TURIN, 20TH—21ST NOVEMBER 2008

GREAT INNOVATIONS IN CARDIOLOGY

4TH JOINT MEETING WITH MAYO CLINIC

4TH TURIN CARDIOVASCULAR NURSING CONVENTION

COFFEE BREAK

MAYO CLINIC

Azienda Ospedaliera
San Giovanni Battista di Torino

www.cardiologiaospedalieramolinette.it

What's New on Adult Stem Cells?

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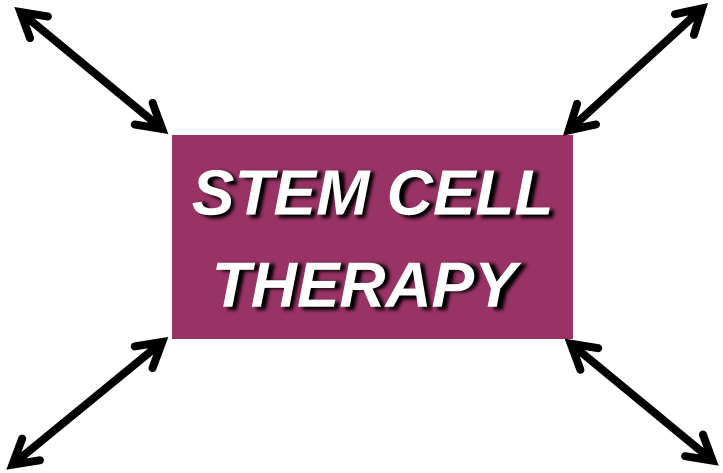
4th Joint Mayo Clinic-Torino Meeting
November 20-21, 2008

JB is a member of an institution which is a founding member of Cardio³ Biosciences

Adult Stem Cells for Cardiac Repair

Framework towards the optimization



PATIENT

- *Disease*
- *Risk Profile*
- *Bone Marrow*

CELL PRODUCT (Biologics)

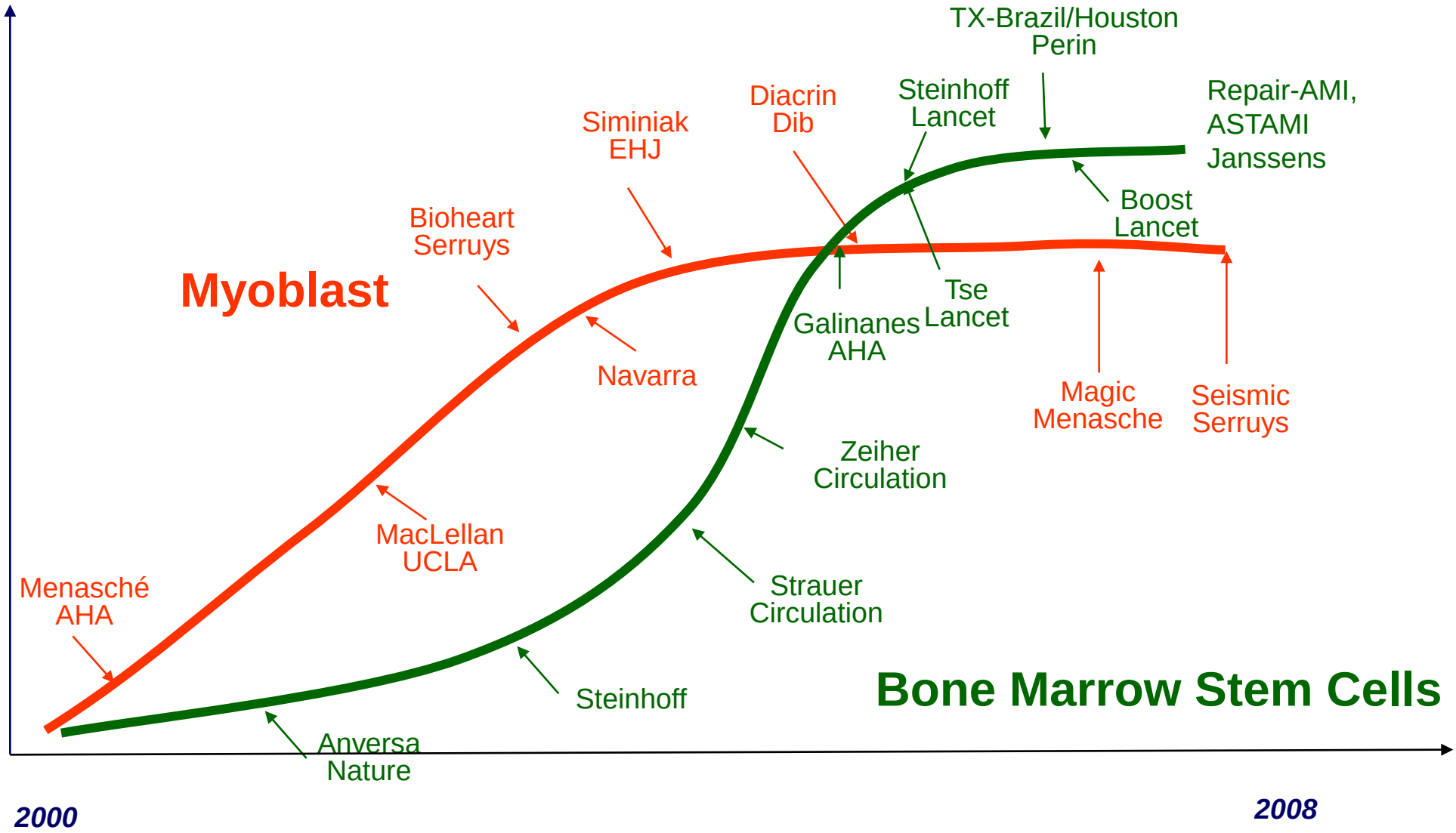
- *Type*
- *Processing*
- *Function and number*

CELL DELIVERY

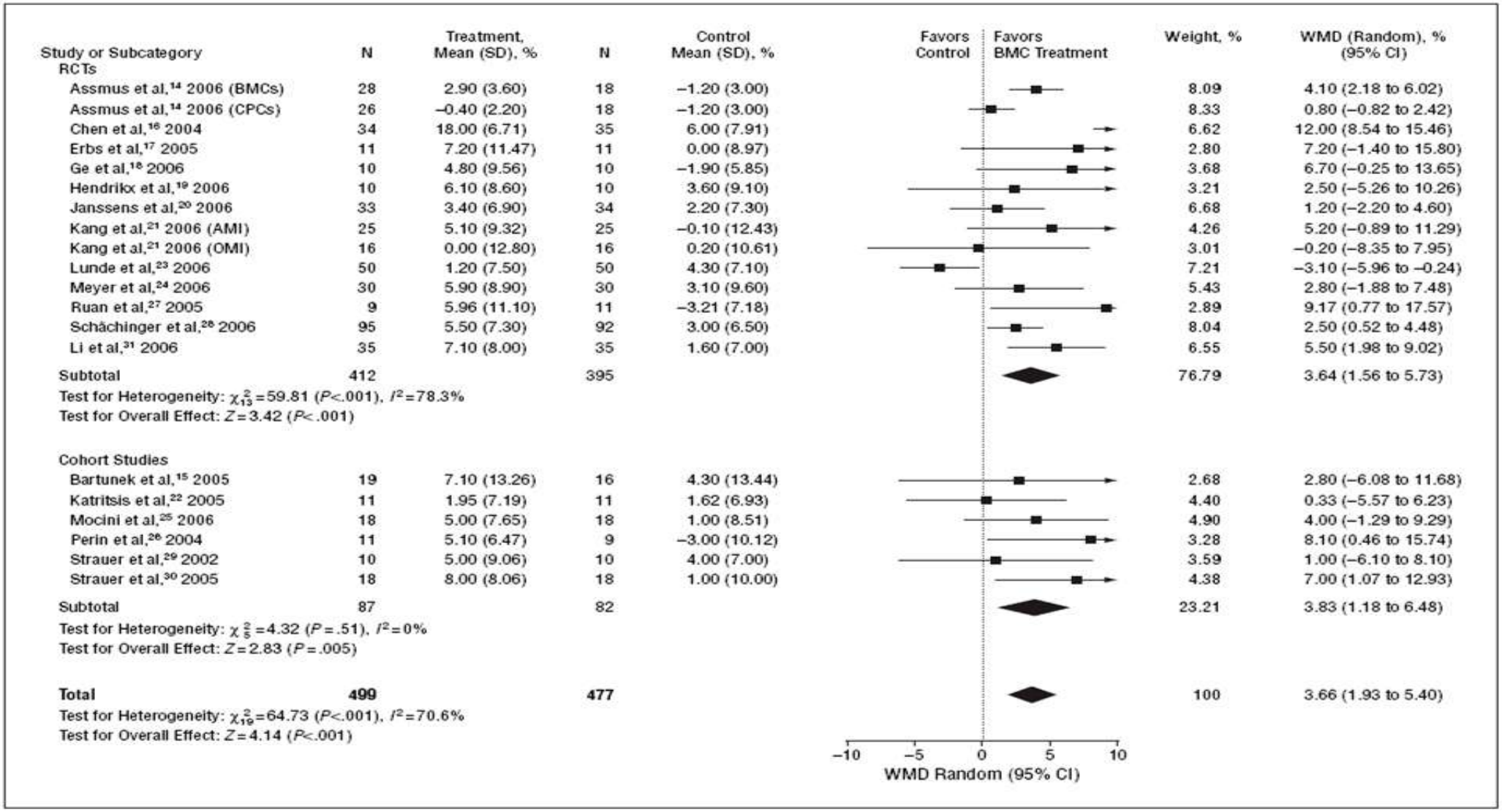
"MECHANISMS AT WORK"

- *Target organ – heart*
- *Remote Homing*
- *Arrhythmias*
- *Coronary vasculature*

Two Clinically Applied Cell Types



Adult Stem Cells Therapy for Cardiac Repair



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: $Z=4.14$ ($P<.001$)						

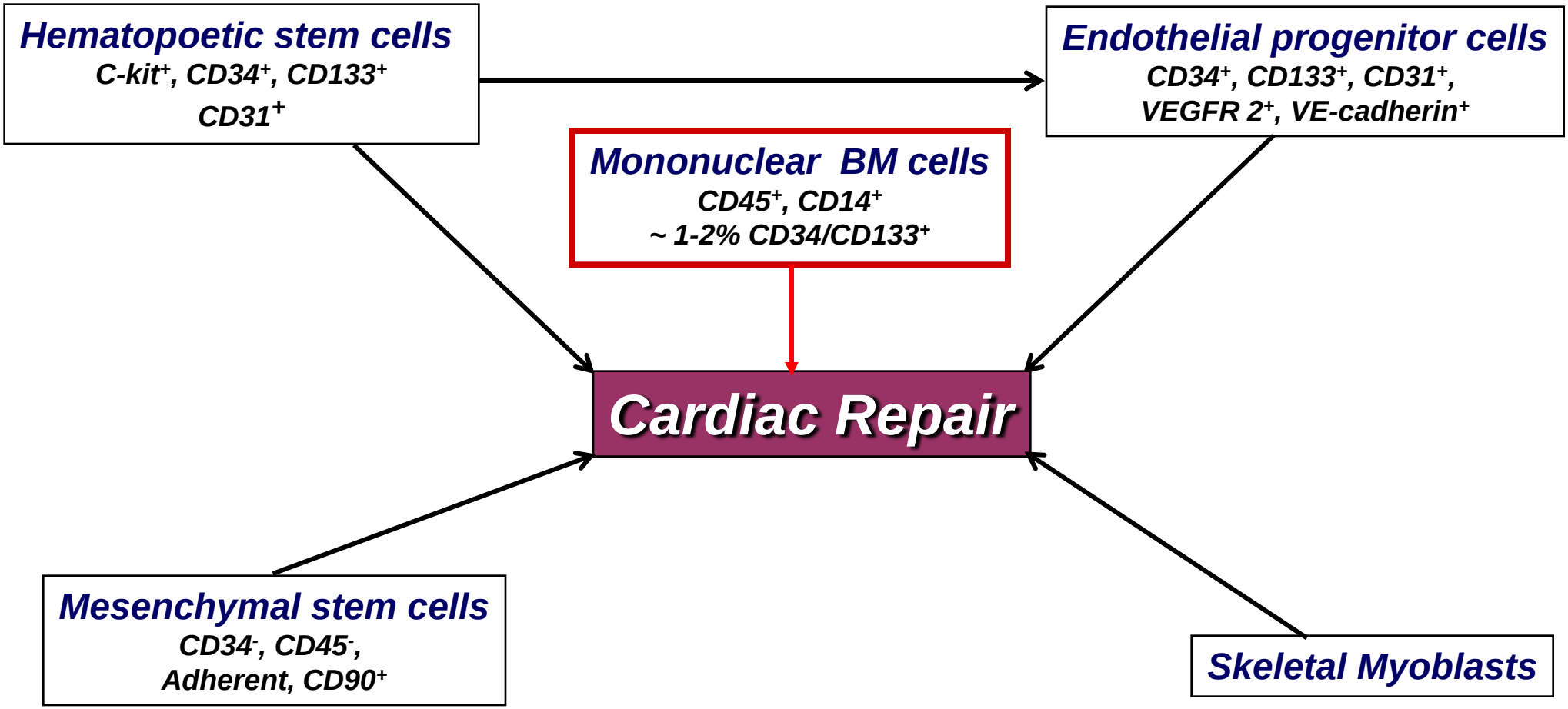
Overall clinical safety was demonstrated over 1 to 2 years period

Functional efficacy is only modest

Abdel-Latif, Arch Intern Med 2007

Adult Stem Cells for Cardiac Repair

Cell Type



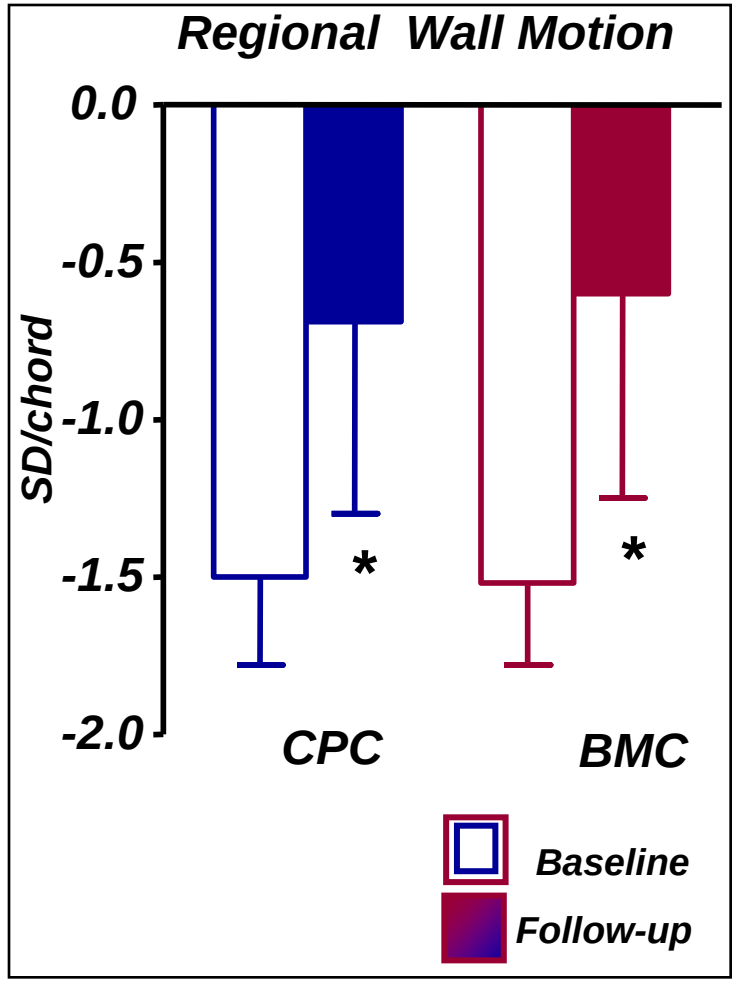
Stem Cell Type and Therapeutic Effect

Cell type

- BM mononuclear fraction delivers functional response
  - No cell type omitted
  - Effect ~ cellular cross talk between multiple cell types

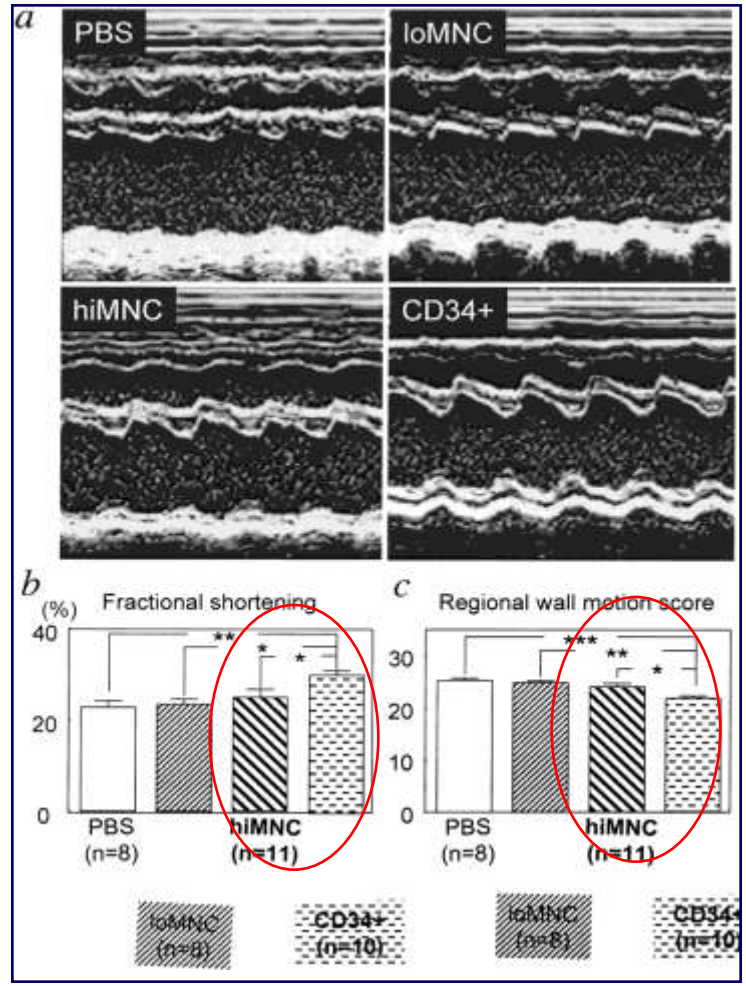
Potential of enriched (hematopoietic) cell populations should be Investigated

Similar functional effects EPC vs MNC



Shachinger V, JACC 2004

CD34⁺ superior to MNC



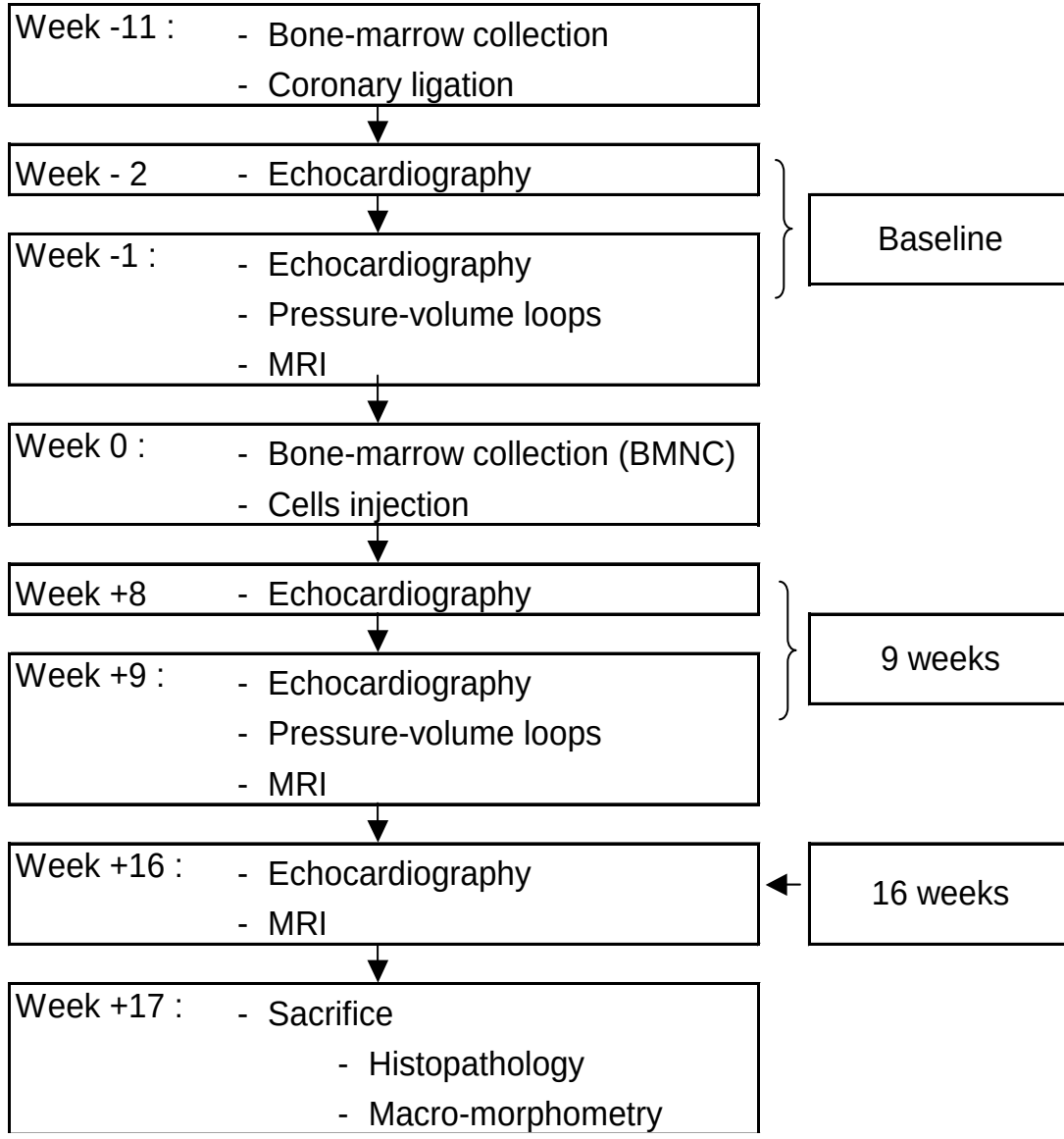
Kawamoto A, Circulation 2006

Selected/Enriched Adult Stem Cells for Cardiac Repair

	Markers	Source	Delivery	Potential	Pre-clinical experience	Clinical Studies
Hematopoietic stem cells	CD133, CD34 CD31	Autologous Allogeneic (umbilical cord)	Coronary Myocardial	Angiogenic Paracrine	Perfusion Function	Rand-DB Ongoing
BM mesenchymal stem cells	CD166, Stro-1, CD44, CD106	Allogeneic Autologous	Myocardial Systemic	Multipotency Paracrine "Off-shelf" use	Function Perfusion	Rand-DB Ongoing
Adipose-derived stem cells	CD49D, Stro-1, CD166, CD44	Autologous	Coronary Myocardial	Pluripotency Angiogenic	Perfusion	Rand-DB Ongoing
Resident cardiac stem cells	C-kit, Sca1, MDR1, Isl1,	Autologous Limited?	Coronary Myocardial	Cardio-myogenic	Function	Planned

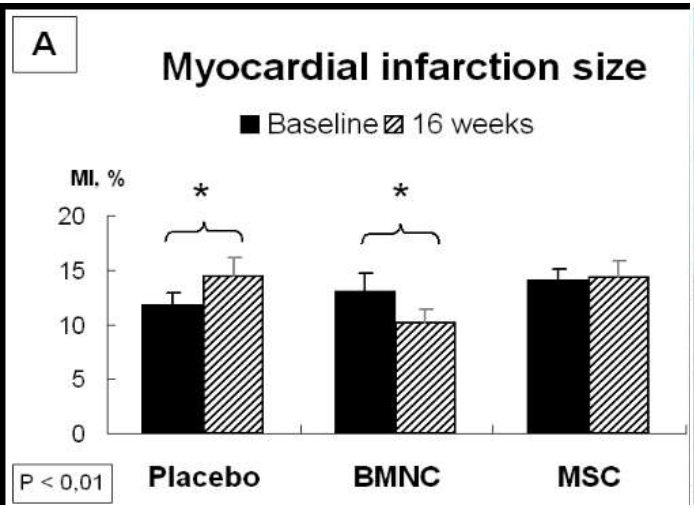
Stem Cell Type and Therapeutic Effect
Direct Comparison MSCs vs BMNCs

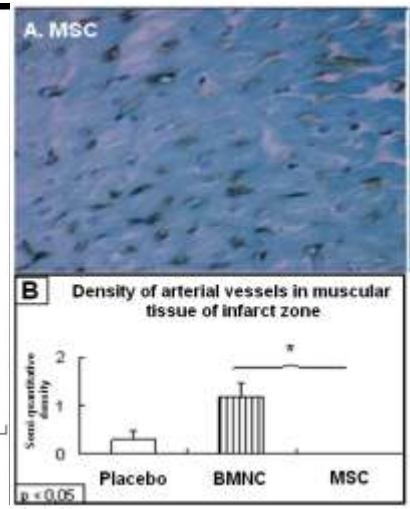
- Blinded, placebo controlled, randomized study
- Direct comparison of autologous BM MNC and autologous culture expanded BM MSCs in the canine model of the chronic myocardial infarction
- Multimodality functional and morphological assessment including echocardiography, MRI and invasive pressure-volume loops
- Histology and gene expression analyses

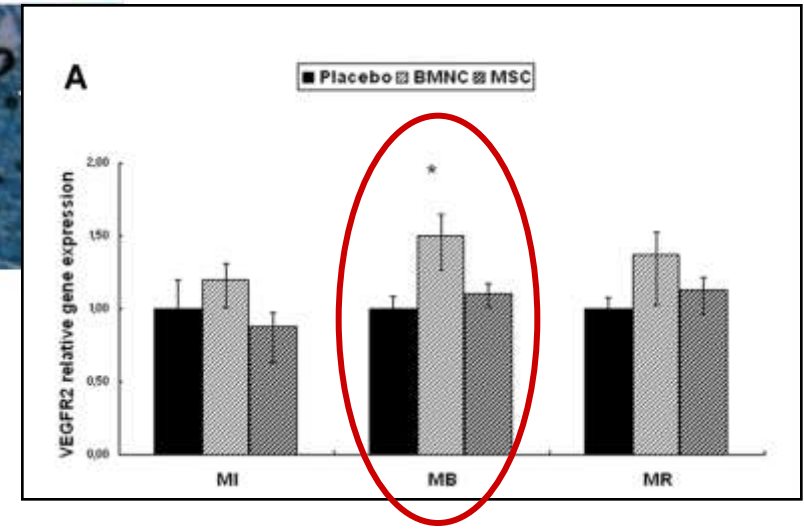


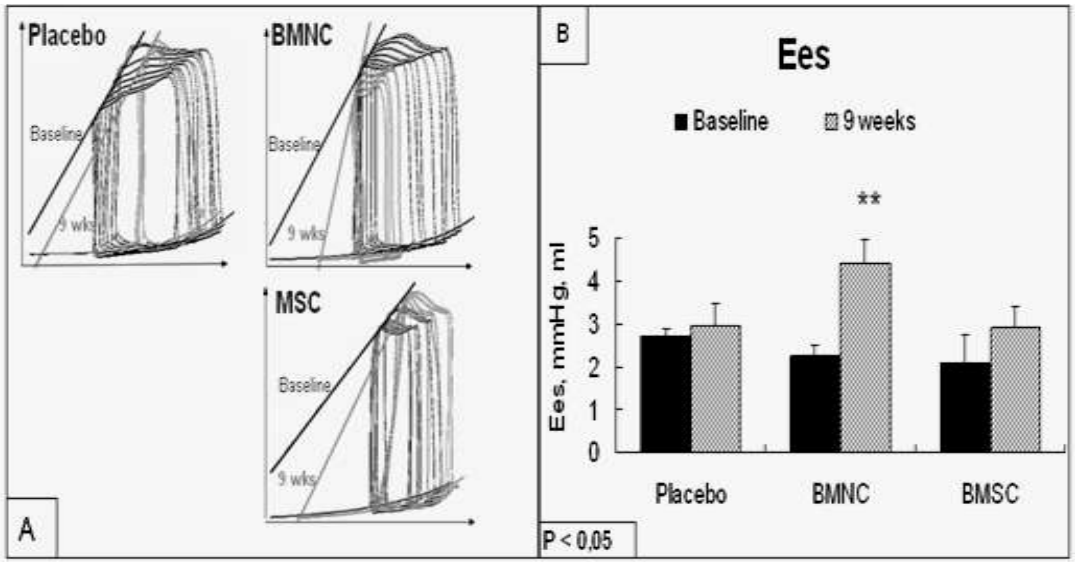
Mathieu M, McEntee K, Erasmus, ULB Brussels, AHA 2008

Stem Cell Type and Therapeutic Effect
MSCs vs BMNCs









Superior effects of MNC vs MSCs :
- Model specific?
- Disease/remodelling specific?
- Implications for the clinical trials?

Mathieu M, Mc Entee K, Erasmus, ULB Brussels, AHA 2008

"Stem Cell Product" and Therapeutic Effect

Cell type

- **Cells mediating the functional responses remain unknown**
- **Potential of enriched cell types should be investigated**

Cell processing

- **Different protocols may profoundly affect cell function and therapeutic response**

Cell function and number

- **Cell function may determine response in individual patient**
- **Dose-dependent effect needs to be demonstrated**

To define cells-mediators of the functional effects

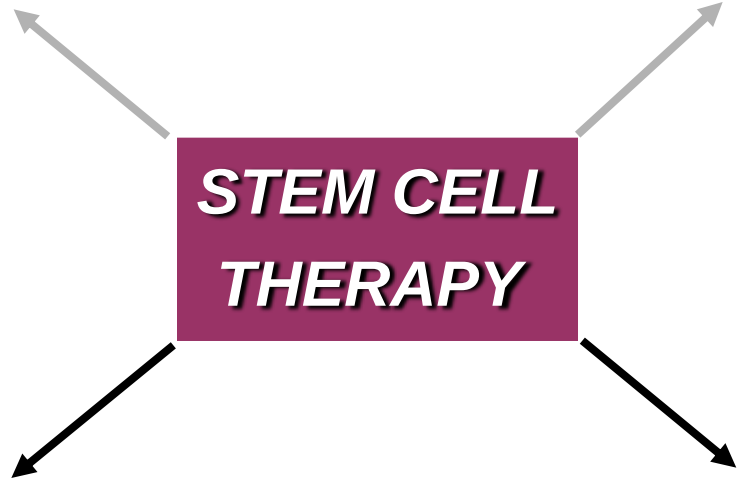
To standardize cell processing assays

To establish release criteria of each specific cell product

BM Stem Cells for Cardiac Repair
What To Consider?

PATIENT
- *Disease*
- *Risk Profile*
- *Bone Marrow*

CELL PRODUCT (Biologics)
- *Type*
- *Processing*
- *Function and number*



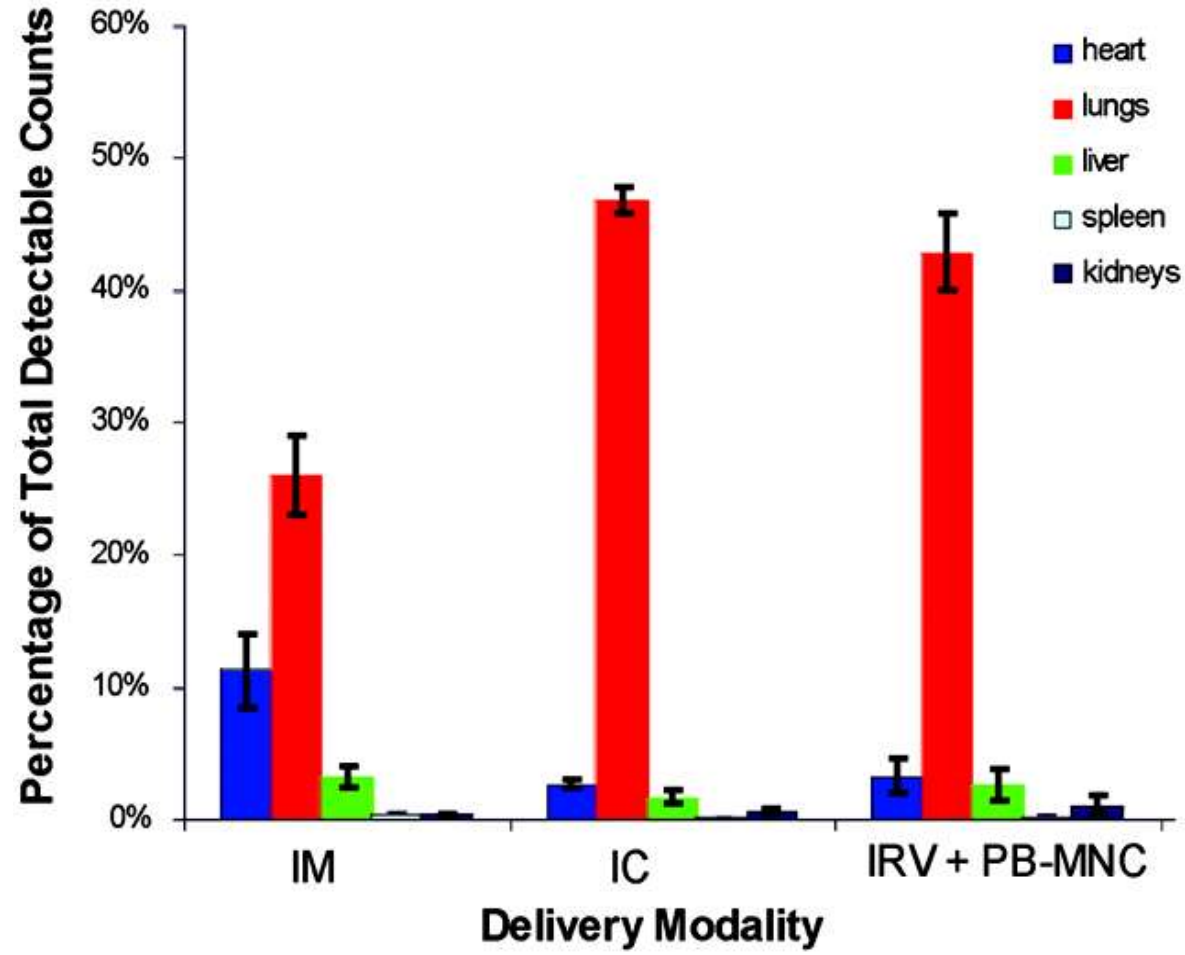
CELL DELIVERY

"MECHANISMS AT WORK"
- ***Target organ – heart***
- ***Arrhythmias***
- ***Coronary vessel wall***
- ***Remote homing***

Framework for Assessing Homing

Targeting

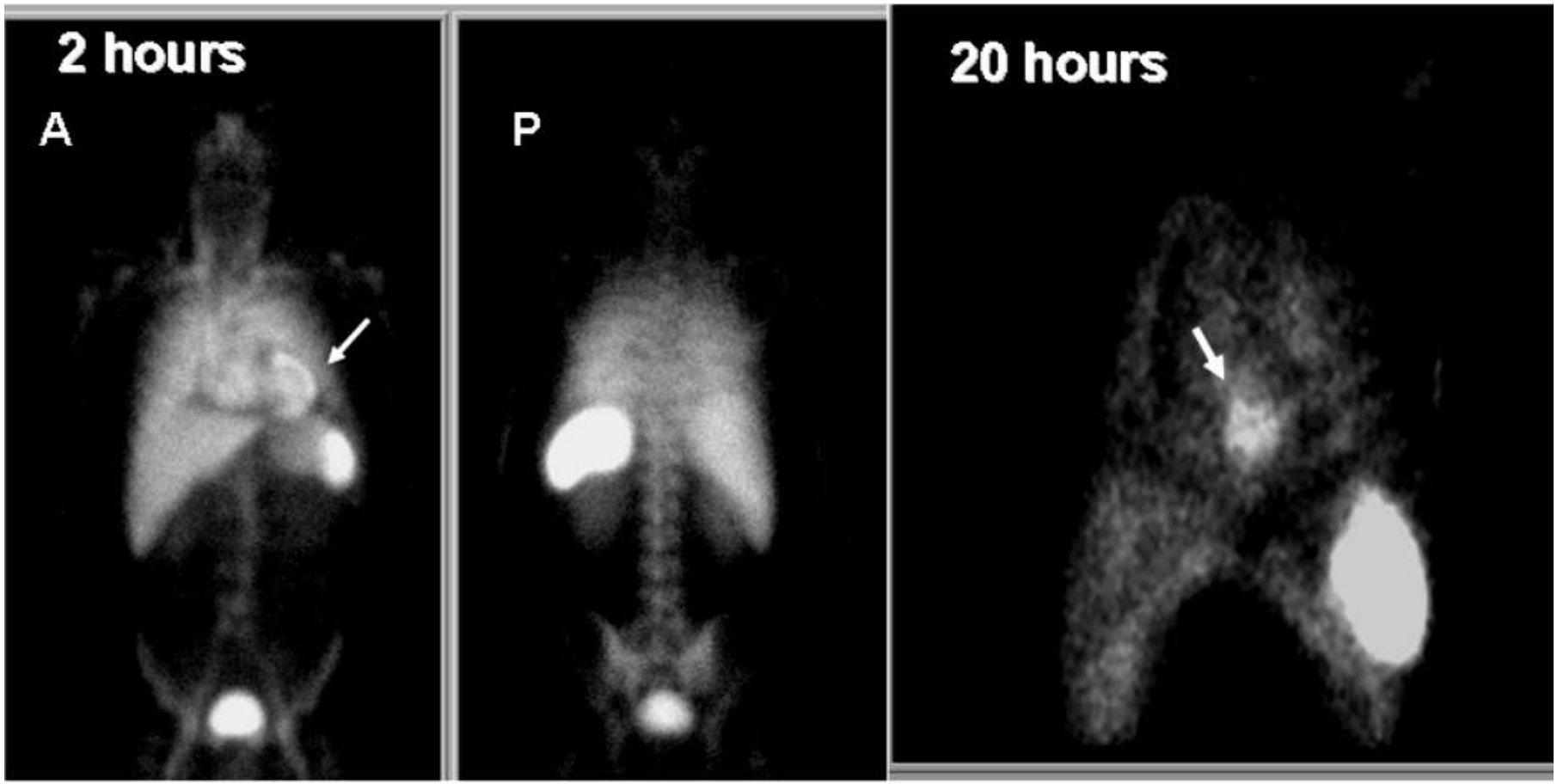
- **2-10% of cells end up in the heart**
- **Consequences of remote homing are unclear**



Hou et al, Circulation 2005

One Day Kinetics after Coronary Cell Transfer

Coronary transfer of BMNC labelled with ^{99m}Tc 5 days after anterior MI



Penicka et al, Heart 2007

Framework for Improving Delivery and Retention

Targeting and retention

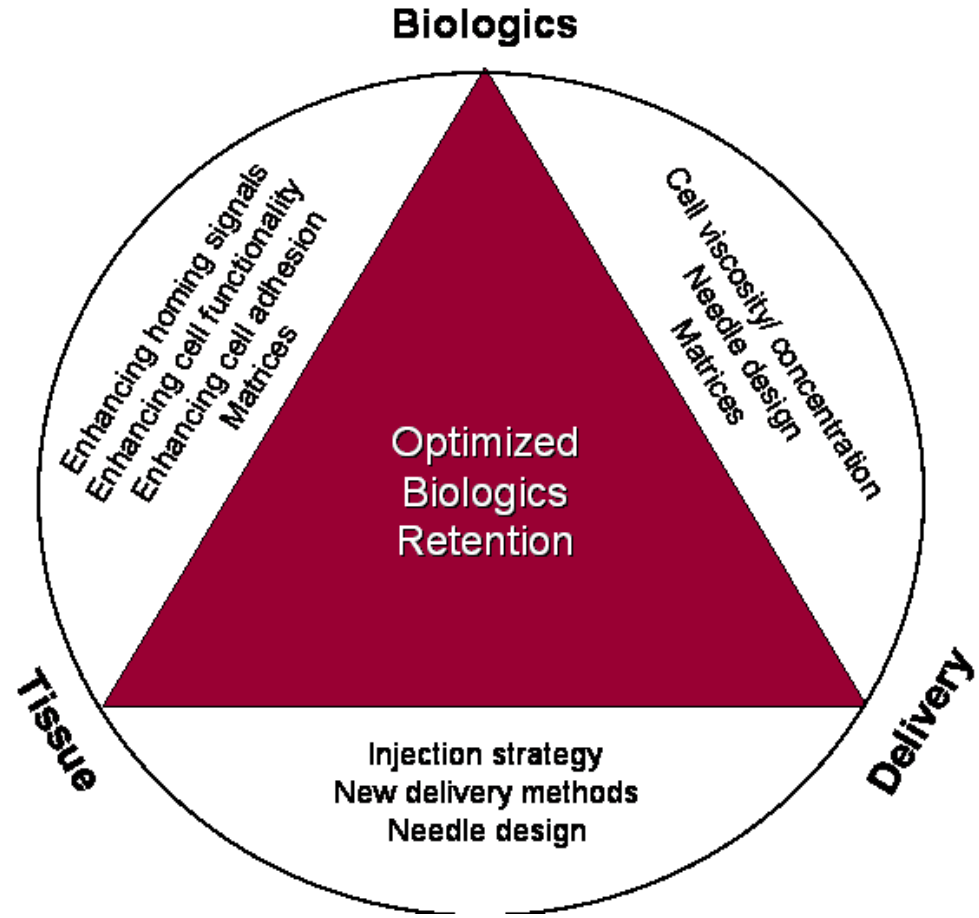
2-10% of cells end up in the heart

Consequences of remote homing are unclear

Decline/(disappearance) early after cells injection

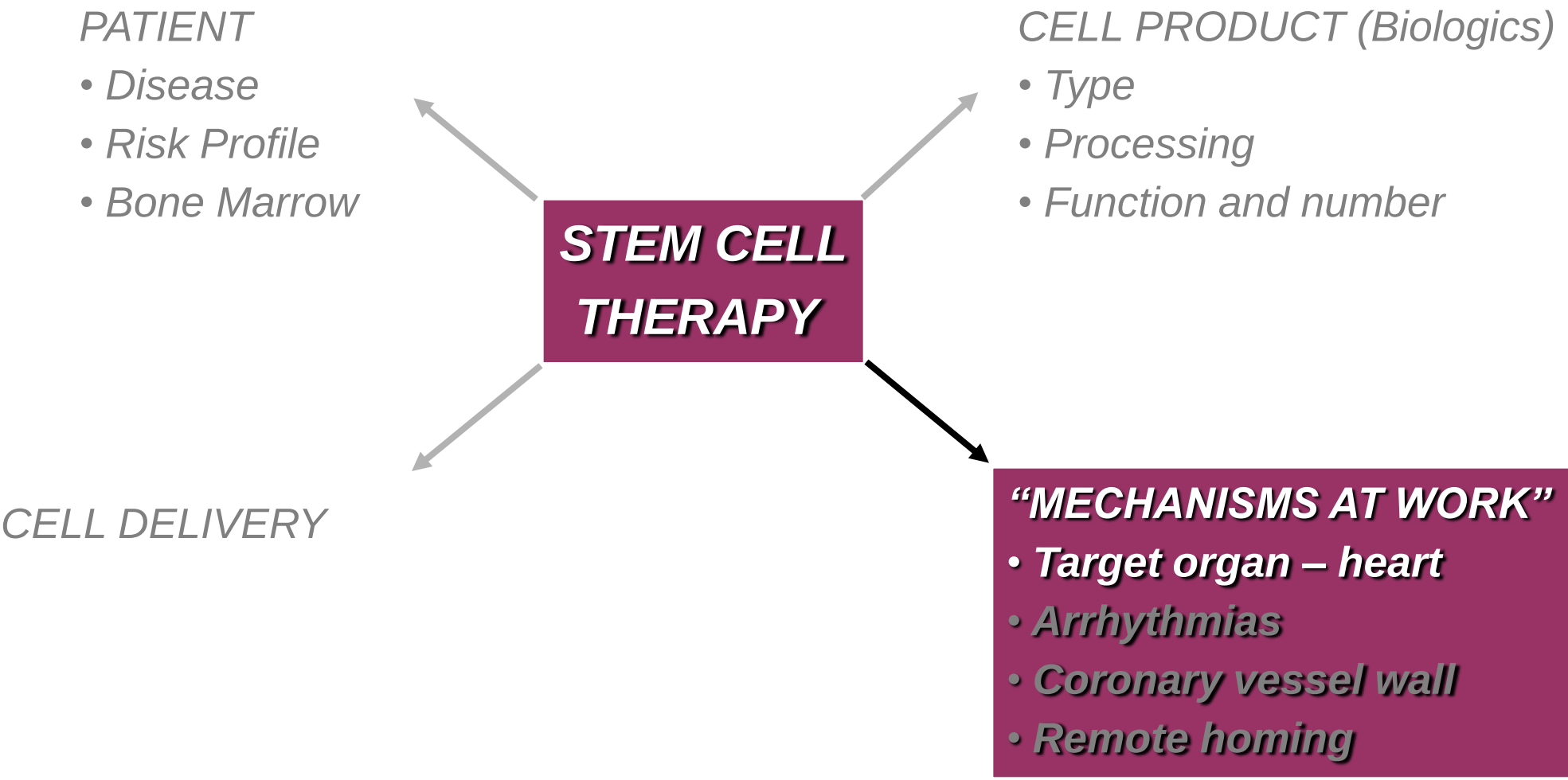
Continuous bed-bench-bed cycle:

- **Biologics – cells enhancement**
- **Tissue priming – augmenting homing signals**
- **Methods / techniques for cell delivery**



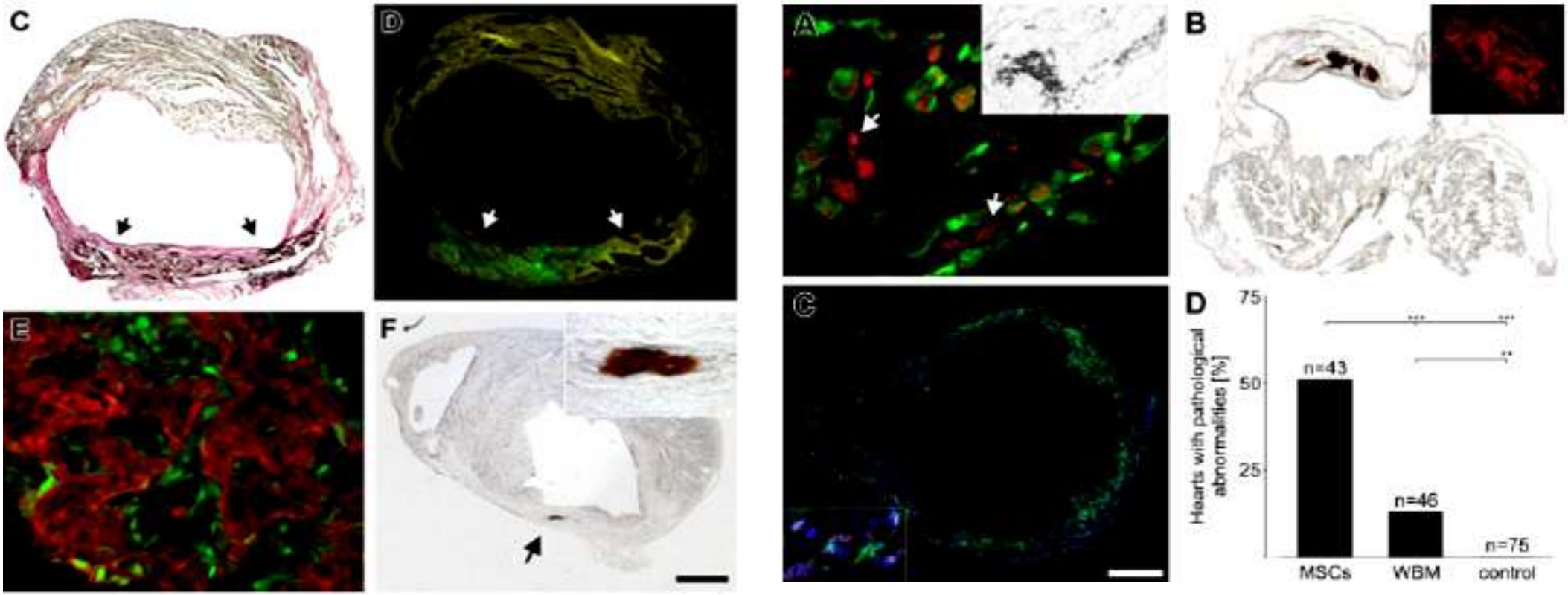
Bartunek et al, Clin Pharmacology and Therapeutics, 2009, accepted

BM Stem Cells for Cardiac Repair
What To Consider?



Stem Cell Type and Therapeutic Effect
Word of Caution on Safety?

- *Mice model of the myocardial infarction (cryoinjury, coronary ligation)*
- *Injection of 2x10^5 MSCs (passage 3) or total BM from EGFP$^+$ transgenic mice*
- *Follow up from 29 to 268 days.*



Osteocalcin, Cy3 *EGFP labeled stem cells*

Breitbach M et al, Blood 2007

Adult Stem Cell Therapy

Damaged adult heart may not be able to recapitulate necessary millieu to stimulate myocardial specification resulting in the limited efficacy or unwanted signalling/differentiation of "naive" or plastic stem cells

Olson, Nat Medicine 2004; Chien, Nature 2004; Wang, J Thorac Cardiovasc Surg 2001, Yoon, Circulation 2004, Breitbach, Blood 2007

From Non-modified Adult Stem Cells to "Second Generation Stem Cell Products"

Goals:

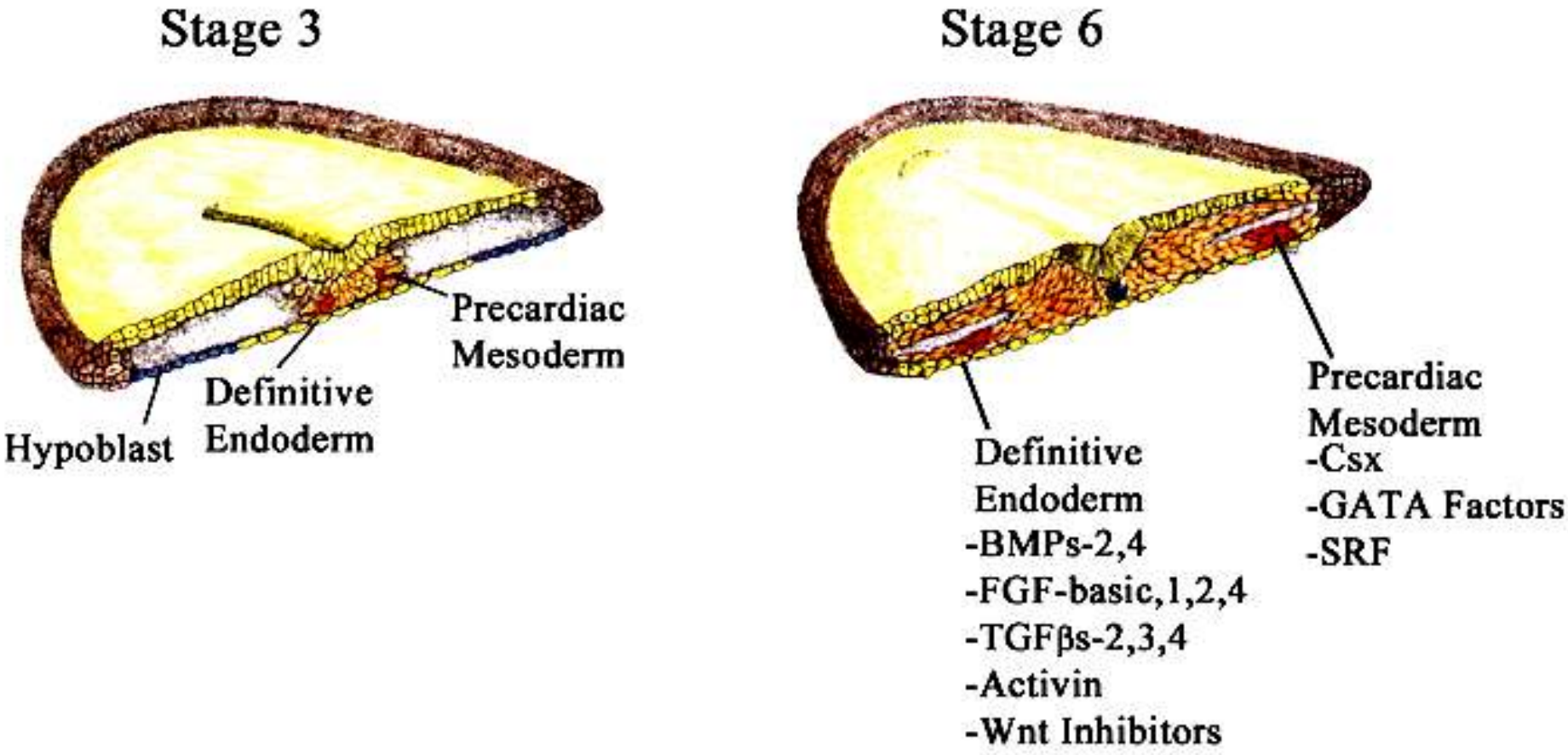
- **to improve cell function**
- **to increase engraftment**
- **to increase integration**
- **to increase cell survival**
- **to guarantee safety**

Strategies:

- **Pharmacological pretreatment**
- **Genetic engineering**
- **Tissue engineering**
- **…..**

"Second Generation Stem Cell Products"
Cardiomyogenic Specification?

"clues" from embryonic development?



"Second Generation Stem Cell Products"
Cardiomyogenic Specification?

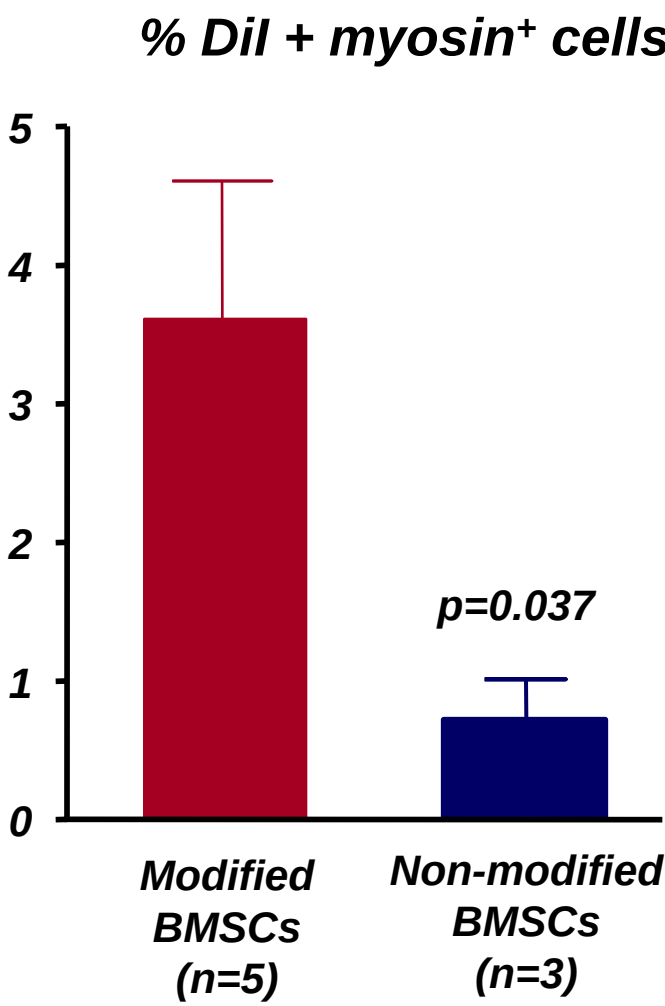
Selected Cardiomyogenic Growth Factors Pretreatment

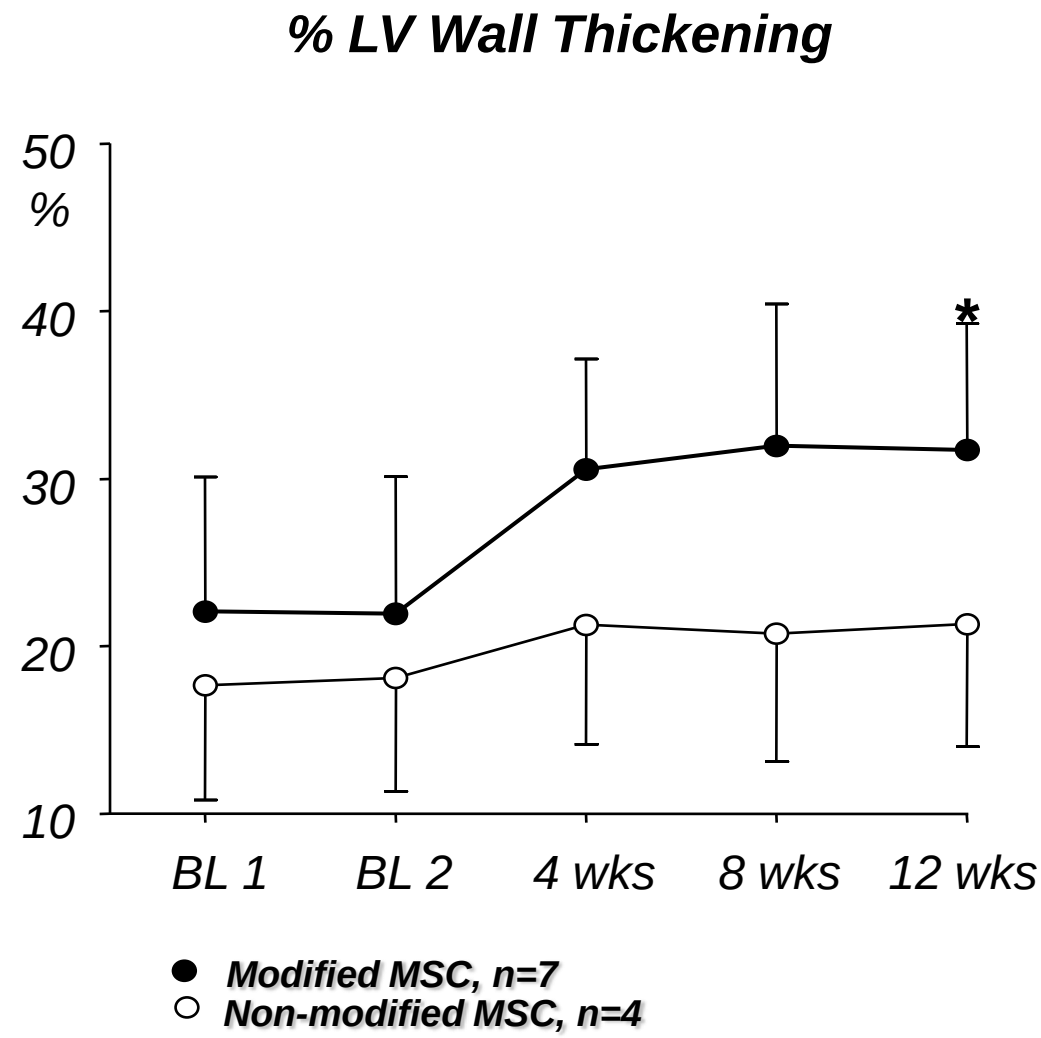
Dog model of chronic myocardial infarction

- *Injection of culture expanded MSCs*
- *Injection of modified MSCs (coctail of growth factors including BMP2, IGF-1 and bFGF)*
- *Follow-up up to 12 weeks including histology*

Bartunek et al, AJP 2007

Non-modified vs Modified BMSCs in a Chronic Dog MI Model
Regional LV function and Cell Retention





Bartunek et al, AJP 2007

"Second Generation Stem Cell Products
Selected Cardiomyogenic Growth Factors Pretreatment

Problems encountered:

- **Only cytoplasmic expression, but no nuclear translocation of cardiac markers**

- **"Terminal' cardiac commitment (functional excitation-contraction coupling) in vitro was not achieved**

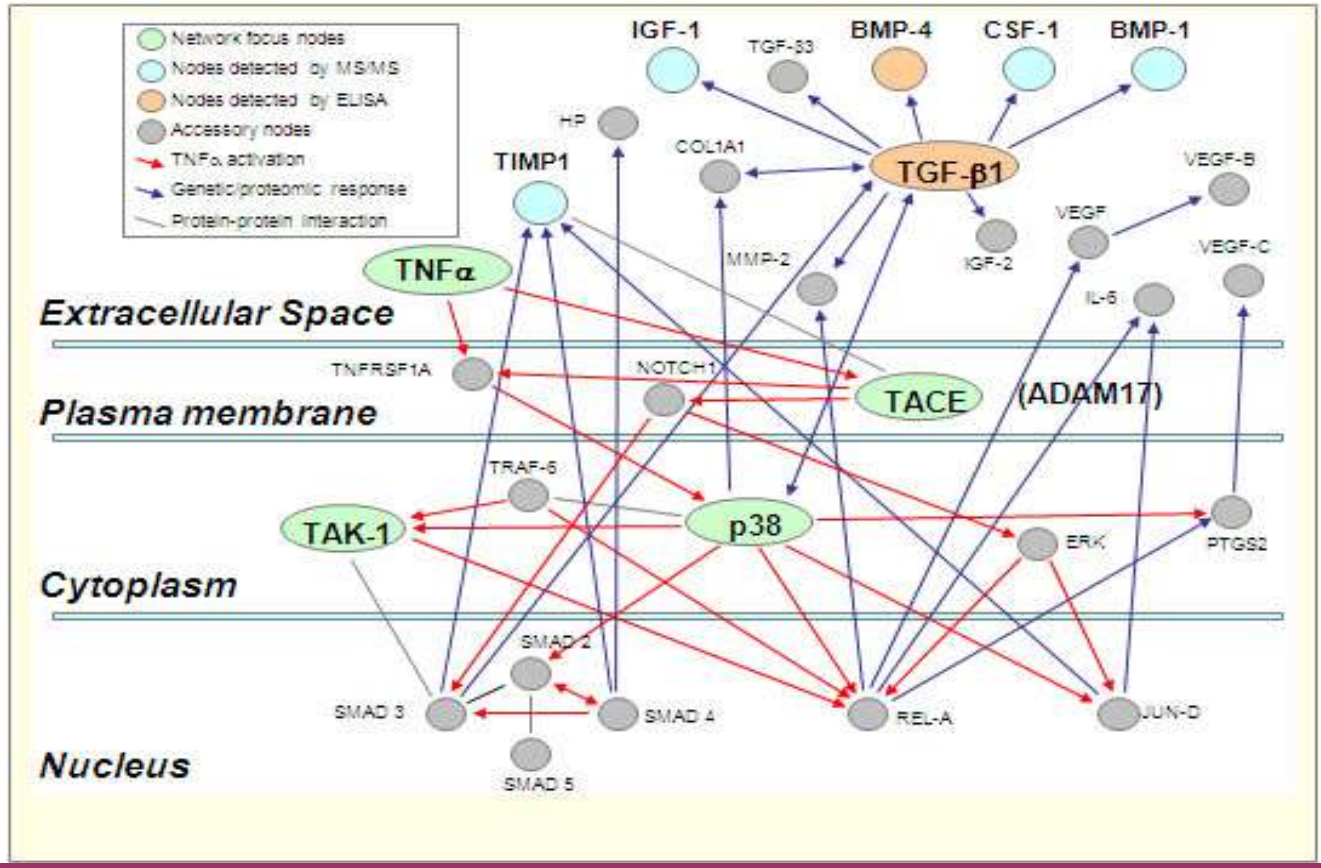
- **Suboptimal reproducibility when treatment applied to mesenchymal stem cells from cardiac patients**

“Second Generation Stem Cell Products
High Throughput Genomic and Proteomic Technology

A. Behfar & A. Terzic

Behfar A et al , J Exp Med 2007; Arell et al Stem Cells 2008; Nelson TJ et al, Stem Cells 2008; Faustino RS et al , Genome Biol 2008; Behfar A et al, Nat Clin Pract Cardiovasc Med 2006

"Second Generation Stem Cell Products
High Throughput Genomic and Proteomic Technology



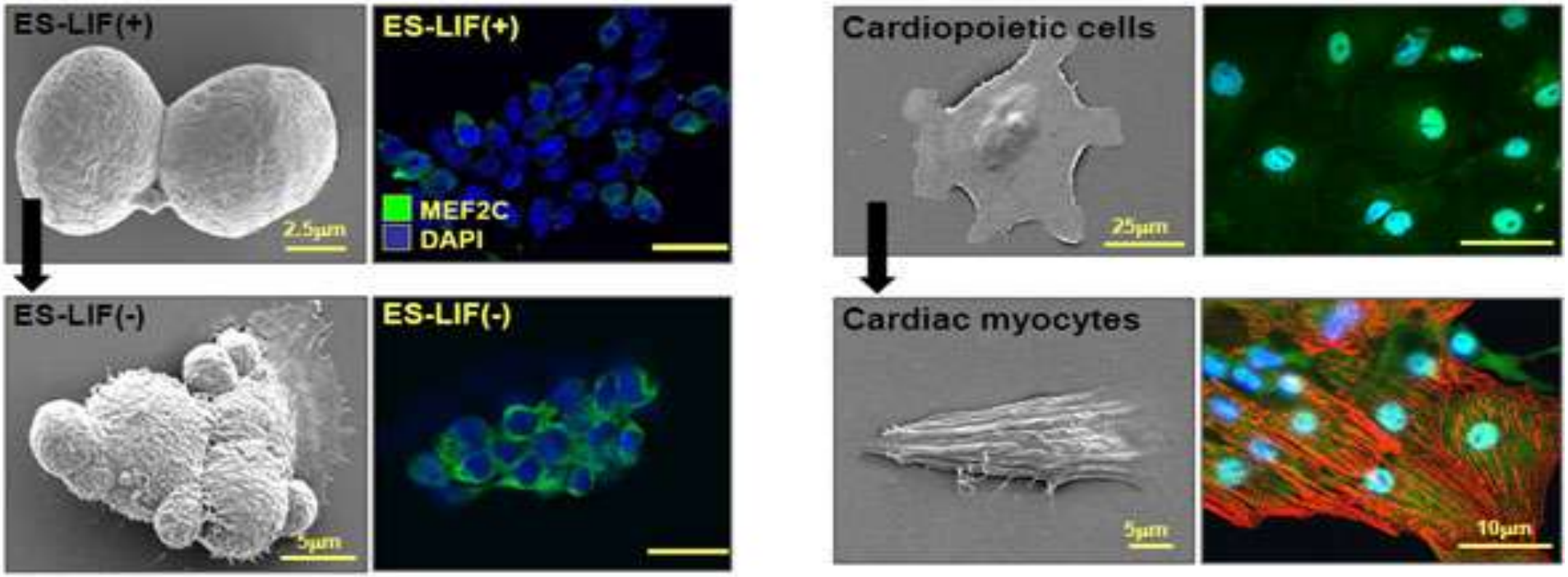
Candidate effectors of cardiac differentiation were identified using the comparative proteomics and genomics on the secretome of murine visceral endoderm-like cells in response to TNF-α

Behfar et al, J Exp Med 2007; Arell et al , Stem Cells 2008

"Second Generation Stem Cell Products
High Throughput Genomic and Proteomic Technology

Cardiogenic Cocktail

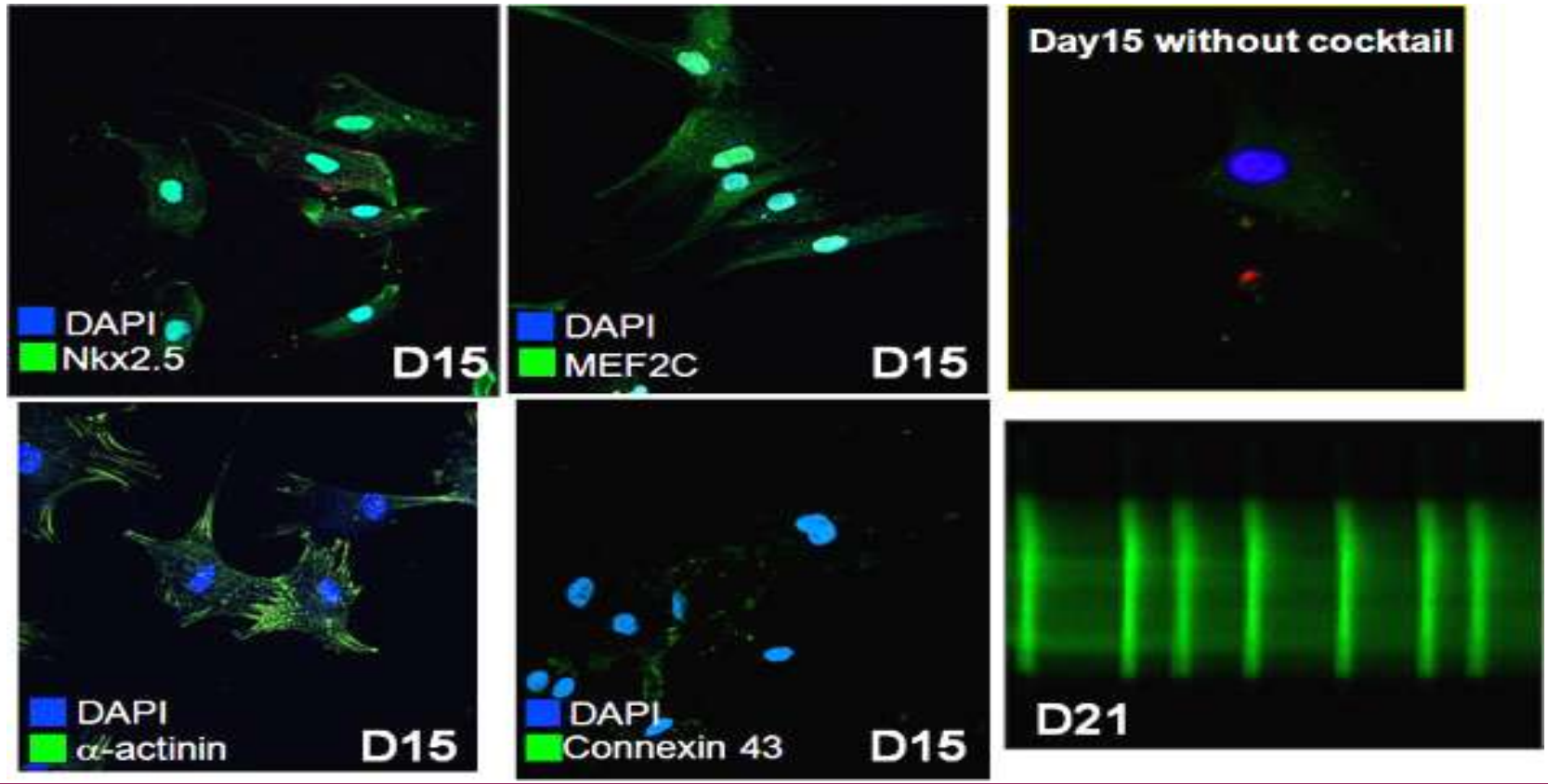
Mouse ESCs → **Guided mouse ESCs**



The cardiogenic coctail secured guided differentiation of mouse embyronic stem cells into cardiopoietic cells

Behfar et al, 2007, J Exp Med 204: 405-420

"Second Generation Stem Cell Products
Cardiogenic Coctail in Adult BM-MSCs from Cardiac Patients

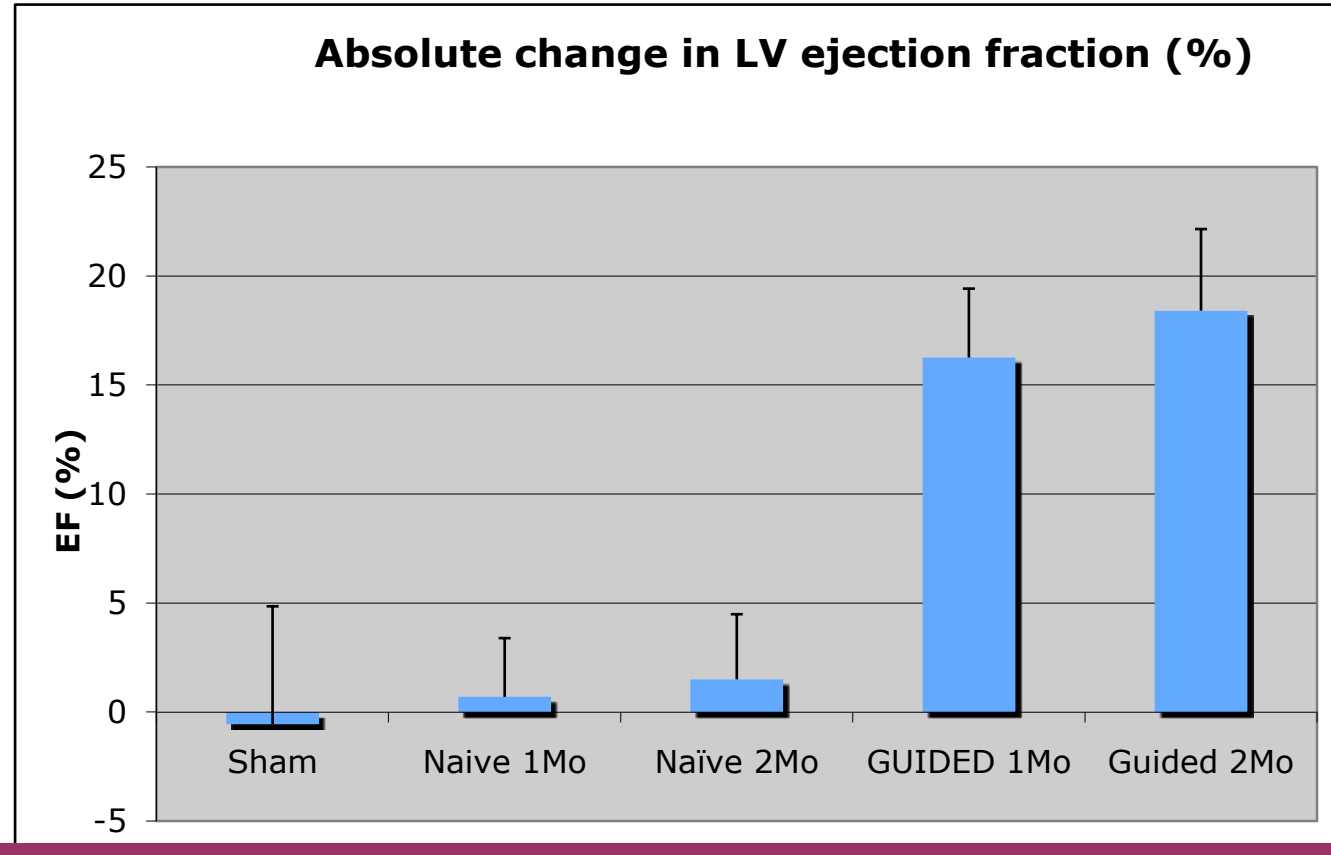


***Guided cardiopoietic BM mesenchymal stem cells**:*

- upregulation and nuclear translocation of cardiac transcription factors,
- sarcomeric organization and expression of the gap junction protein connexin 43
- rhythmic calcium transients.

Behfar & Terizc, personnal communication

"Second Generation Stem Cell Products
Guided Cardiopoietic BM-MSCs in the Mice Model of the Chronic MI



Guided cardiopoietic BM mesenchymal stem cells:

- superior effects on functional improvement in the chronically infarcted myocardium as compared to nonmodified MSCs
- paralleled by the superior effects on neovascularization and cardiac differentiation
- no toxicity observed

Behfar & Terzic, personnal communication

Cardio³ BioSciences C-Cure™ I Clinical Trial
Protocol Number: C3BS-C-07-02
EudraCT Number: 2007-007699-40

Goal:

To test the safety and efficacy of guided, autologous bone marrow-derived cardiopoietic mesenchymal stem cells

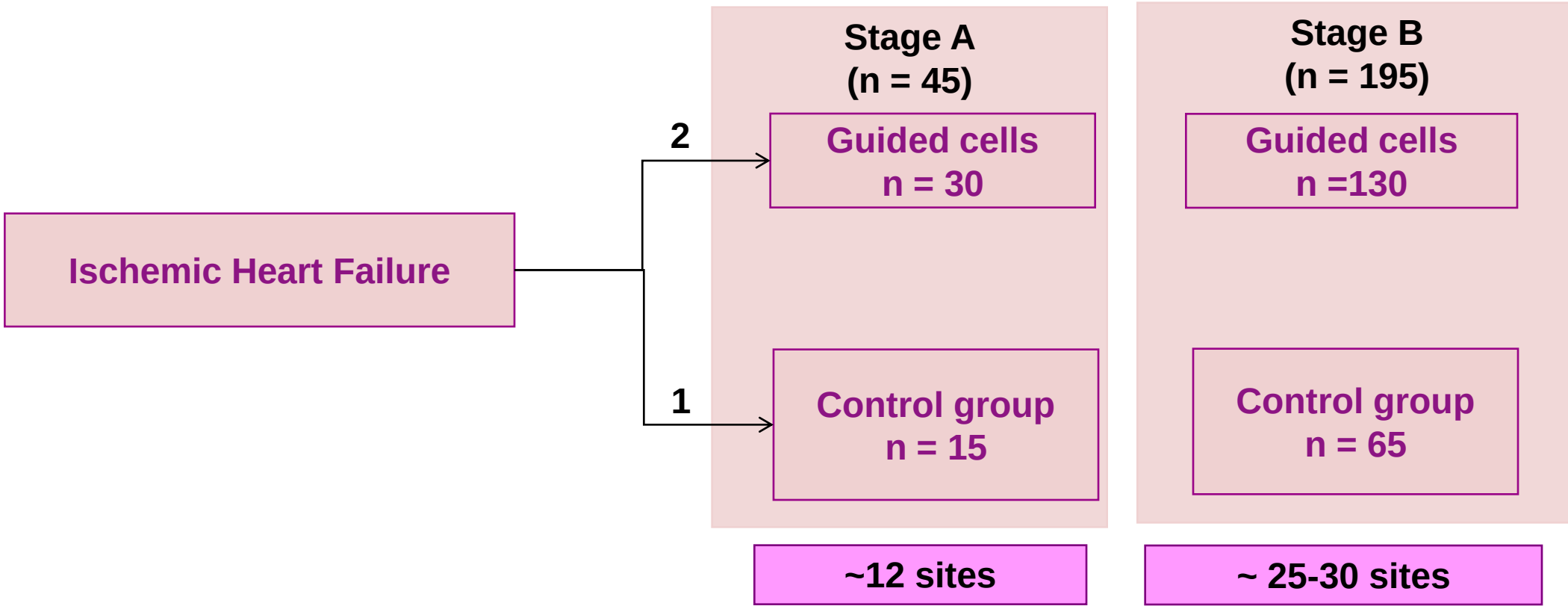
in ischemic cardiomyopathy

Co-PIs: Jozef Bartunek and Andre Terzic

Sponsor: Cardio³ Biosciences, Braine L'alleud, Belgium

Cardio³ BioSciences C-Cure™ I Clinical Trial

- **A multicenter, prospective, open-label, sequential design with 2 parallel arms**
- **Blinded core lab analyses**
- **2:1 randomization**



Adult Stem Cells Therapy
SWOT 2008

Strengths

- Interplay between BM and heart after injury
- Multipotency of BM stem cells
- Variety of stem cell types
- Promising functional effects on cardiac repair in experimental setting

Weaknesses

- **Limited effects of naive stem cells**
- **Fate of cells: survival, transdifferentiation and integration**
- **Optimal cell type?**
- **Mechanism?**
- **Timing? Delivery? Homing?**
- **What Patient?**

Opportunities

"New age" in cardiac interventions:

- "The" fundamental solution for the myocyte loss
- Heart failure treatment and prevention

Threats

- **"Hype" effect**
- **Large scale randomized trials could be negative**
- **Intellectual conflict of interest**
- **Unforeseen safety problems**
- **Proliferation of uncontrolled small trials**

Cardiovascular Center, Aalst

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Myreille Mathieu

University of Gent, Belgium

Bart Vandekerkhove
Frank Timmermans

King's College London, UK

Jonathan Hill

Primary and Secondary Endpoints

	Baseline	1-mo	3-mo	6-mo	9-mo	1-yr	18-mo	2-yrs
LVEF (Muga)	✘			☑				
LVEF and volumes (Echo)	✘	✘	✘	✘	✘	✘	✘	✘
6-min walking distance	✘			✘		✘		✘
Quality of Life	✘			✘		✘		✘
Humoral Markers	✘			✘		✘		✘
Spiroergometry	✘			✘				
Clinical events	✘	✘	✘	✘	✘	✘	✘	✘
Arrhythmias	✘	✘	✘	✘	✘	✘	✘	✘
Resource utilization	✘	✘	✘	✘	✘	✘	✘	✘