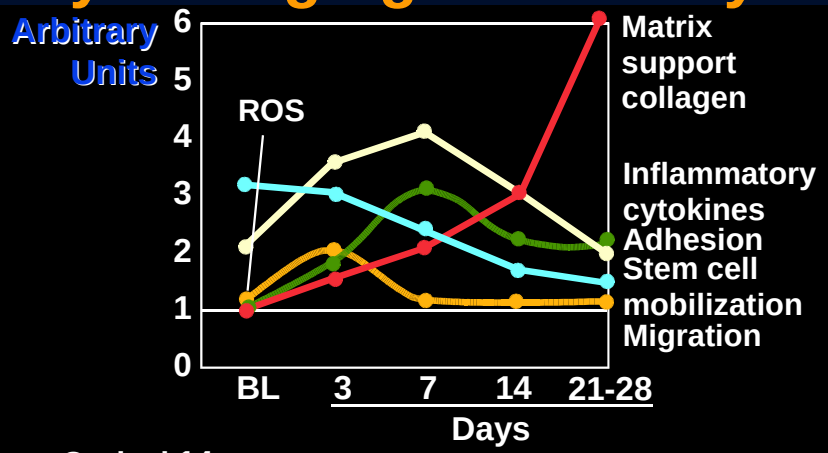
- Intracoronary
- Intravenous/homing dependent
- Transvenous (coronary sinus)
- Direct intramyocardial injection: OHS or catheter based

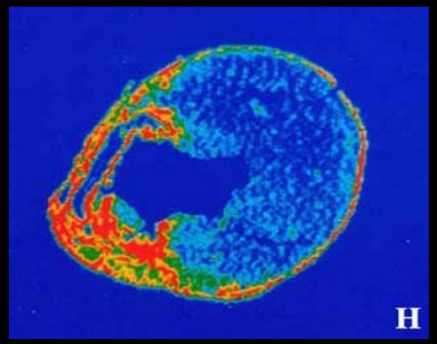
Improvement in LVEF with Primary PCI

	Lytics	PCI	No Pts.
PAMI (NEJM ’93)	56	56	395
Zwolle (JACC ’94)	44	50	301
Mayo Clinic (NEJM ’93)	50	53	108
Weighted Ave	50.7	53.3	

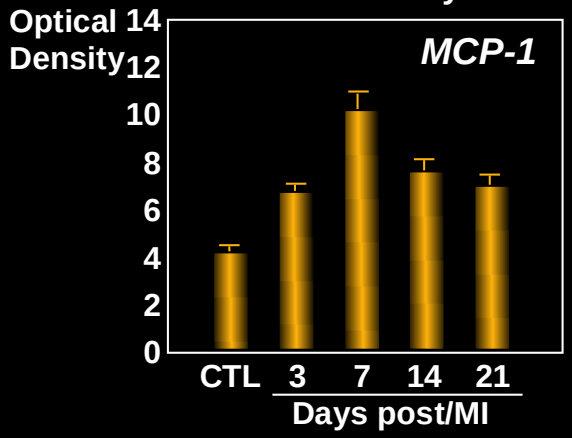
Post-Infarction Inflammatory Milieu

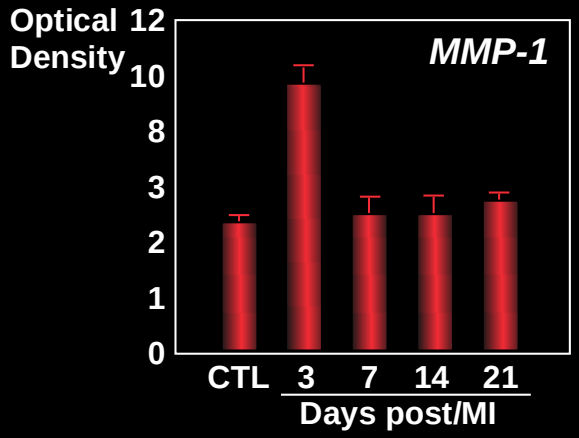
Rapidly Changing/Potentially Hazardous to Introduced Cells





*permanent LAD ligation in rat, imaging at 7 days

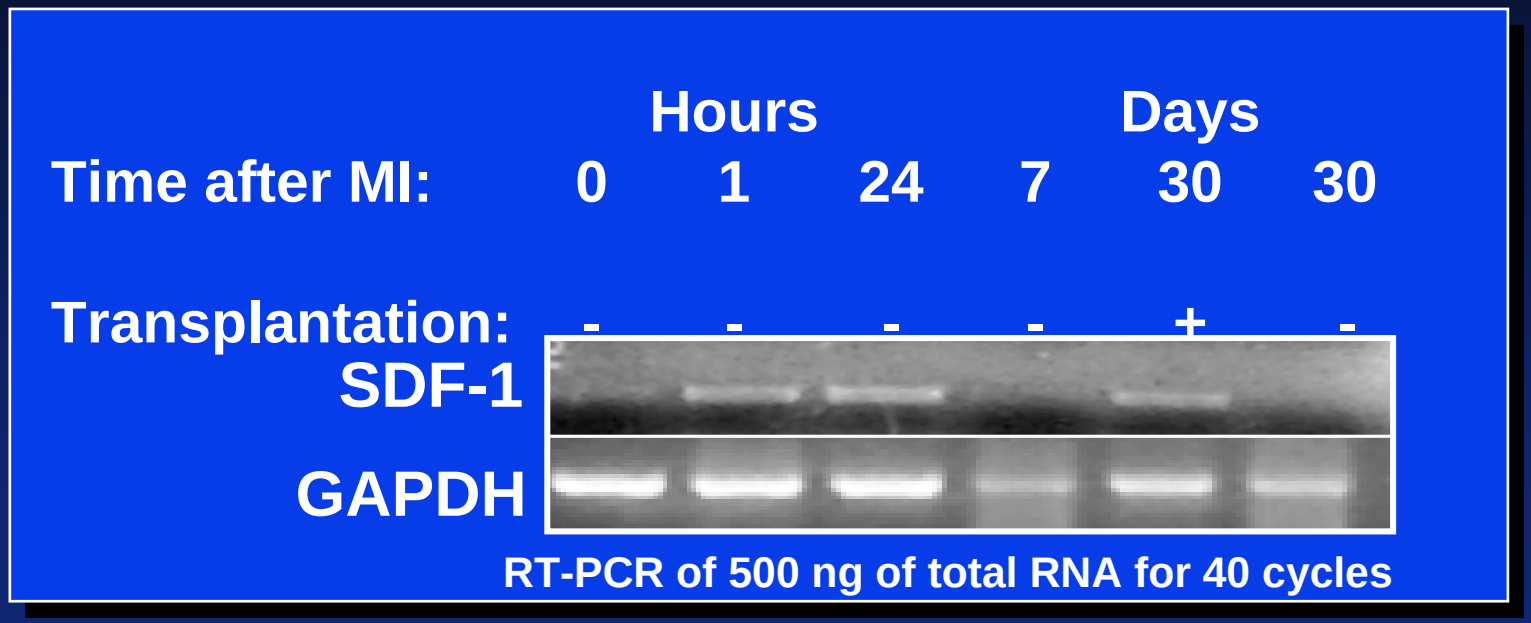




Bartunek, Nat CP CVM 3:S52, '06
Lu, Biochem Biophy Rev Com 320:907.'04
Niam, Circ Res 94:1543,'04

SDF-1 Mediates Stem Cell Homing following MI

Limited Temporal Upregulation After Infarction

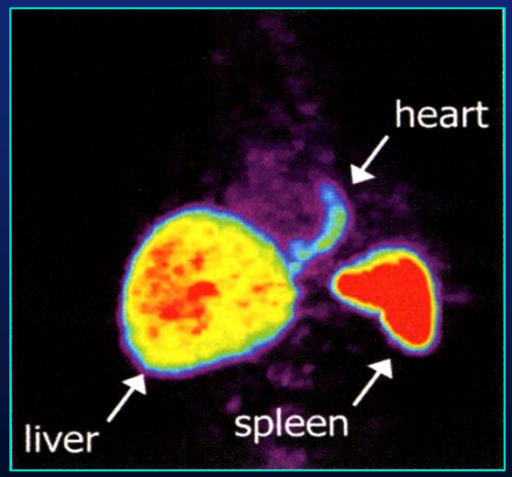


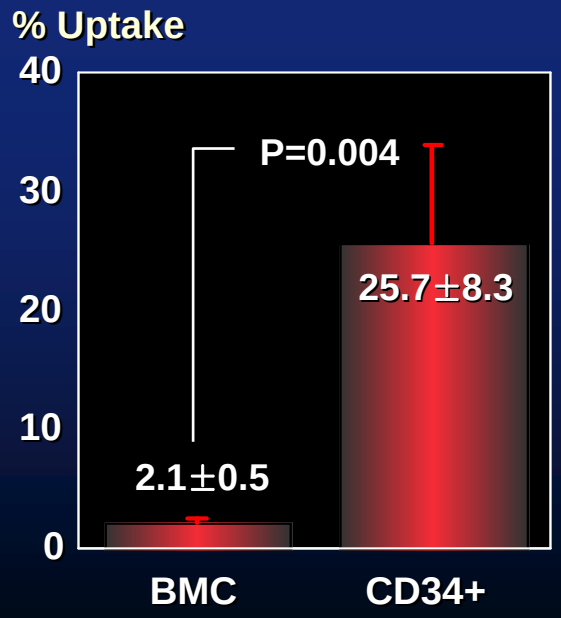
Askari et al. Lancet. 362:697, 2003

Cell Retention After IC Injection for Acute MI

Limited Retention

- BMC or CD34+ enriched (CliniMACSplus/CD34+ Ab from Miltenyi Biotech) cells 18F-FDG labeled)
- Cell transfer 5-10 days after PPCI (n=3pts/grp)
- 3D PET scanning 50-75 min after IC cell transfer

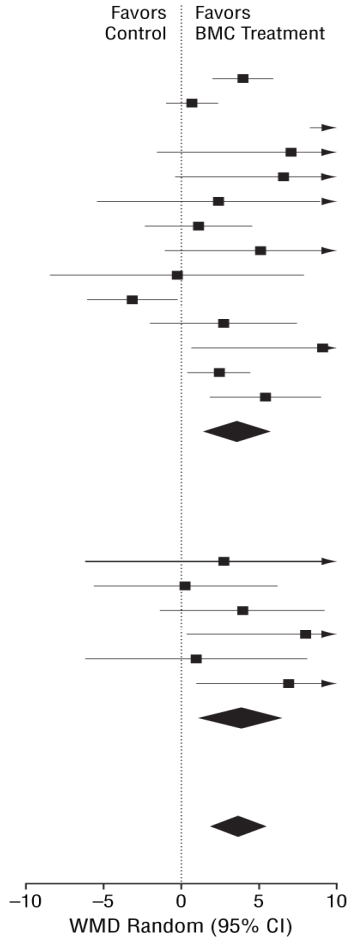




Hofmann and Drexler, Circ 111:2198 '05

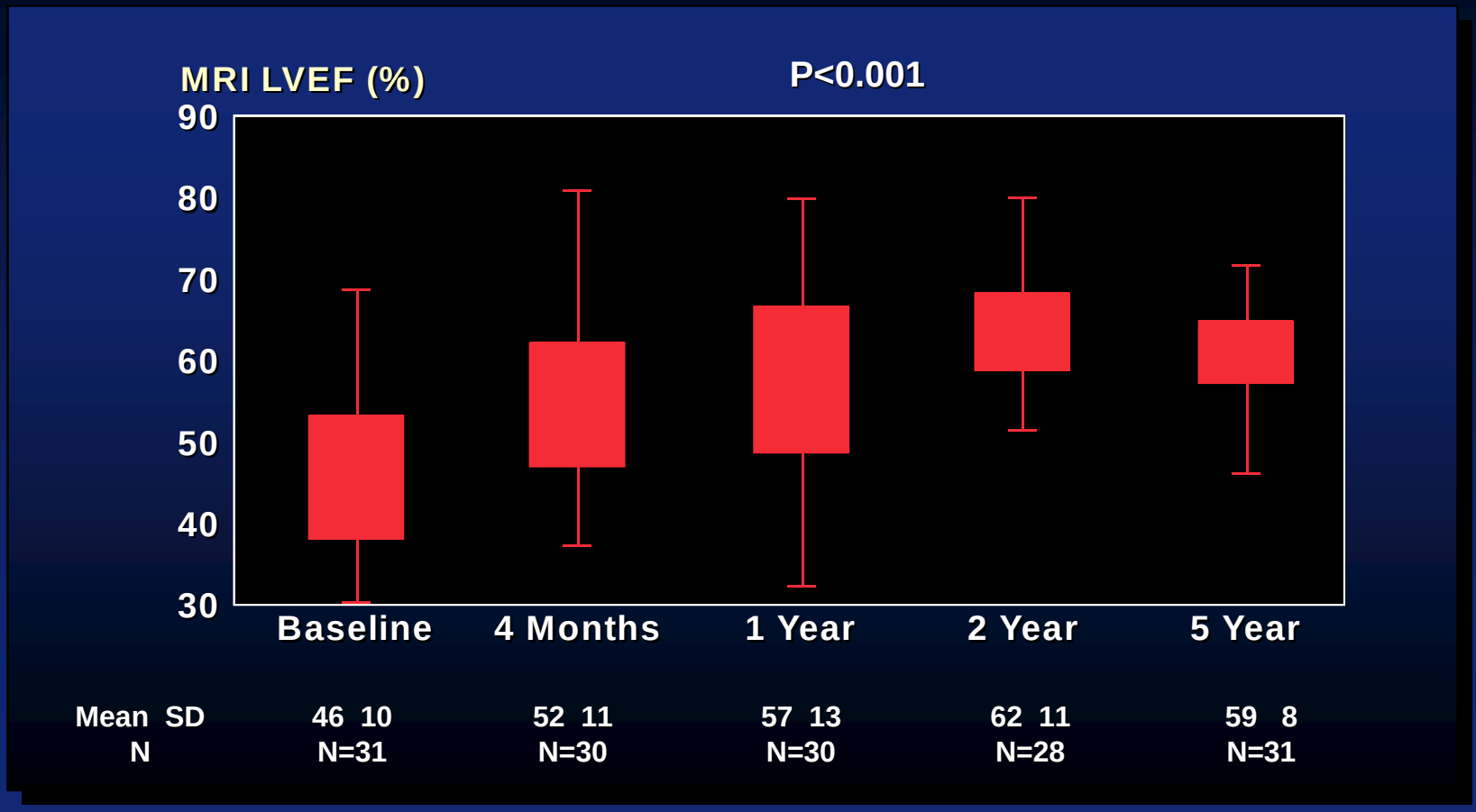
Stem Cells for AMI-Metaanalysis

Study or Subcategory	N	Treatment, Mean (SD), %	N	Control Mean (SD), %	Weight, %	WMD (Random), % (95% CI)
RCTs						
Assmus et al,[14] 2006 (BMCs)	28	2.90 (3.60)	18	−1.20 (3.00)	8.09	4.10 (2.18 to 6.02)
Assmus et al,[14] 2006 (CPCs)	26	−0.40 (2.20)	18	−1.20 (3.00)	8.33	0.80 (−0.82 to 2.42)
Chen et al,[16] 2004	34	18.00 (6.71)	35	6.00 (7.91)	6.62	12.00 (8.54 to 15.46)
Erbs et al,[17] 2005	11	7.20 (11.47)	11	0.00 (8.97)	2.80	7.20 (−1.40 to 15.80)
Ge et al,[18] 2006	10	4.80 (9.56)	10	−1.90 (5.85)	3.68	6.70 (−0.25 to 13.65)
Hendrikx et al,[19] 2006	10	6.10 (8.60)	10	3.60 (9.10)	3.21	2.50 (−5.26 to 10.26)
Janssens et al,[20] 2006	33	3.40 (6.90)	34	2.20 (7.30)	6.68	1.20 (−2.20 to 4.60)
Kang et al,[21] 2006 (AMI)	25	5.10 (9.32)	25	−0.10 (12.43)	4.26	5.20 (−0.89 to 11.29)
Kang et al,[21] 2006 (OMI)	16	0.00 (12.80)	16	0.20 (10.61)	3.01	−0.20 (−8.35 to 7.95)
Lunde et al,[23] 2006	50	1.20 (7.50)	50	4.30 (7.10)	7.21	−3.10 (−5.96 to −0.24)
Meyer et al,[24] 2006	30	5.90 (8.90)	30	3.10 (9.60)	5.43	2.80 (−1.88 to 7.48)
Ruan et al,[27] 2005	9	5.96 (11.10)	11	−3.21 (7.18)	2.89	9.17 (0.77 to 17.57)
Schächinger et al,[28] 2006	95	5.50 (7.30)	92	3.00 (6.50)	8.04	2.50 (0.52 to 4.48)
Li et al,[31] 2006	35	7.10 (8.00)	35	1.60 (7.00)	6.55	5.50 (1.98 to 9.02)
Subtotal	412		395		76.79	3.64 (1.56 to 5.73)
Test for Heterogeneity: $\chi^2_{13}=59.81$ ($P<.001$), $I^2=78.3\%$						
Test for Overall Effect: $Z=3.42$ ($P<.001$)						
Cohort Studies						
Bartunek et al,[15] 2005	19	7.10 (13.26)	16	4.30 (13.44)	2.68	2.80 (−6.08 to 11.68)
Katritsis et al,[22] 2005	11	1.95 (7.19)	11	1.62 (6.93)	4.40	0.33 (−5.57 to 6.23)
Mocini et al,[25] 2006	18	5.00 (7.65)	18	1.00 (8.51)	4.90	4.00 (−1.29 to 9.29)
Perin et al,[26] 2004	11	5.10 (6.47)	9	−3.00 (10.12)	3.28	8.10 (0.46 to 15.74)
Strauer et al,[29] 2002	10	5.00 (9.06)	10	4.00 (7.00)	3.59	1.00 (−6.10 to 8.10)
Strauer et al,[30] 2005	18	8.00 (8.06)	18	1.00 (10.00)	4.38	7.00 (1.07 to 12.93)
Subtotal	87		82		23.21	3.83 (1.18 to 6.48)
Test for Heterogeneity: $\chi^2_5=4.32$ ($P=.51$), $I^2=0\%$						
Test for Overall Effect: $Z=2.83$ ($P=.005$)						
Total	**499**		**477**		100	3.66 (1.93 to 5.40)
Test for Heterogeneity: $\chi^2_{19}=64.73$ ($P<.001$), $I^2=70.6\%$						
Test for Overall Effect: $Z=4.14$ ($P<.001$)						



Abdel-Latif, Arch Intern Med. 2007;167:989-997

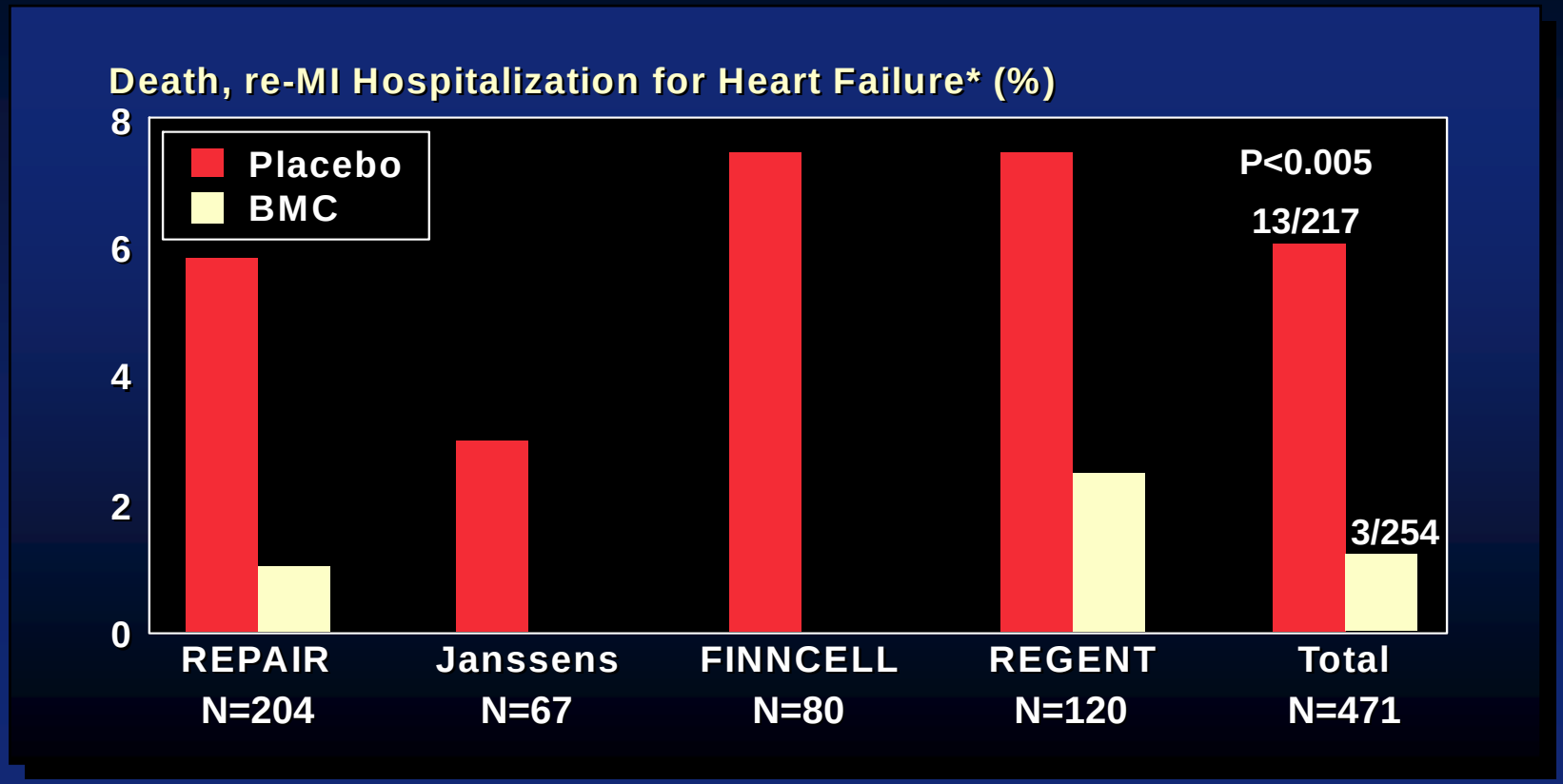
Improvement of MRI - Determined LVEF is Sustained 5 Years After Progenitor Cell Therapy



TOP-CARE AMI, unpublished data

SGE; 1009-1, 6

Death, Re-MI, Heart Failure at 4-6 Months in Randomized, (Placebo)-Controlled BMC Trials



*after hospital discharge

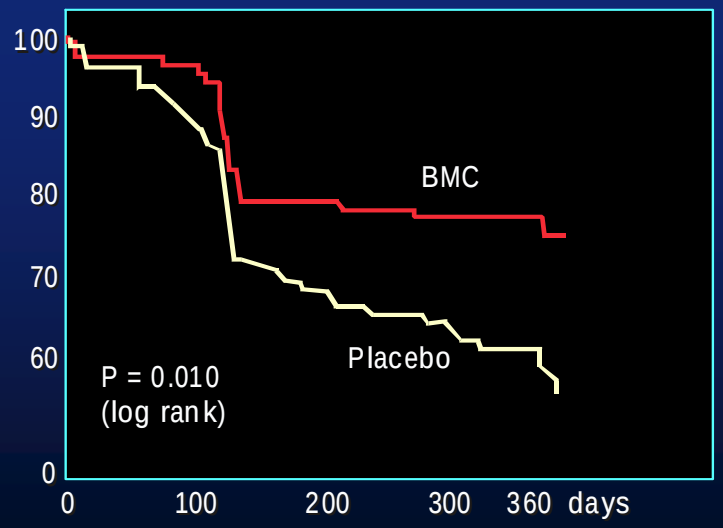
SGE; 1009-1, 4

Stem Cell Therapy

Repair-AMI

Event-free survival (%)

(death, myocardial infarction, revascularization)



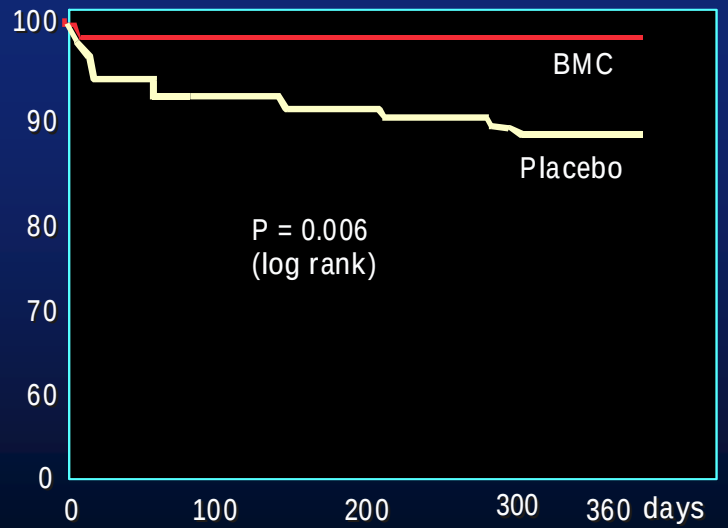
Number exposed to risk

Placebo	103	91	68	63	55
BMC	101	97	80	77	66

Figure 1
Study flow diagram

Event-free survival (%)

(death, myocardial infarction, rehospitalization for heart failure)



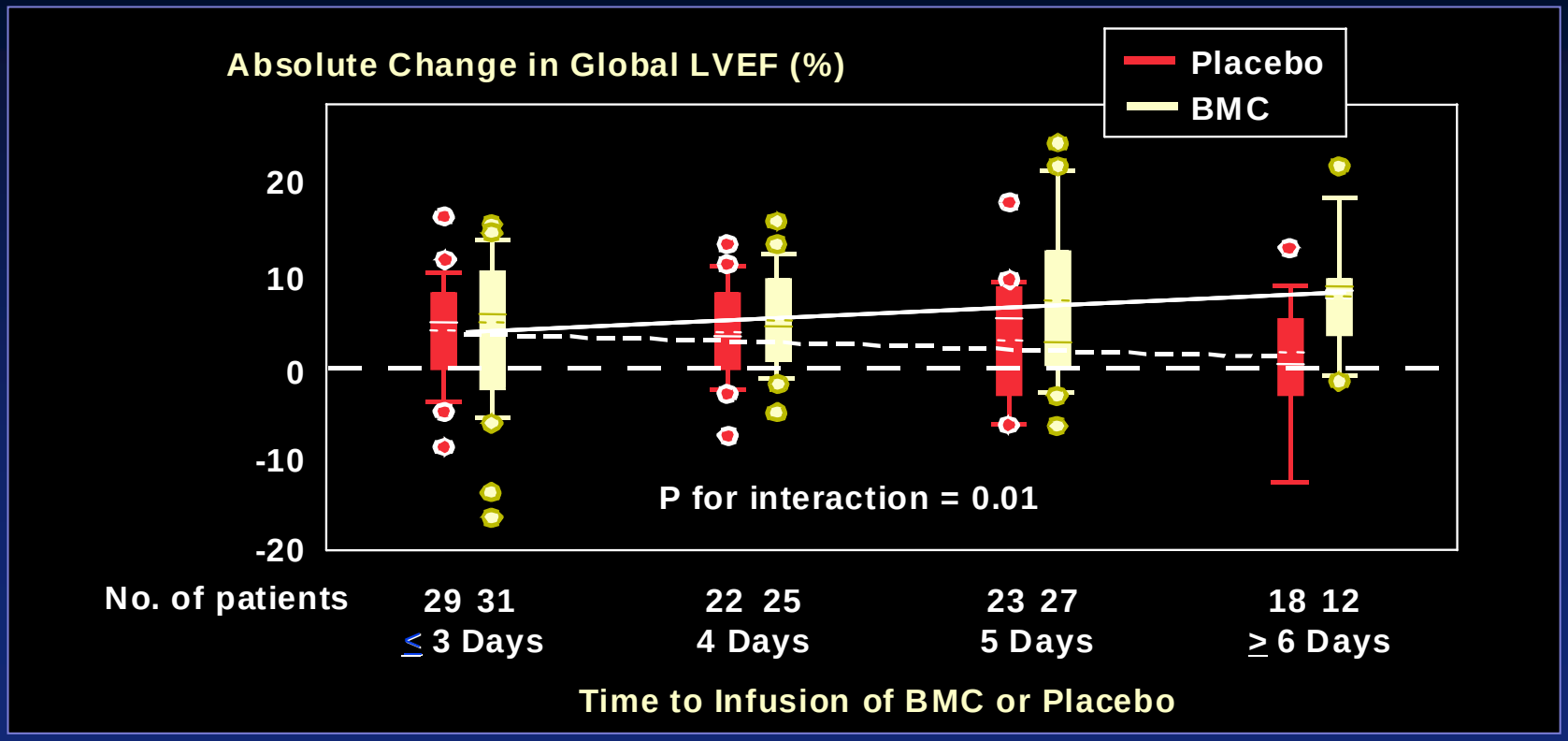
Placebo	103	93	89	85	79
BMC	101	99	99	98	85

V. Schachinger et al., European Heart Journal (2006) 27, 2775–2783

SGE; 1008-1, 03

Cellular Cardiac Repair

IC Progenitor Cells for Acute MI-Impact of Infusion Timing



REPAIR-AMI, NEJM 335:1210, '06

Cellular Cardiac Repair

TIME Trial

Patients:

Anterior MI <48 hrs with EF<45% and no prior MI or CABG

Randomized Treatments:

IC BMMNC (150 million cells) vs placebo (2:1), and
Treatment at 3 vs 7 days (1:1)
(factorial design, n=120)

Primary and Key Secondary Endpoints:

1) Change in global and infarct area function by MRI, baseline to 6 months

NHLBI CV Cell Therapy Network: Cleveland Clinic, Minneapolis Heart Institute, Texas Heart Institute, U. Florida, Vanderbilt

Cellular Cardiac Repair

LATE TIME Trial

Patients:

Anterior MI <48 hrs with EF<45% and no prior MI or CABG

Randomized Treatments:

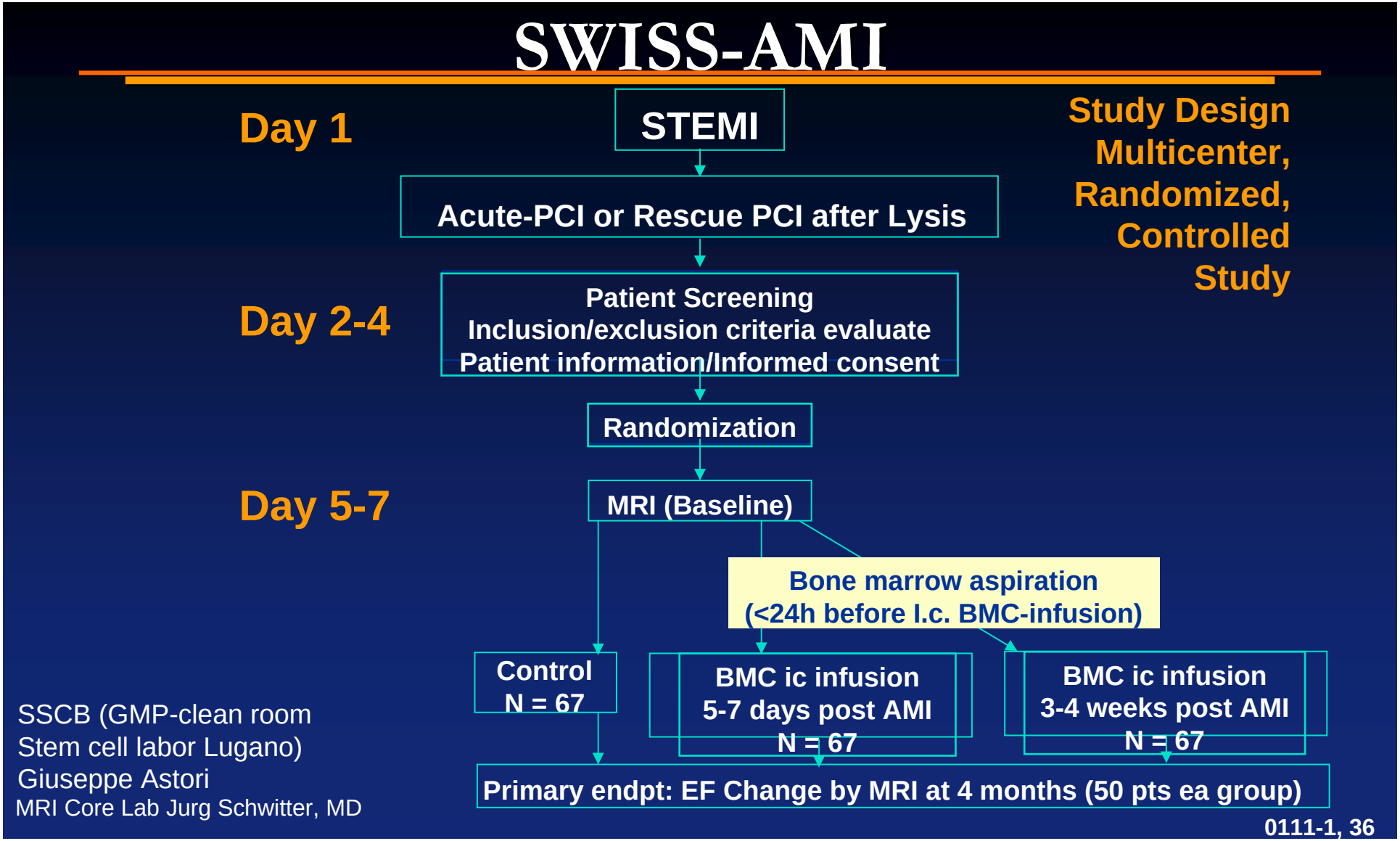
IC BMMNC (150 million cells) vs placebo (2:1)
(n=87)

Primary and Key Secondary Endpoints:

1) Change in global and infarct area function by MRI, baseline to 6 months

NHLBI CV Cell Therapy Network: Cleveland Clinic, Minneapolis Heart Institute, Texas Heart Institute, U. Florida, Vanderbilt

SWISS-AMI

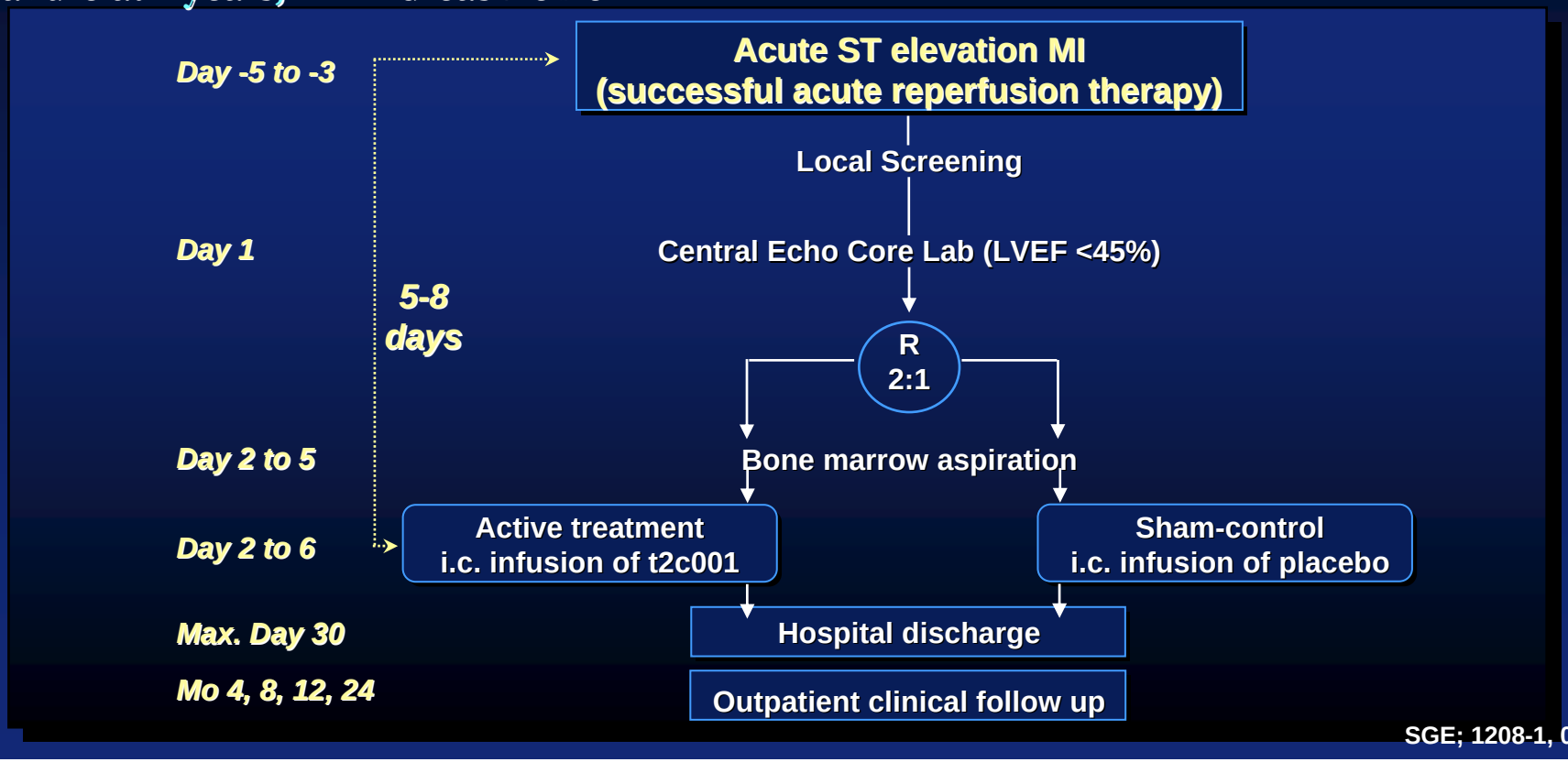


Study Design
Multicenter, Randomized, Controlled Study

SSCB (GMP-clean room Stem cell labor Lugano)
Giuseppe Astori
MRI Core Lab Jurg Schwitter, MD

Phase III REPAIR-AMI2-EU (n=1450)

Double-blind, placebo (sham)-controlled, randomized (2:1), multicenter trial; central cell processing facility; Primary endpoint: composite death, reMI, or hospitalization for heart failure at 2 years; PI Andreas Zeiher



SGE; 1208-1, 09

Stem Cells – Ideal Characteristics

- Large numbers easily accessible
- Autologous or immune privileged
- Pleuripotent
- Relevant cytokine producing
- Ischemic resistant

Cell Source Options

Autologous

Enriched

Resident Cardiac Progenitor
Skeletal Satellite
Selected MMNC (CD34, CD133, MAPCs, MIAMIs)
Peripheral EPCs
Adipose-derived EPCs

Non-Enriched

Unselected BMMNC

Allogenic

Adult

Mesenchymal

Embryonic

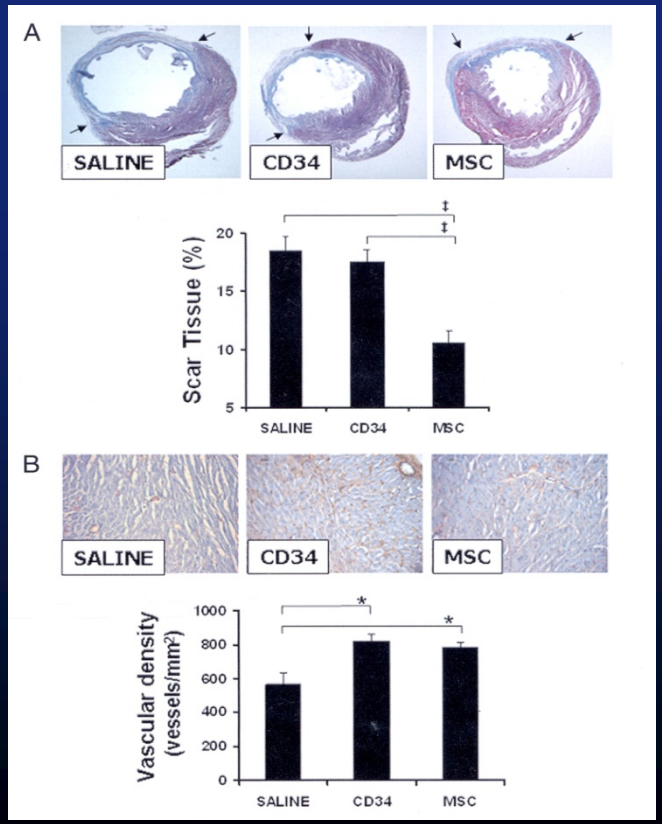
Embry SC
Cord-derived
Amniotic fluid-derived

- Even fibroblasts improve passive mechanics
- For abbreviations, see Science 315:760, 2007

Cardiac Stem Cell Therapy

Which Cells are Best?

Athymic nude rat
CAD occlusion
model randomized
at 7 days to MSC
(1.2 x 10^6 cells)
$CO34^+$ ($6x10^5$ cells)
intracardial
injection



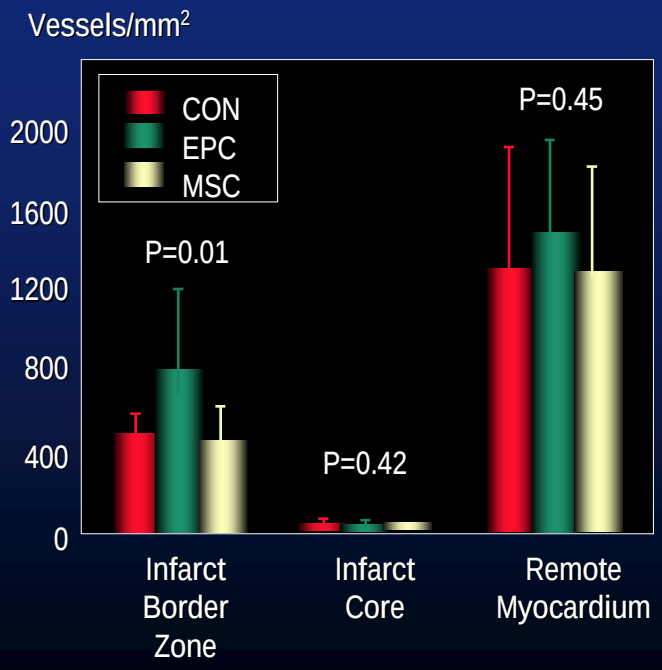
Armiñan, JACC 2010;55:2244

Cardiac Stem Cell Therapy

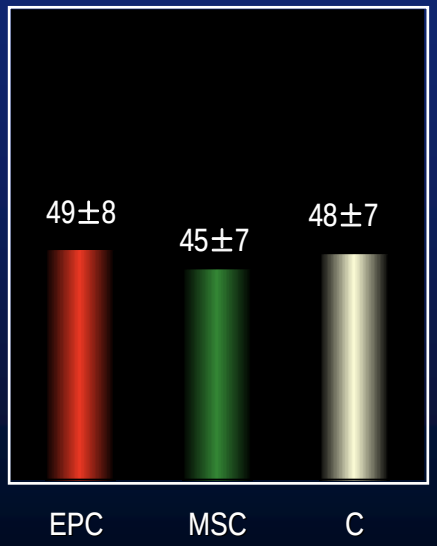
Which Cells are Best?

Porcine LCX occl model
Randomized at 7 days
To: EPC (3 x 10^7 cells),
MSC (2 x 10^6 cells) or
control IC injection

Vascular Density



LVEF at 7 weeks p=ns

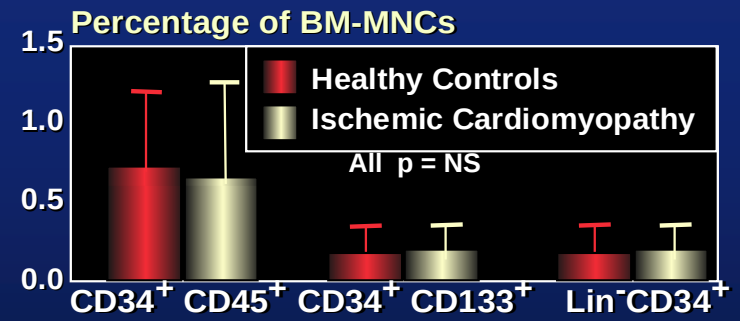


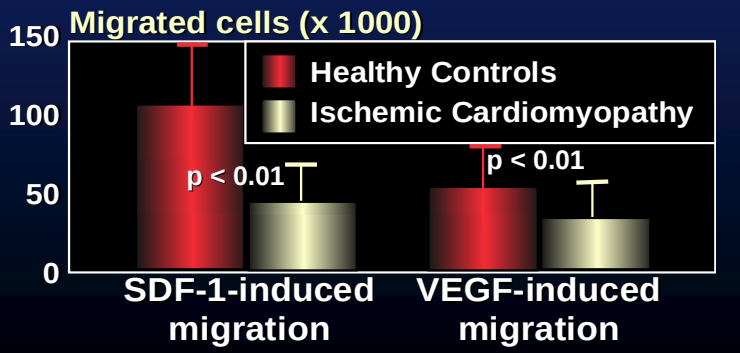
Dubois, JACC 2010;55:2232

Cellular Cardiac Repair

Stem Cell Numbers and Function are Diminished in Target Populations

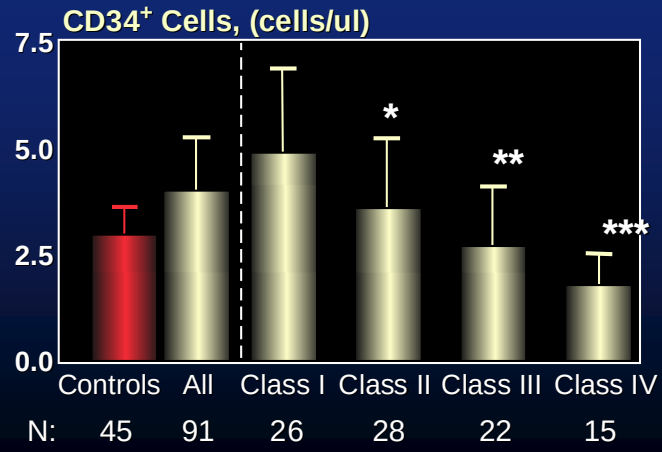
Ischemic Heart Disease





Heeschen, Circ 109:1615,'04

CHF



*p < 0.05 vs. class I
**p<o.05 vs. class II
***p<0.005 vs. class II

Valgimigli, Circ 110:1209,'04

REGENT

Study Design and Patient Characteristics

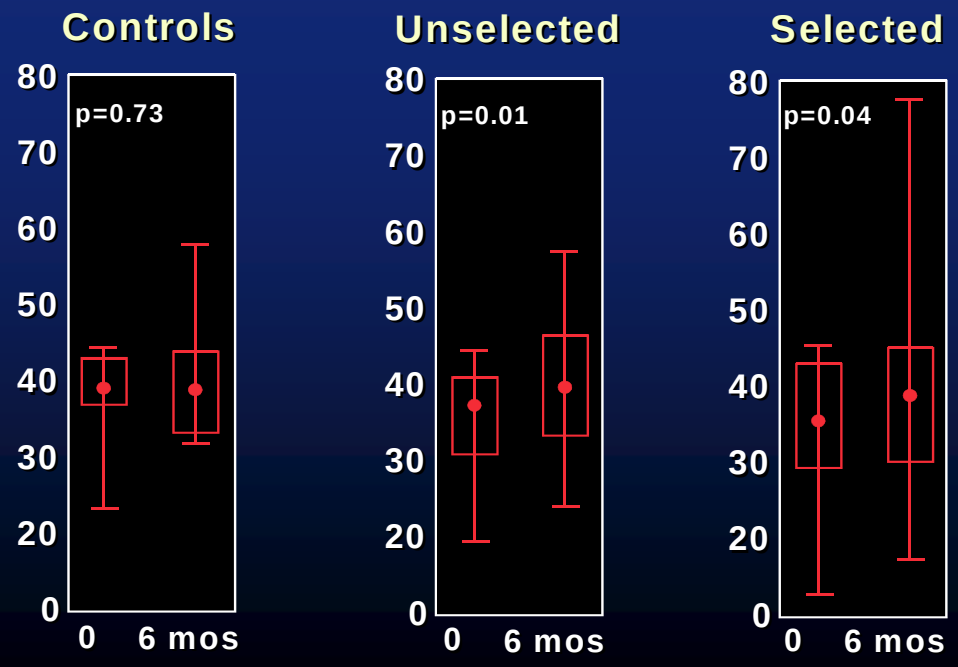
200 consenting STEMI pts age 18-75
Reperfused with PCI < 12 hr from onset
LVEF < 40%
BMMNC obtained using Ficoll gradient from 50-70 ml marrow cells ($1.8x10^8$) cells)
$CD34^+$ $CXCR4^+$ cells selected using immunomagnetic separation (MidiMACS, milteny:Biotec GmloH)
After Ficoll, from 100-120 ml marrow cells
Randomized to selected, non-selected cells or placebo given ic 3-12 days post MI
Primary endpoint: Δ EF baseline→6 months

Age	57±12 yrs
Diabetes	21%
LAD infarct	100%
Hrs to reperfusion	5 ± 12 hrs

Tendera, EHJ 30:1313 '09

REGENT

LVEF (MRI)

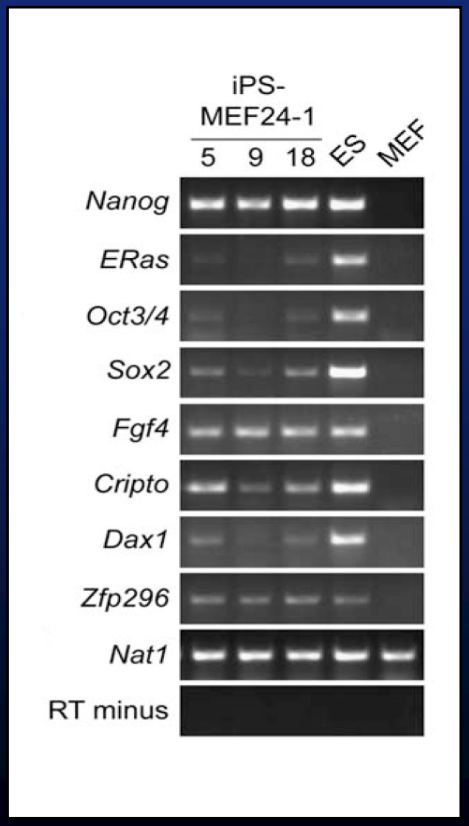


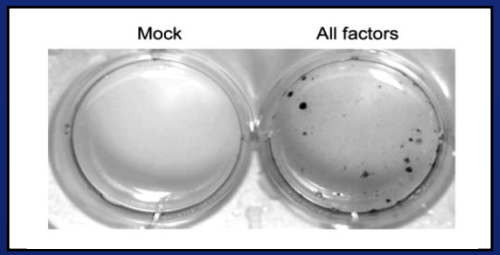
Tendera, EHJ 30:1313 '09

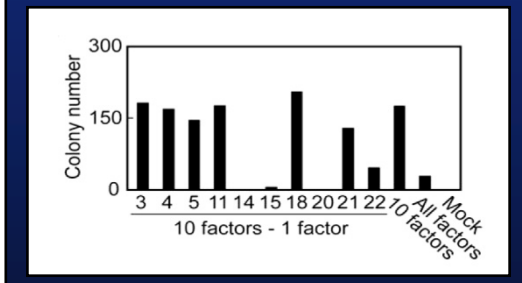
SGE; 1009-1, 12

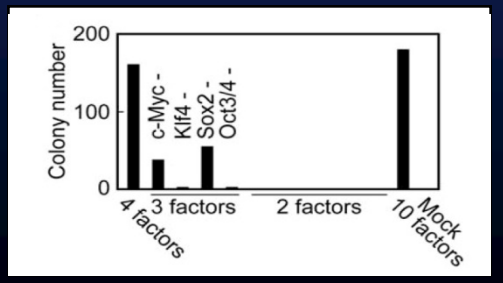
Induction of Pluripotent Stem Cells (iPSC) from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

- 24 candidate genes transfected alone or in groups to mouse embryonic fibroblasts
- Pluripotent state detected by resistance to G418
- Sequential withdrawal of identified genes
- Oct 3/4, Sox 2, c Myc, Klf4 together required.



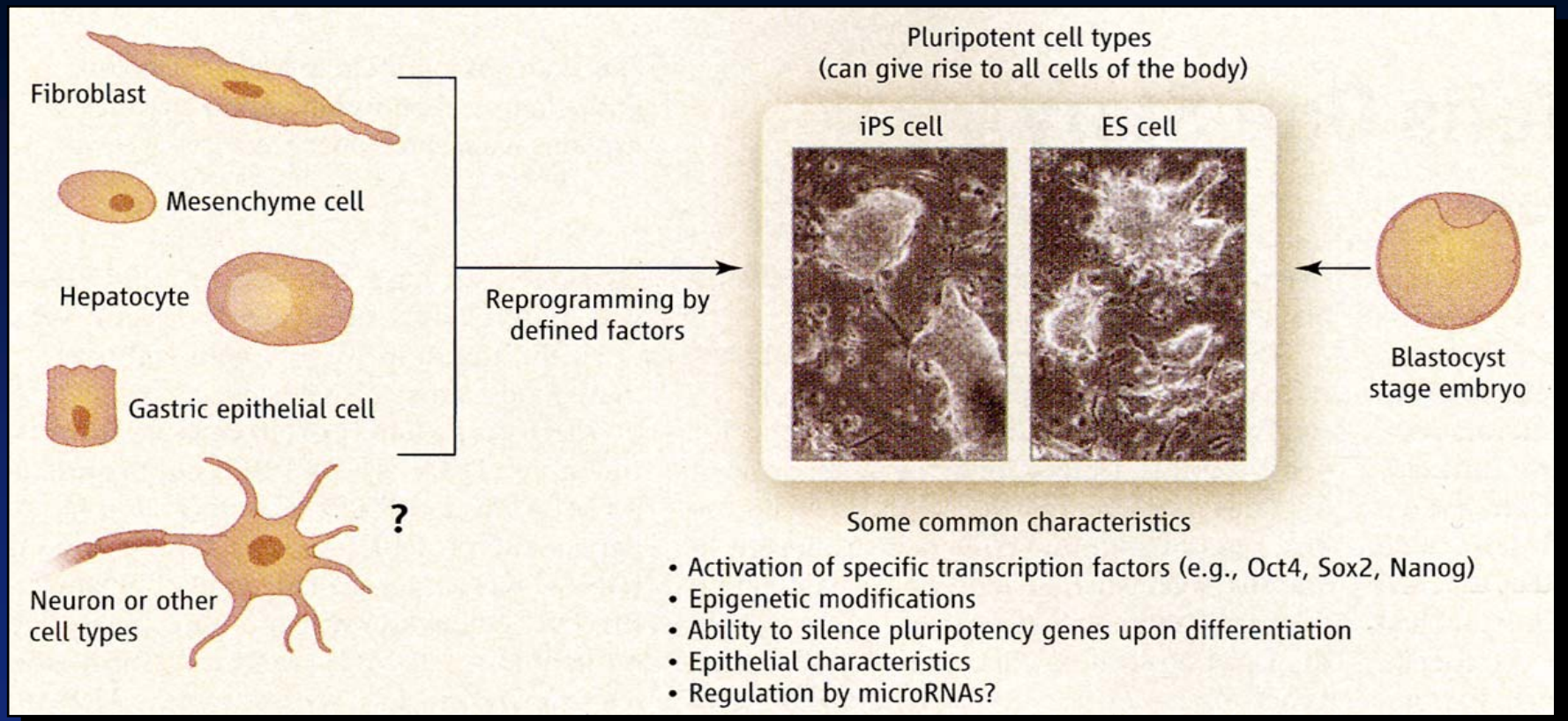






Takahashi et al. Cell 126;663 '06

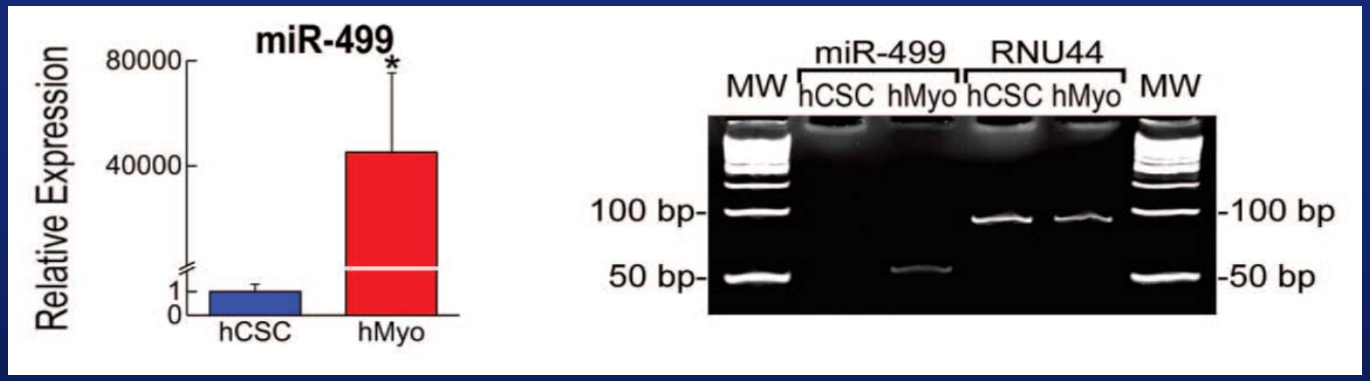
Induced Pluripotent Stem Cells

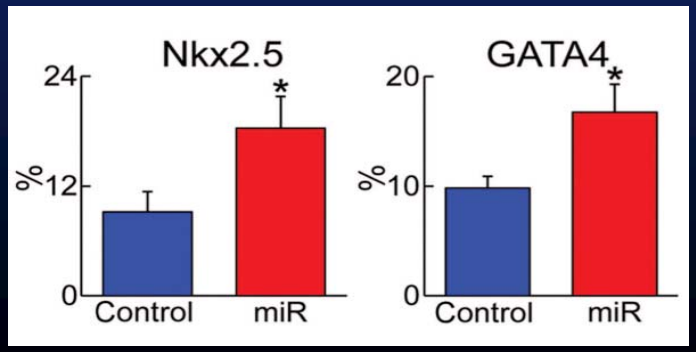


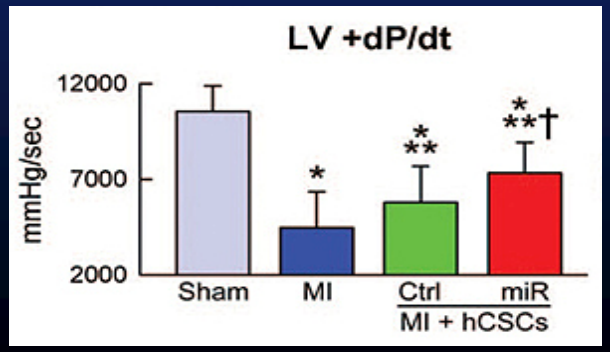
Bang, Science 2008

Stem Cell Therapy

miR – 499 Drives CSC Differentiation







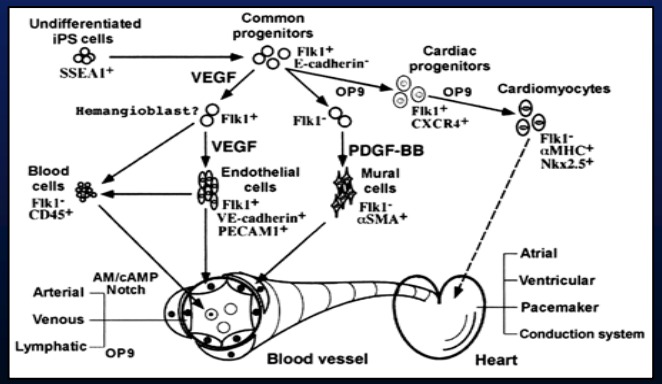
Hosoda and Anversa, Circulation 2011;123:1287-96

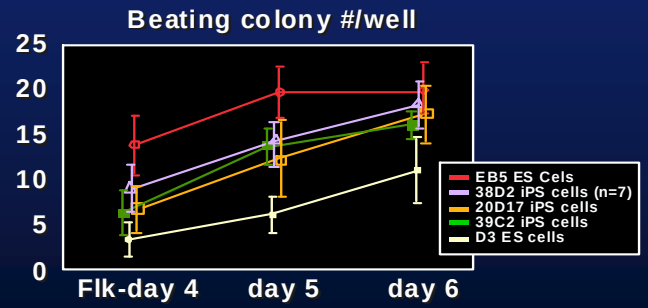
Cellular Cardiac Repair

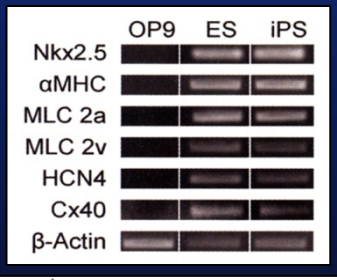
Directed and Systematic Differentiation of Cardiovascular Cells From Mouse Induced Pluripotent Stem Cells

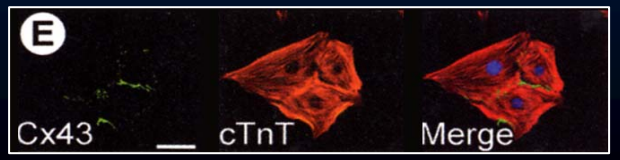
Genta Narazaki, MS; Hideki Uosaki, MD; Mizue Teranishi, BS; Keisuke Okita, PhD;
Bongju Kim, PhD; Satoshi Matsuoka, MD, PhD;
Shinya Yamanaka, MD, PhD; Jun K. Yamashita, MD, PhD

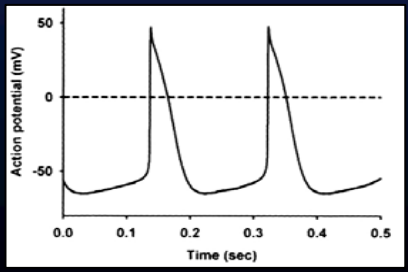
Circulation 118:498_ 08











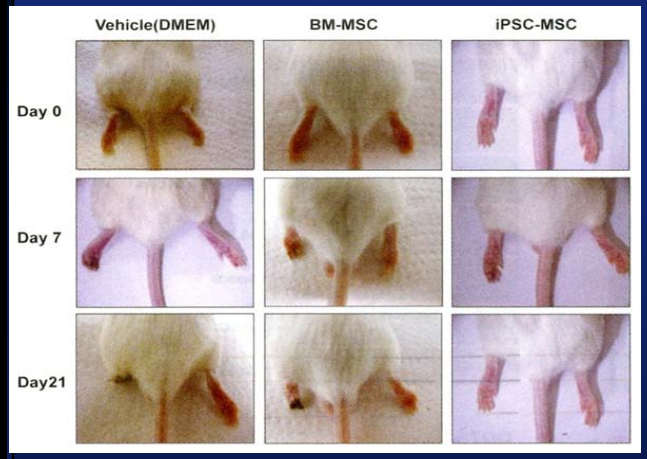
Issues with iPSC

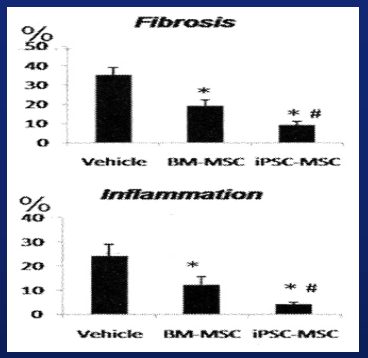
- Multiple protocols (Adv, plasmids, transposons…)
- C-Myc appears to be oncogenic -> 2 or 3F iPS lines (Oct 3/4, Sox2, Klf4 [Oct4 req'd],) or with proteins alone
- Reprogramming activates p53 -> apoptosis/senescence
- Generally low efficiency
- Many intermediately reprogrammed states often d/t epigenetic memory, different methylation patterns
- Need to remove viral transgenes to decrease risk of teratoma formation
- Efficiency → cardiomyocytes (eg sequentially BMP4 → bFGF, Activin A → VEGF, DKKI → bFGF) also low

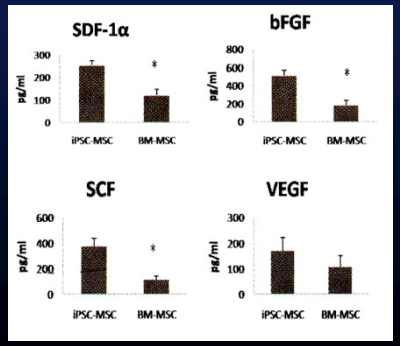
Shi, Cell Stem Cell 3:568 '08, Zhou Cell Stem Cell 4:381 '09, Kamp Circ Res 105:617 '09

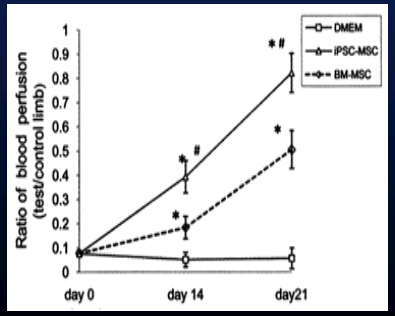
iPSC for Limb Ischemia

- iPSC generated from human fibroblasts by lentivirus transduction of Oct 4, Sox 2, Nanog and Lin 28
- iPSC → MSC with protocol including bFGF, PDGF, EGF
- Sorted for $CD24^{-}$ $CD105^{+}$, cloned and expanded$^{\Delta}$
- IM injection into SCID mouse FA excision model
- Designed to overcome limitations of nMSCs - limited proliferation, differentiation, cytokine exp (esp with aging) and of undifferentiated iPSC - teratomas









$^{\Delta}$ > 45 passages: random chromosomal aberrations first noted

Lian, Circ 121:1113 '10

SGE; 0410-7, 03

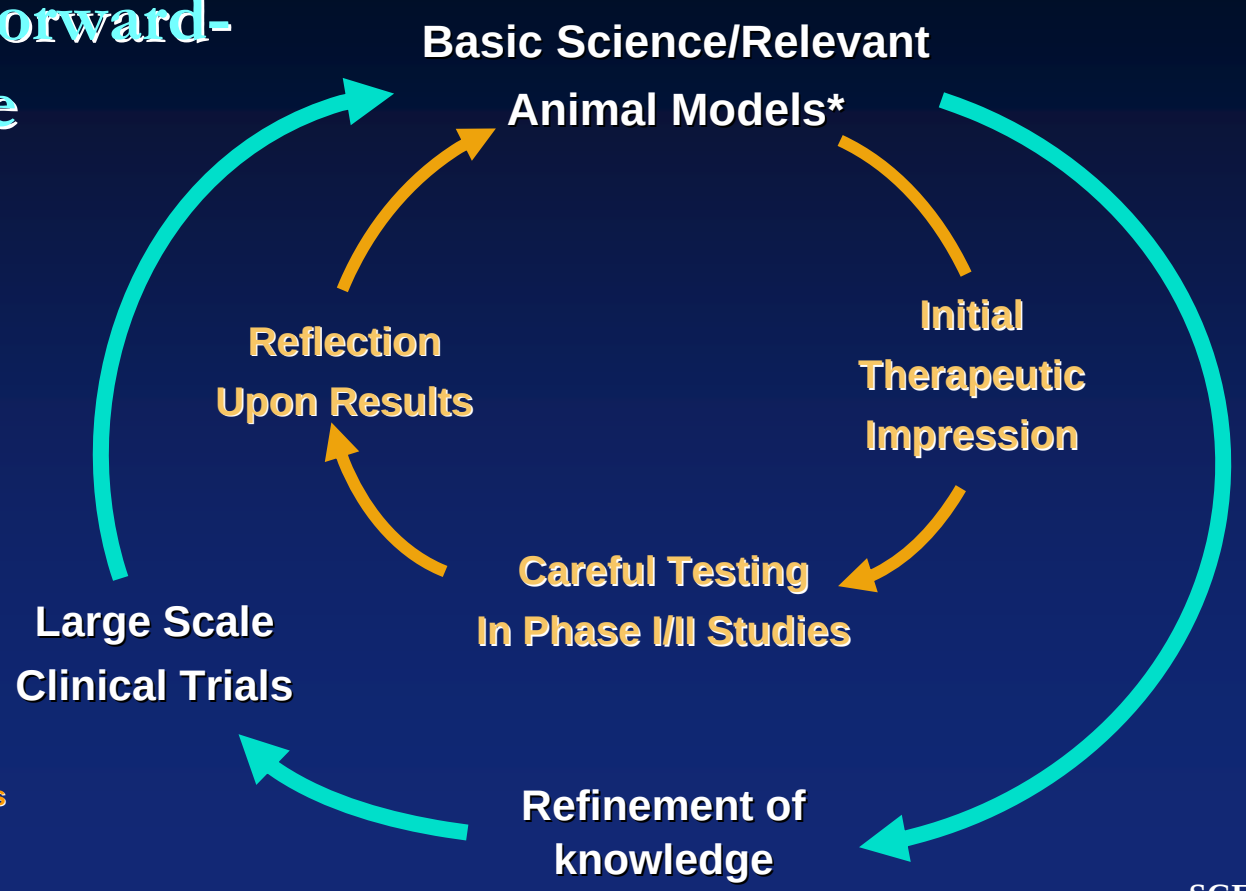
Acute MI

Interim Lessons Learned:

- Non-selected BMMN cells given ic 3-10 days after moderate-large MI appear safe and improve measures of LV function to about the same degree as does reperfusion therapy
- The optimal timing of cell administration (and possible benefit of late administration) remain unknown
- We have little insight as to the optimal cell or cells for treatment in this setting
- However, method of SC preparation and storage affects results

Cellular Cardiac Repair

The Way Forward- An Iterative Approach



* Limited by impact on stem cells by human co-morbidities: age, Diabetes, hyperlipidemia, etc.

SGE; 0207-2, 38