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Intracoronary transfer of human umbilical cord matrix-derived Mesenchymal Stem Cells (hUCM-MSC) for the preservation of left ventricular function after acute myocardial infarction

Assessment of invasive hemodynamic effects and potential underlying mechanisms in a large animal pre-clinical model

PhD Dissertation

Luís Raposo, MD | Lisbon | June 2023

Supervisors

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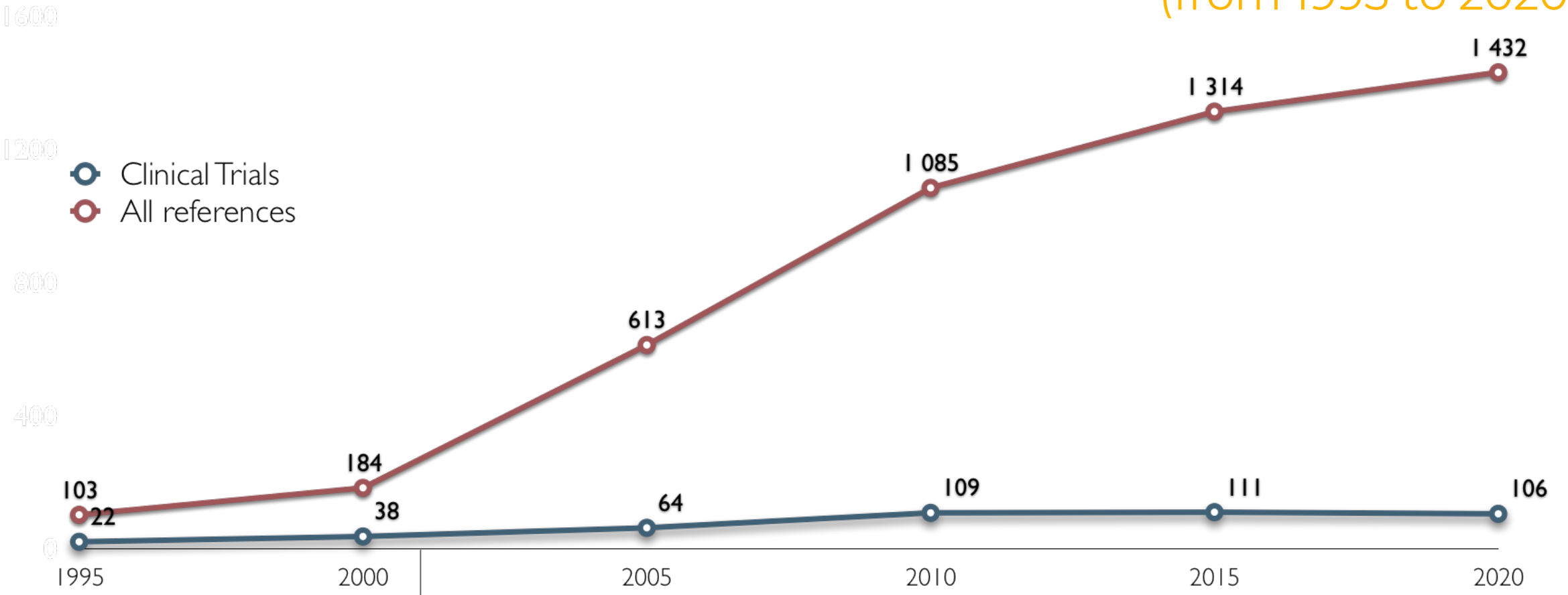
Faculdade de Medicina Universidade do Porto

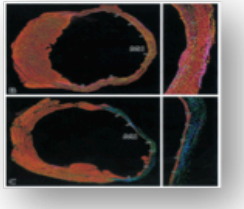
Background

PubMed® References on "Stem Cell Therapy" and "Cardiovascular disease" (from 1995 to 2020)



Orlic *et al.* **Bone marrow cells regenerate infarcted myocardium.**
Nature. 2001 Apr 5;410(6829):701-5.

Intramyocardial injection of Lin-/cKit+ autologous bone-marrow derived cells in the peri-infarct area of experimental MI in rats led to less scar formation.



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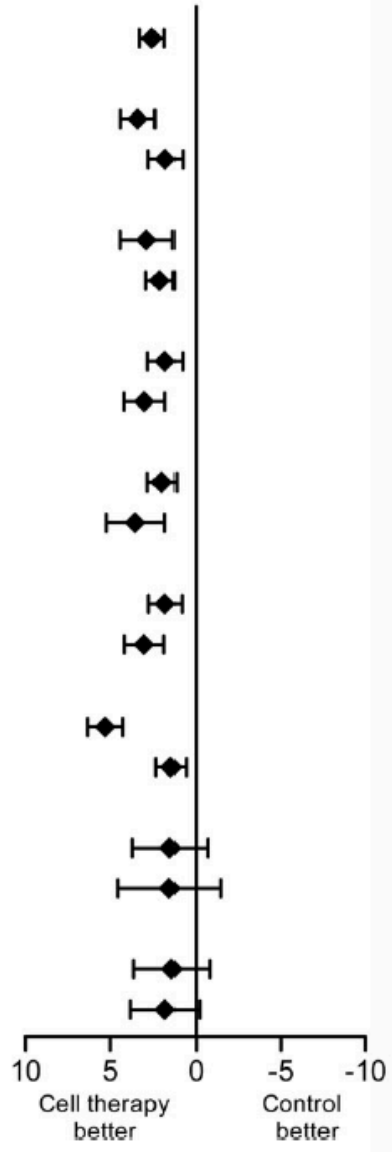
The evolving concepts of regenerative and Cell therapies for heart disease

1st Generation	2nd Generation	3rd Generation	Emerging strategies
Skeletal myoblasts	**Allogeneic MSCs (adipose, BM, UC…)**	Embryonic stem cells (ESC)	**Whole organ matrix decellularization**
Autologous Bone Marrow	Cardiac Stem Cells (cKit+ CSC)	Induced Pluripotent Cells (iPS)	Biomaterials
Circulating blod derived EPCs (CD133)	Cardiosphere Derived Cells (cKit+ CDC)	**Exossomes & Microvesicles** (non-coding mRNAs, cytokines, groth factors…)	
Bone Marrow Fractions (MNC, EPS, MSC)	Combined Cell Therapy and Hybrid strategies		
Autologous adipose derived cells (ADSC)	**Repeated cell administrations**		
Cell & target organ preconditioning (hypoxia, shock waves…)			
	Lineage commitment with cardiopoietic cocktails and gene transfection		

Most cell therapy studies in AMI have used autologous bone marrow derived cells

Characteristics	N	Treatment effect (95% CI)	p-value*
Overall ¥	1494	2.55 (1.83, 3.26)	
Age (years)			
<55	676	3.38 (2.36, 4.39)	0.03
≥55	809	1.77 (0.80, 2.74)	
Diabetes			
Yes	229	2.87 (1.32, 4.41)	0.39
No	1263	2.10 (1.29, 2.90)	
Symptoms to PCI (hours)			
<6	764	1.79 (0.81, 2.77)	0.13
≥6	509	2.98 (1.79, 4.16)	
IRA			
LAD	1239	1.98 (1.16, 2.80)	0.11
RCA/LCX	235	3.53 (1.82, 5.23)	
LVEDVI (ml/m²)			
<100	988	1.79 (0.84, 2.73)	0.11
≥100	326	3.00 (1.85, 4.15)	
LVEF (%)			
<40	503	5.30 (4.27, 6.33)	<0.001
≥40	991	1.45 (0.60, 2.31)	
Infarct size (g)			
<20	169	1.50 (-0.69, 3.69)	0.84
≥20	355	1.54 (-1.46, 4.54)	
MVO			
Presence	350	1.41 (-0.79, 3.61)	0.67
Absence	196	1.80 (-0.21, 3.80)	



Effect of intracoronary BMC on left ventricular remodelling and Ejection Fraction in STEMI patients

Patient level Meta-analysis of 16 RCTs

N=1,494 pts (986 BMC vs 657 controls)

Parameter	Variation vs. control	*p*
FEVE (%)	+2.55% (1.8 to 3.3)	<0.001
VTSi (ml/m²)	-3.17 (-4.8 to -1.47)	<0.001
VTDi (ml/m²)	-2.6 (-3.84 to -1.35)	<0.001

Timing of IC injection

within 1 day (1RCT)

3 to 28 days (15 RCTs)

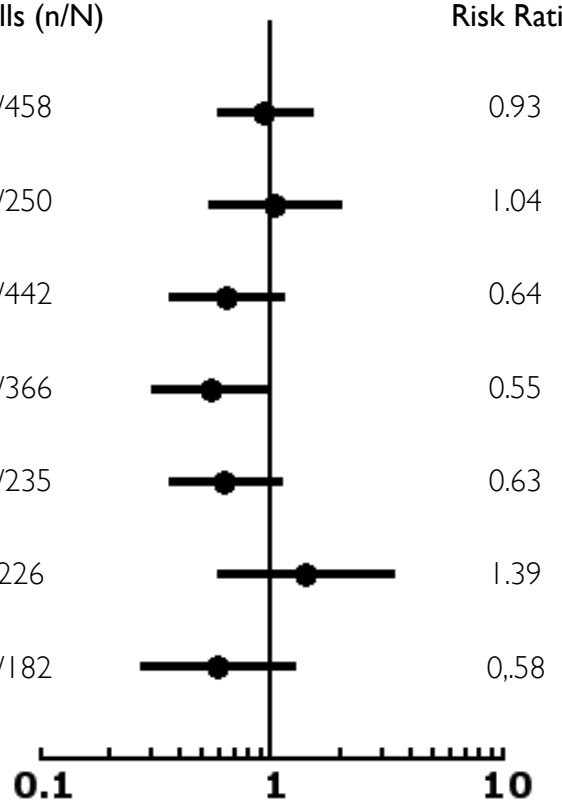
European Heart Journal (2014) 35, 989–998

Most cell therapy studies in AMI have used autologous bone marrow derived cells

Meta-Analysis & Review of **41 RCTs** of autologous Bone Marrow derived Cells

N=1564 active treatment vs N=1168 controls

Outcome	Cells (n/N)	No Cells (n/N)	Risk Ratio	95% CI	*p* value	Heterogeneity (I^2)
Total Mortality	34/538	32/458	0.93	0.58-1.5	0.77	0%
Cardiovascular Mortality	23/277	18/250	1.04	0.54-1.99	0.91	7%
Myocardial Infarction	20/624	25/442	0.64	0.36-1.12	0.12	0%
Admission for HF	18/459	27/366	0.55	0.30-1.0	0.05	0%
Death/MI/HF	24/262	33/235	0.63	0.36-1.1	0.10	12%
Arrhythmia	11/231	7/226	1.39	0.58-3.37	0.46	0%
Restenosis	10/213	14/182	0,.58	0.27-1.25	0.17	0%

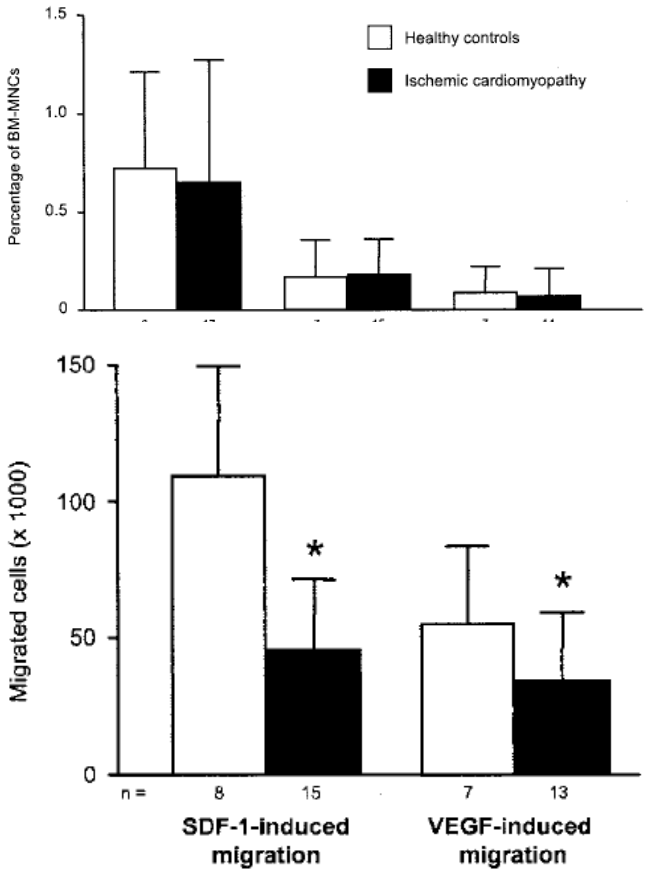


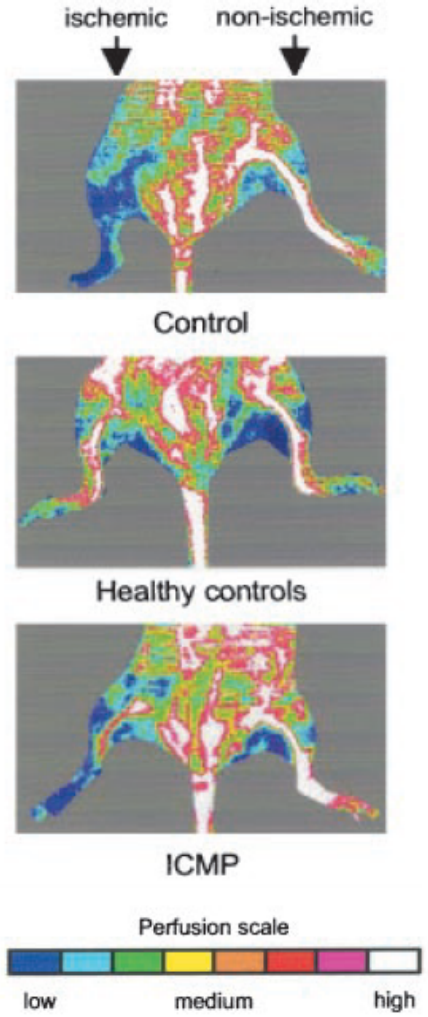
Favors Cell Therapy Favors Standard Therapy

Cochrane Database of Systematic Reviews 2015, Issue 9. Art. No.: CD006536.
DOI: 10.1002/14651858.CD006536.pub4.

Impaired functional potency has been considered a limitation for translational effects of autologous cells

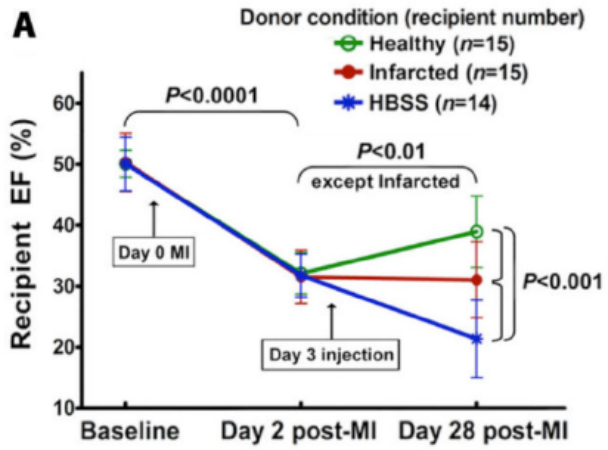
Profoundly Reduced Neovascularization Capacity of Bone Marrow Mononuclear Cells Derived From Patients With CIHD

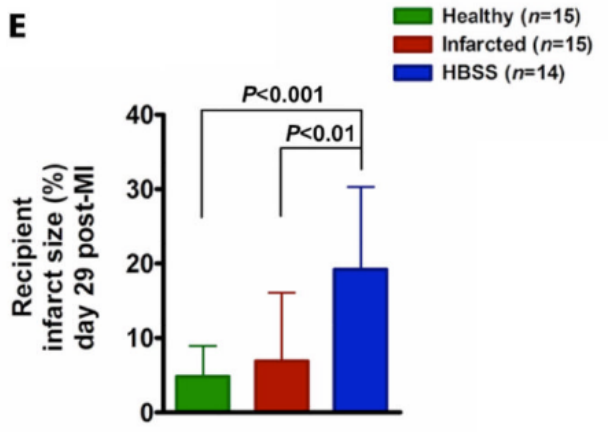




Circulation. 2004;109:1615-1622

Donor Myocardial Infarction Impairs the Therapeutic Potential of Bone Marrow Cells by an IL-1-Mediated Inflammatory Response



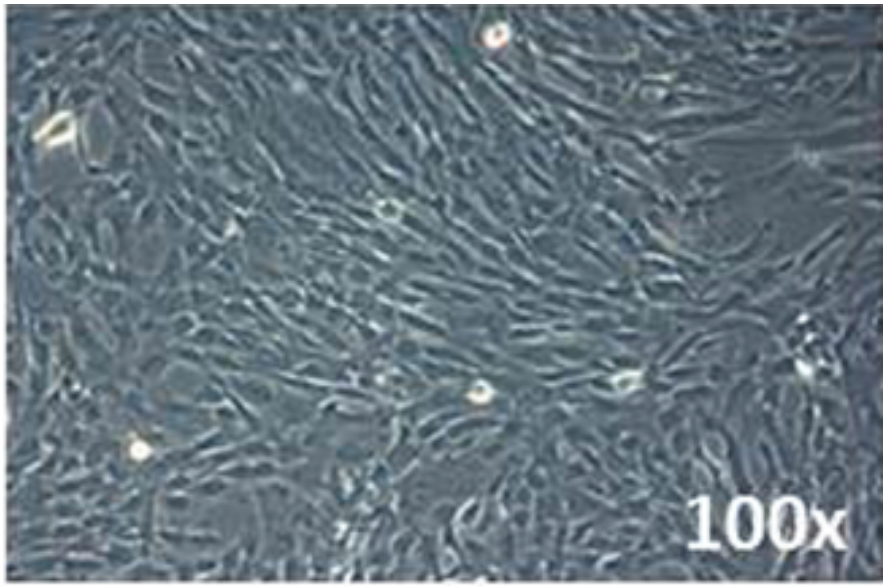


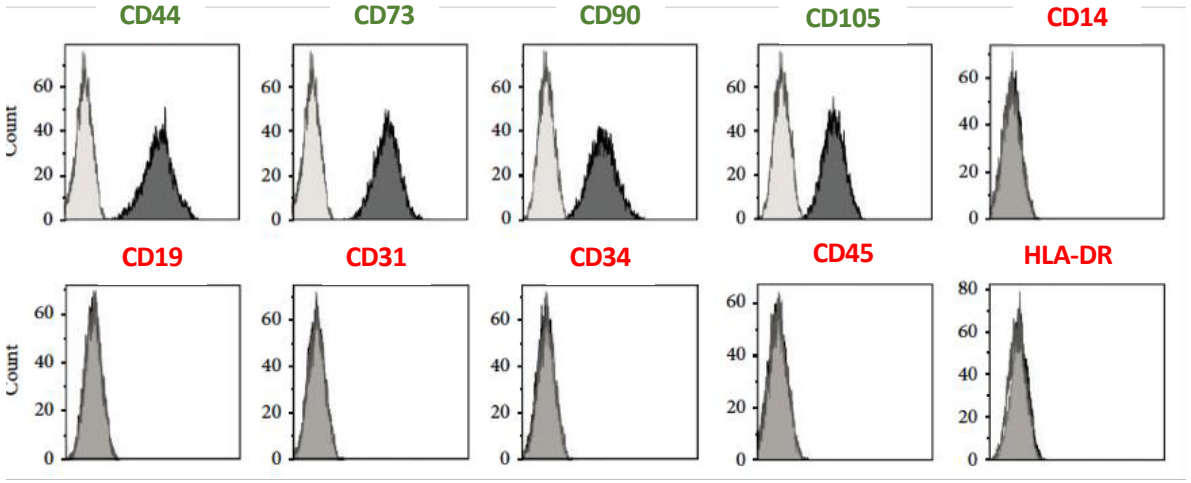
Sci Transl Med. 2011 September 14; 3(100): 100ra90.

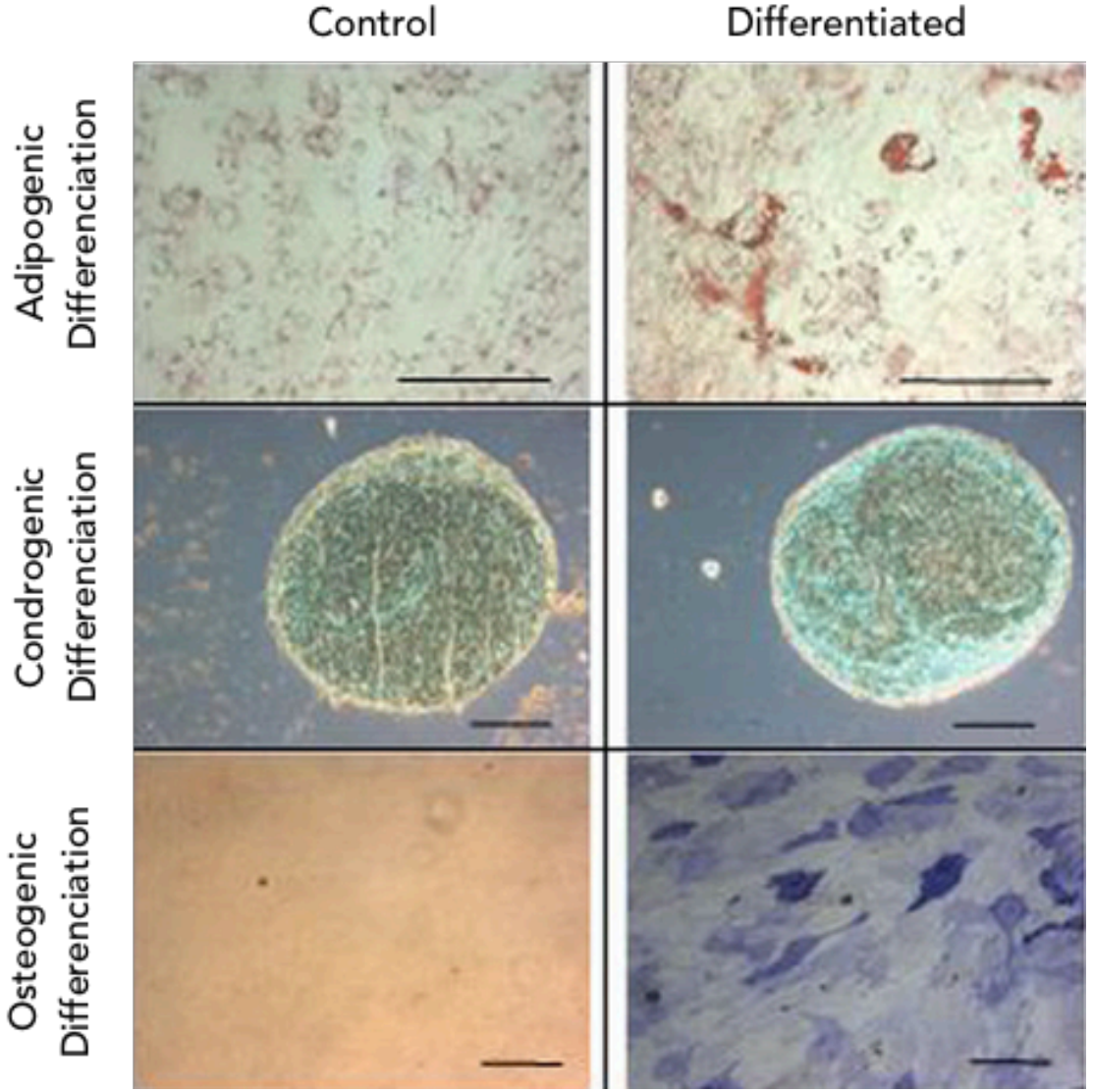
Limitations of autologous cell therapy protocols

- Biological potency affected by patient comorbidity, including AMI itself
- Need for invasive harvest.
- Unavailable for acute administration (reperfusion adjuvant)
- Standardization of protocols and impact of cell manipulation
- Inconsistency/heterogeneity of cell product (number and composition)

Mesenchymal Stromal Cell as ideal candidates to allogeneic cell therapy







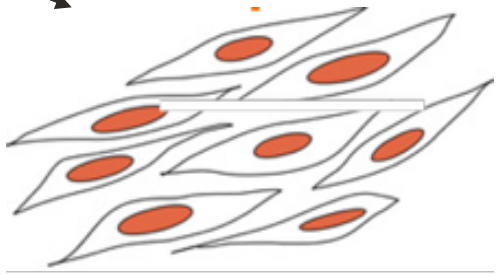
Mesenchymal Stromal Cell as ideal candidates to allogeneic cell therapy

Fibroblasts (iPS)

Peripheral Blood

Bone Marrow

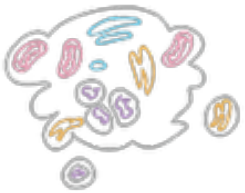
Adipose tissue



Foetal Tissues
Placenta
Cord Blood
Wharton's jelly
UC perivascular MSC

Mesenchymal Stem Cells

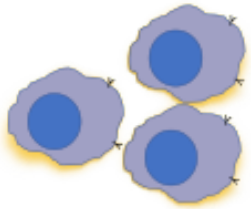
IL-6
LIF
MMP
TIMP
THBS-1
miR-25-3p
miR-210
miR-23a3p



Inhibition of programmed cell death

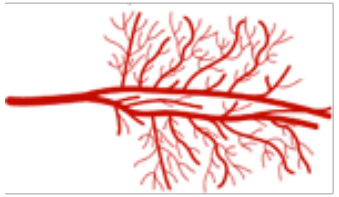
HGF
IGF
TNF*alfa*
miR-212-5p

Proliferation & rejuvenation of Residente cells



Inflammation & imuno-modulation

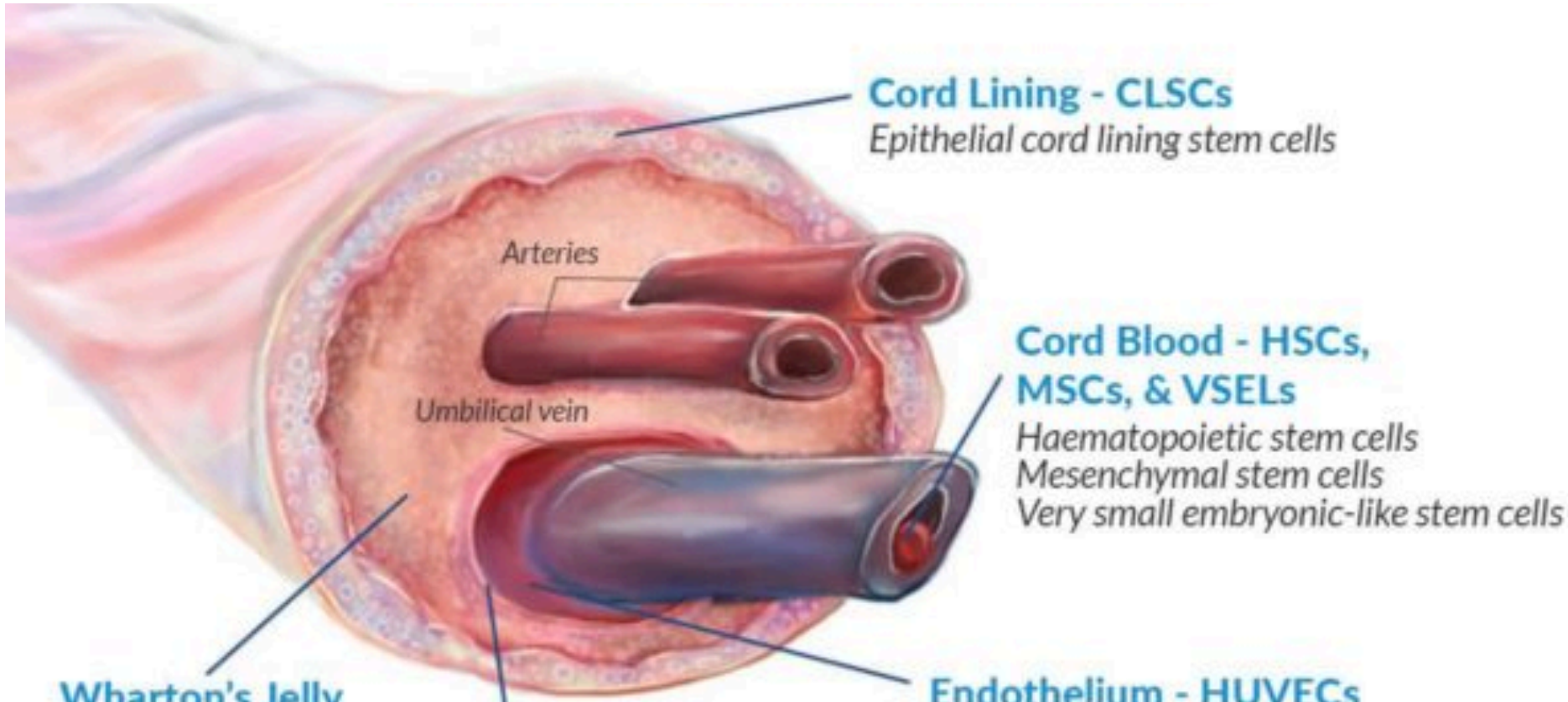
IL-10
TGFß
LIF
soluble HLA-G
IL-1r antagonist
miR-182
miR-212-5p



Angiogenesis

VEGF
FGFs
P1GF
HGF
ANGPT-1/2
bFGF
PDGFß
miR-519D

The Human Umbilical cord as a Source of precursor cells



Potential advantages of Human Umbilical cord matrix derived MSC

- Can be obtained in very large numbers (virtually unlimited)
- Do not need invasive harvest & little ethical concerns
- May be available as an *off-the-shelf* product (suitable for the acute setting)
- Enhanced capacity of cardiopoietic induction.
- Better secretory profile than adult MSC (less prone to comorbidity / senescence)

Most human studies with MSC in AMI have used adult-derived cells, administered days after reperfusion

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Author (year)	Source of MSCs	Comparison	MSCs Route, condition	Timing	Dosage and volume
Acute myocardial infarction					
Chen (2004)	Autologous	Saline	IC, Fresh	18 ± 0.5 days post-PCI	8–10 × 10^9,1 dose – NR
Chullikana (2015)	Allogeneic	Plasmalyte A	IV, Fresh from cryopreserved	2 days post-PCI	2 million cells/kg, 1 dose – 0.5 mL/kg
Gao (2013)	Autologous	Standard treatment	IC, Fresh	Post-PCI Mean 17 ± 1 days	3 ± 0.5 × $10^6$1 dose – 10 mL
Gao (2015)	Allogeneic	Heparanized saline	IC, Fresh	Within 5–7 days of PCI	6 × 10^6, 1 dose – 10 mL
Hare (2009)	Allogeneic	Placebo	IV, NR	Patients randomized 1–10 days post AMI	0.5, 1.6, 5 × 10^6 3 dose escalation cohorts - NR
Houtgraaf (2012)	Autologous	Placebo	IC, Fresh	24 hours post-PCI	17.4 ± 4.1 × 10^6 1 dose - NR
Lee (2014)	Autologous	Standard treatment	IC, Fresh	BM aspiration done 4 ± 2 days post-admission; culture took 25.0 ± 2 days	7 ± 1 × 10^7 1 dose - NR
Musialek (2015)	Allogeneic	No comparison	IC, Fresh from cyropreserved	Within 5–7 days of PCI	30 × 10^6 1 dose – 30 mL
Rodrigo (2013)	Autologous	Historical control	IM, Fresh	21 ± 3 days post-MI/PCI	31 ± 2 × 10^6 1 dose – 5 mL
Wang (2014)	Autologous	Saline	IC, Fresh	14 days post-PCI	2 × 10^8 1 dose – 2 mL
Yang (2010)	Autologous	No comparison	IC, Fresh	NR	1 ± 2 × 10^7 1 dose - NR

Stem Cells Transl Med. (2018) 7(12):857–66

No studies have been performed using hUCM-MSC in relevant preclinical models of AMI with reperfusion

Characteristics of experimental pre-clinical studies of UC-MSC in Acute Myocardial Infarction

Study Reference	Experimental Model	Study Design	Placebo	Blinding of endpoint evaluation
Latifpour *et al.*[154] Cardiology, 2011	Surgical LAD ligation Rabit Transepicardic injection	Non-randomized Positive and negative controls	Yes	Yes (for Echocardiography) Not reported for pathology and immunohistochemistry analysis
Zhang *et al.*[155] Coron Artery Dis. 2013	Surgical LAD ligation Mini pig Transepicardic injection	Randomized Placebo controlled	Yes	Not reported for any of the endpoints
Lim *et al.*[156] Stem Cell Res Ther. 2018	Surgical LAD ligation Mini pig Repeated intravenous injection	Non-randomized Placebo controlled	Yes	Not reported for any of the endpoints

Cytotherapy. (2021) 23 (11):974–9

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Cytotherapy

Supports *open access*

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Cytotherapy

Volume 23, Issue 11, November 2021, Pages 974-979

8
CiteScore

6.196
Impact Factor

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Review

Human umbilical cord tissue-derived mesenchymal stromal cells as adjuvant therapy for myocardial infarction: a review of current evidence focusing on pre-clinical large animal models and early human trials

Luís Raposo [1 2 3], André P. Lourenço [4 5], Diana S. Nascimento [6 7 8], Rui Cerqueira [4 5], Nuno Cardim [2 3], Adelino Leite-Moreira [4 5]

Study Aims

Study Purposes

General Objective

- To contribute to the evidence supporting the use of an allogeneic **GMP-compliant hUCM-MSC cell product** suitable for human trials and, eventually, clinical use.

Specific Objectives

- To establish a fully percutaneous large-animal pre-clinical model of AMI with reperfusion suitable for preclinical experimentation.

- To investigate the **safety** and **efficacy** of intracoronary delivery of hUCM-MSC as **adjuvant therapy** in a relevant preclinical large-animal (swine) model of acute myocardial infarction with reperfusion.

Scientific hypothesis & Primary endpoint

Study hypothesis

- Intracoronary delivery of a **xenogeneic GMP-compliant hUCM-MSC** product, early after reperfusion is **superior to placebo** for preserving left ventricular function in the setting of experimental myocardial infarction.

Primary endpoint

- **LV ejection fraction** evaluated at 8-Weeks follow-up after reperfused acute myocardial infarction, using high-fidelity invasive pressure-volume measurements.

Exploratory Secondary Endpoints

Efficacy	
Histopathological examination	*Semi-automated quantification of infarct size (MiQuant®)*
	Fibrosis and cardiomyocyte hypertrophy in the remote zone
Skinned cardiomyocytes	*Length-tension relationships (active and passive tension)*
Differential gene expression	*Whole genome RNA-sequencing*
Transthoracic echocardiogram	*Morphometric and functional parameters*
Biomarkers	*NT-proBNP and Galectine-3 (ELISA)*
Safety	
Assessment of coronary flow	*Doppler-derived real time coronary flow velocity*
Animal wellbeing and mortality	

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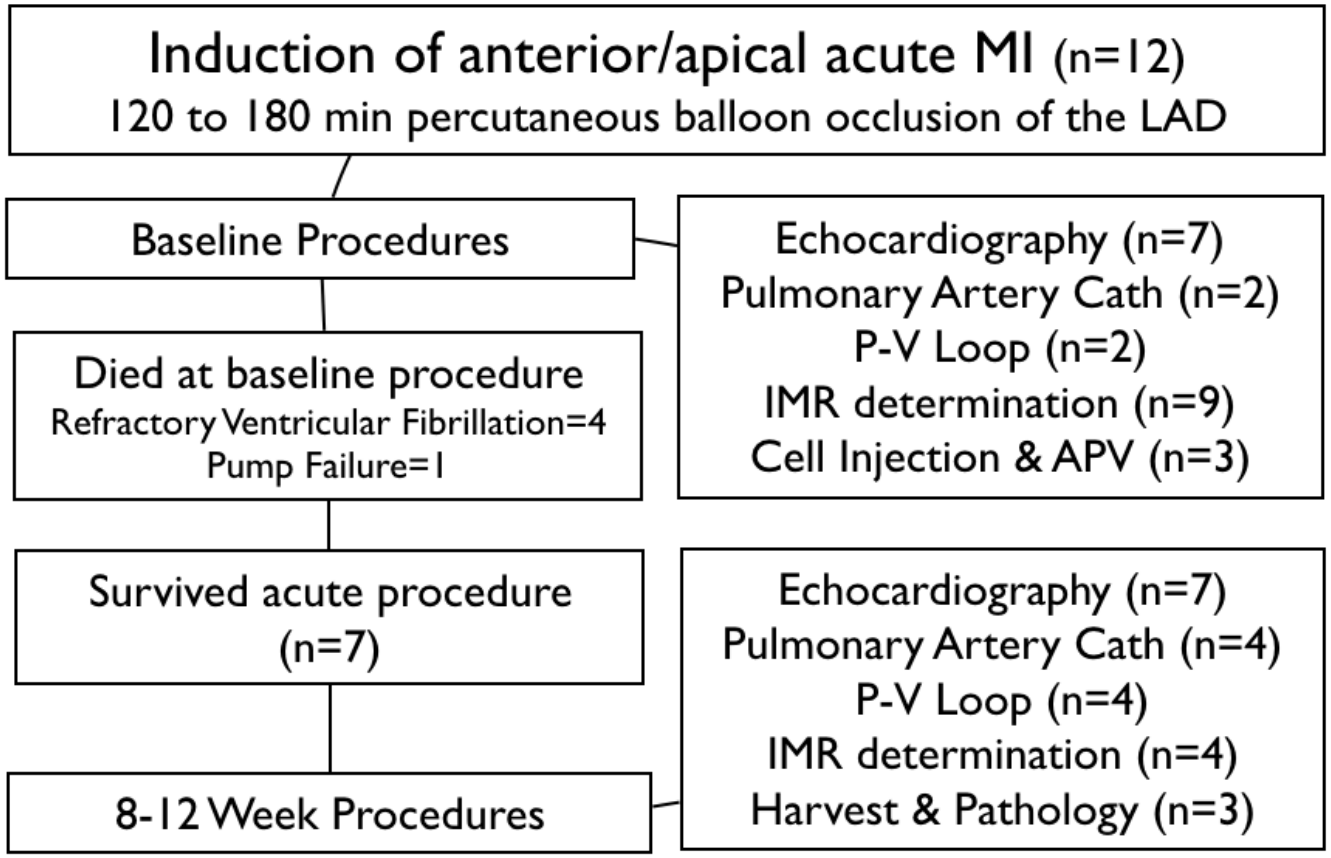
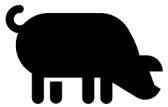
Methodology

Animal model and study procedures

Model Implementation Stage



Animal Species



- Vietnameese pot-belied pigs
- Male
- Aged ≥1 year
- Non-syngeneic

Distinctive model characteristics

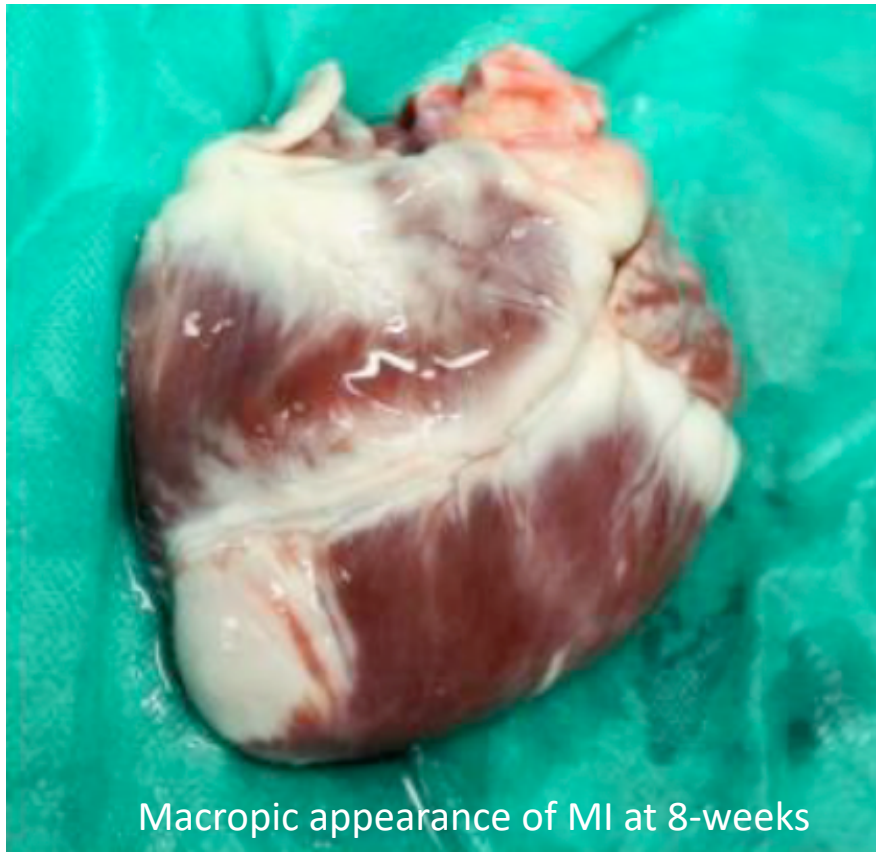
- Fully percutaneous approach
- AMI with reperfusion stage
- ACEi and BB treatment during FUP[1]

Cell dose & infusion rate

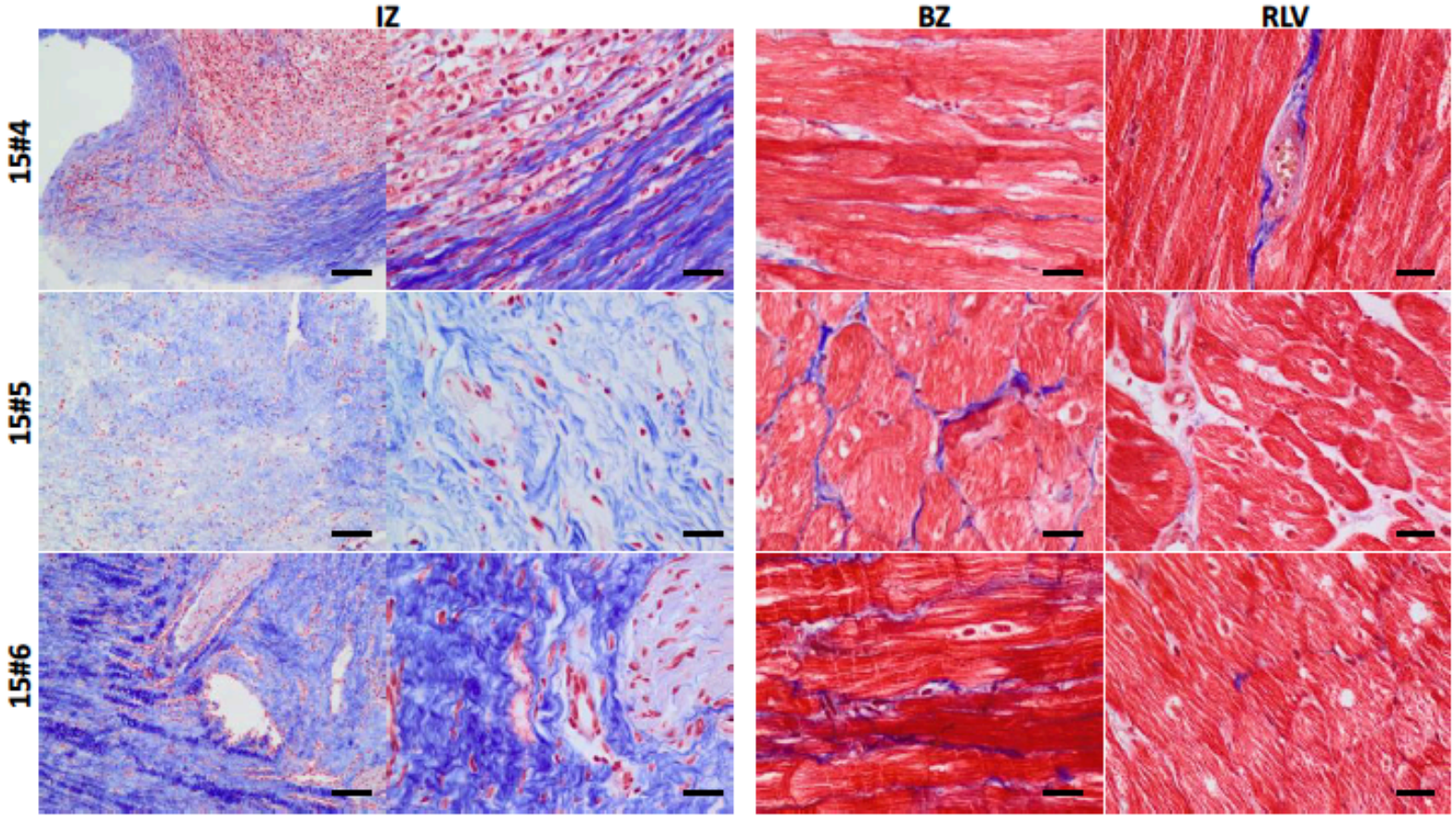
- $0.5x10^6$/Kg of body weight[2]
- Injectate Concentration: $0.5x10^6$/ml
- Infusion rate: 1 ml/min

[1] Comp Med. (2009) 59(3):272–9
[2] Circ Res. (2013) 113(2):153–66

Experimental model Implementation Stage



Macropic appearance of MI at 8-weeks

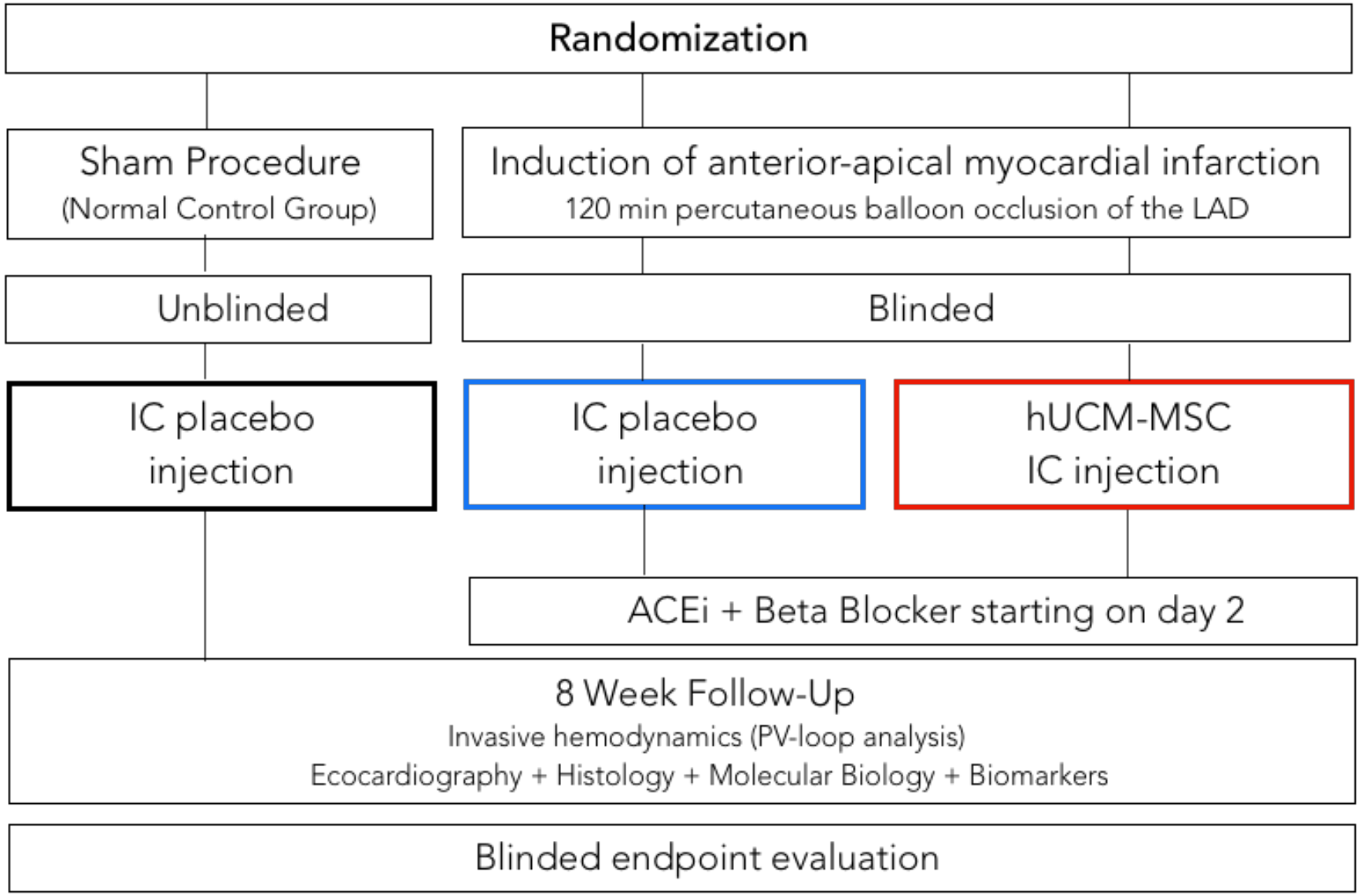


	INFARCT SIZE- AREA	INFARCT SIZE- MIDLINE-LENGTH
15#4	2.7%	0%
15#5	10.1%	13.1%
15#6	18,8%	17,5%

Representative sections of the (IZ), border zone (BZ) and remote LV region (RLV) stained with Trichrome Masson. Scale (IZ): 100 µm (left column) and 25 µm.

Randomized controlled experiment

Study design



Summary of Statistical analysis

Sample Size estimation

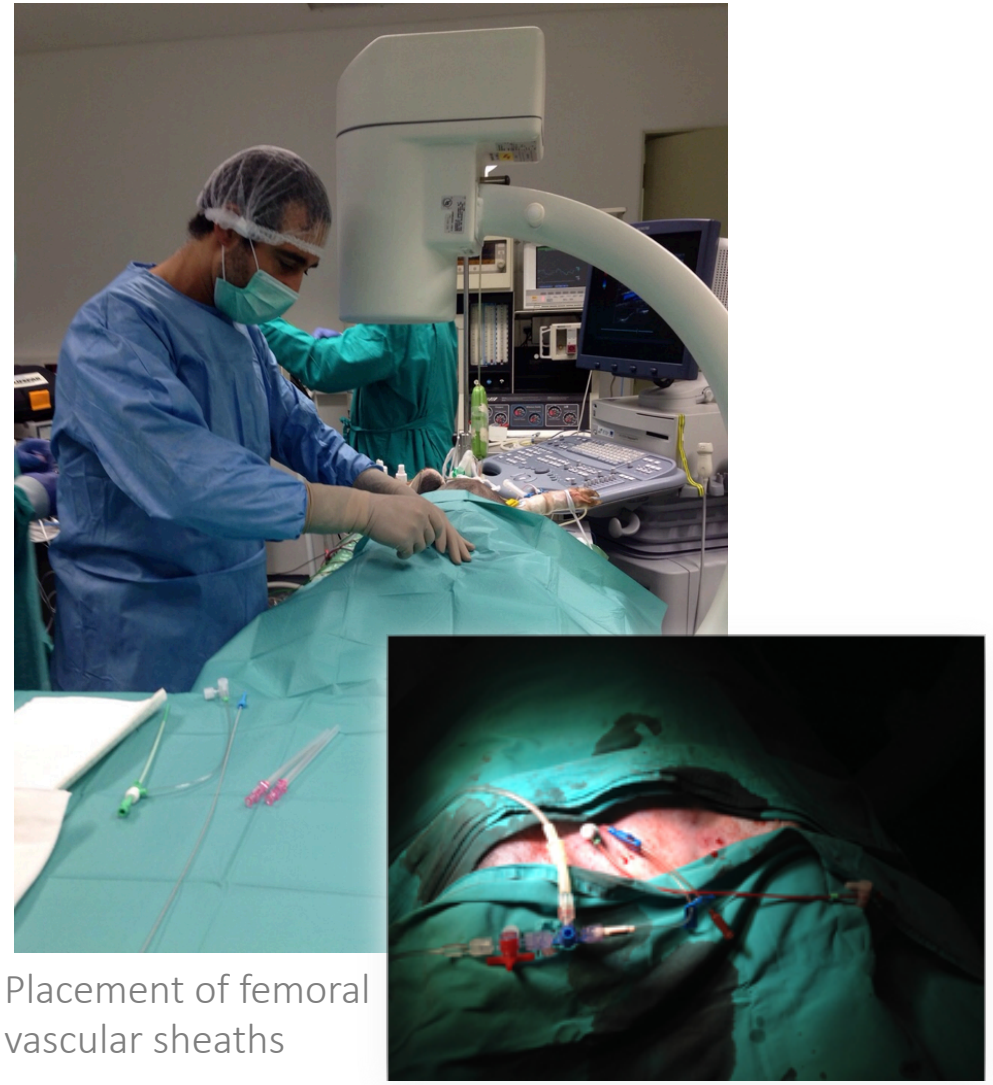
- **20% difference** in P-V loop derived ejection fraction in the active treatment group compared with vehicle
- 80% power, with significance level set at 0.05 for a 2-sided comparison of means in one-way ANOVA
- Estimated n=7 animals per group for final analysis (25% acute mortality accounted for).

Descriptive and inferential

- Shapiro-Wilk's test for normality
- One-Way ANOVA between groups with normal distributions (& Tukey's or Fischer's LSD for *post hoc* comparisons between groups)
- Kruskal-Wallis's test for non-parametric data (& FDR method of Benjamini and Hochberg adjustment for multiple comparisons)
- End-systolic and end-diastolic P-V relationships compared by ANCOVA with end-systolic elastance and chamber stiffness constant beta as dependent variables and other coefficients as covariates.

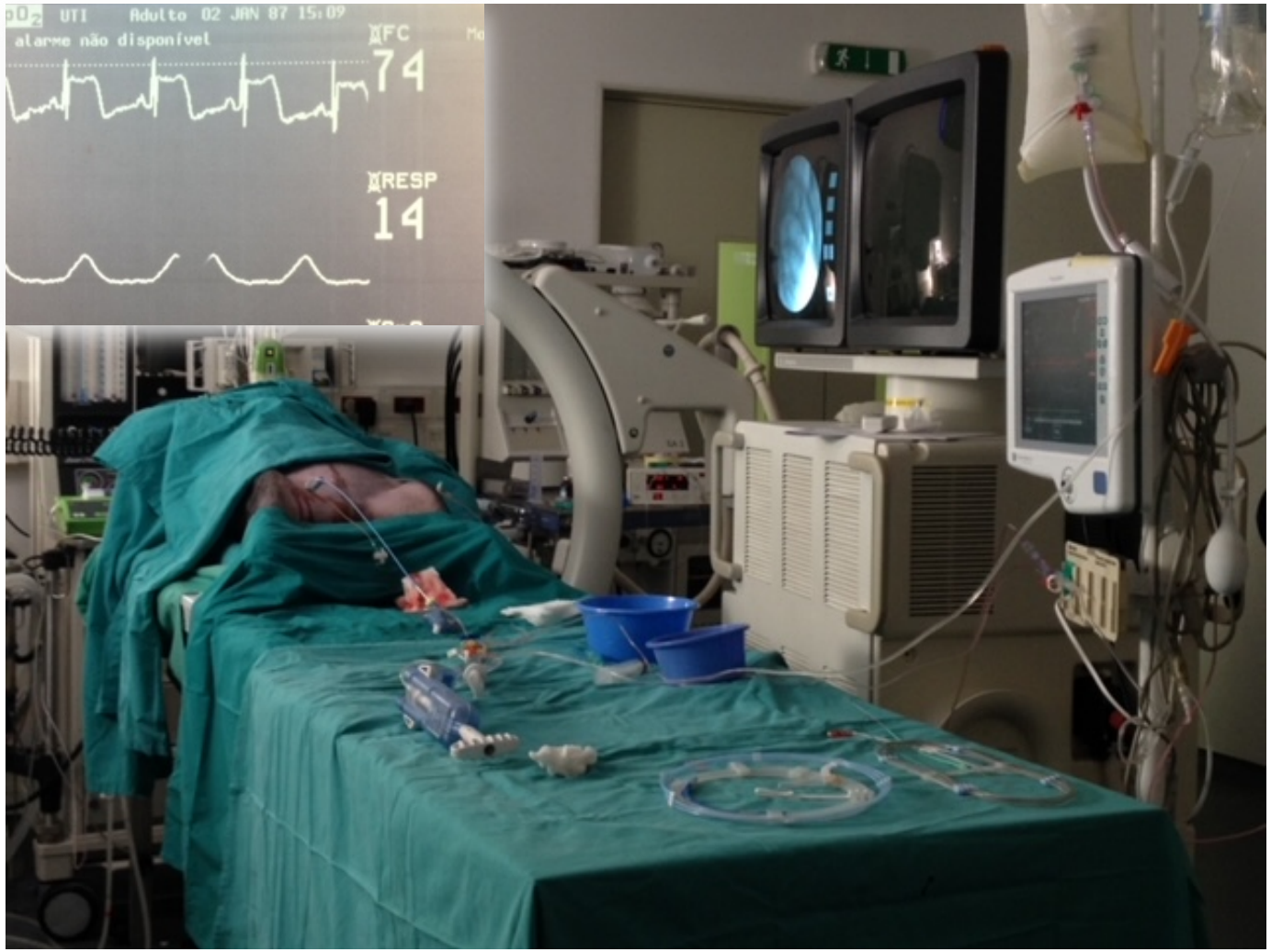
Study Procedures
Experimental setup

Overview of the operating room

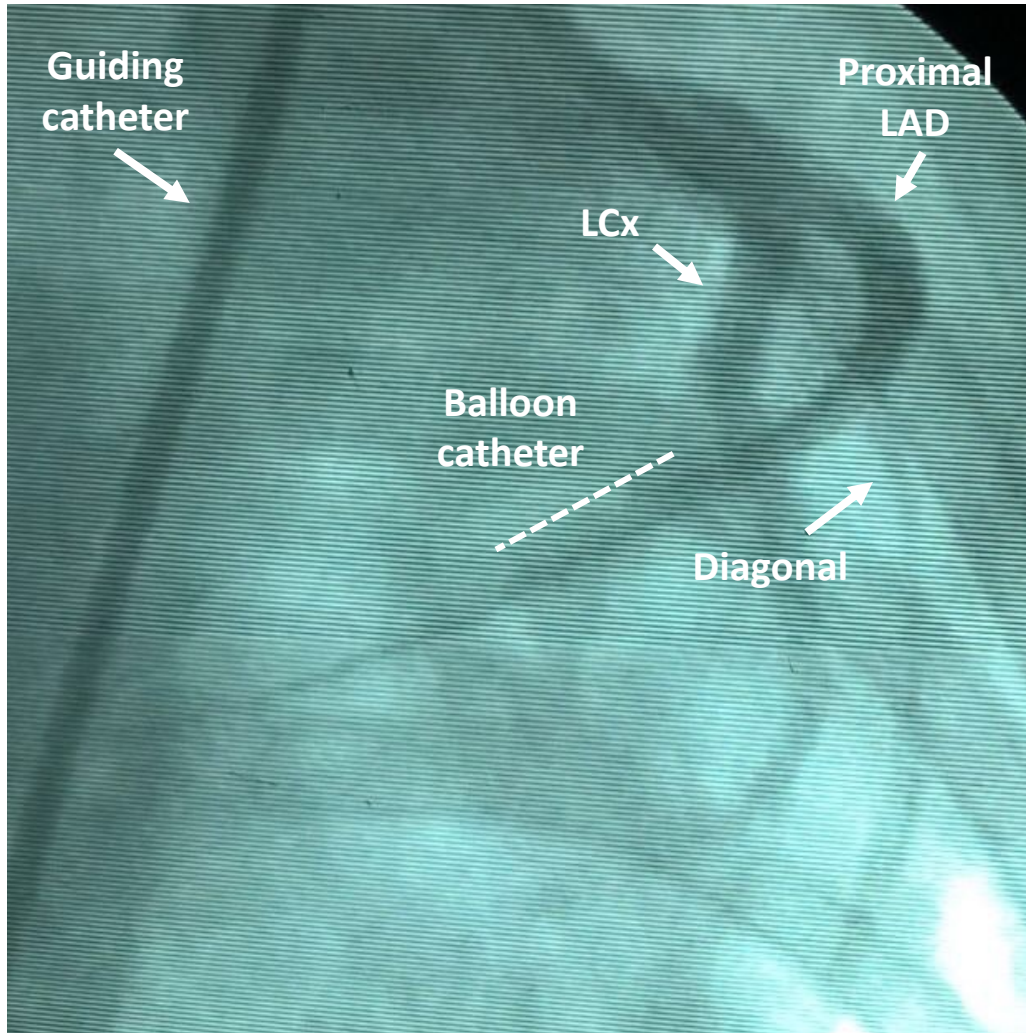


Placement of femoral vascular sheaths

Study Procedures
Induction of myocardial infarction



Animal during the MI induction procedure



Balloon oclusion of the mid LAD during 120 minutes

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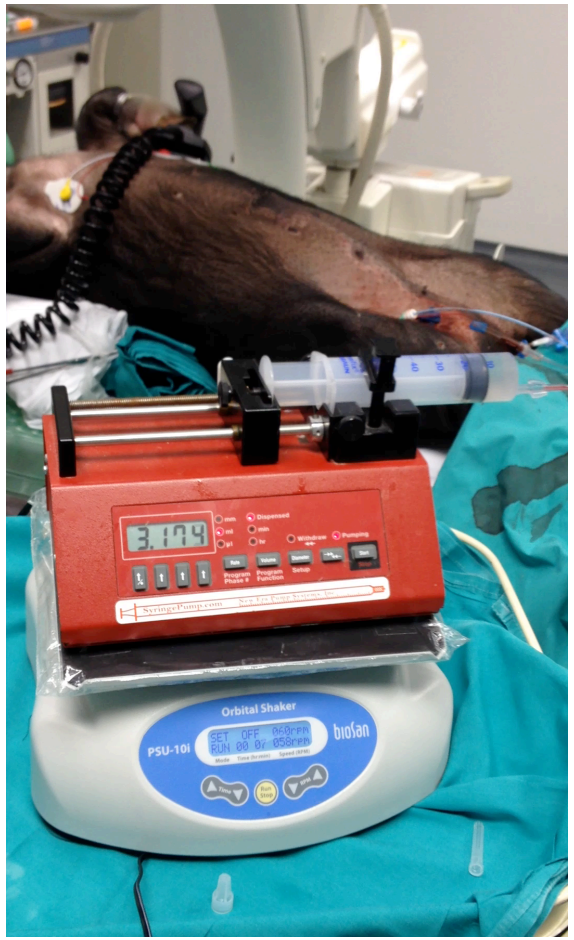
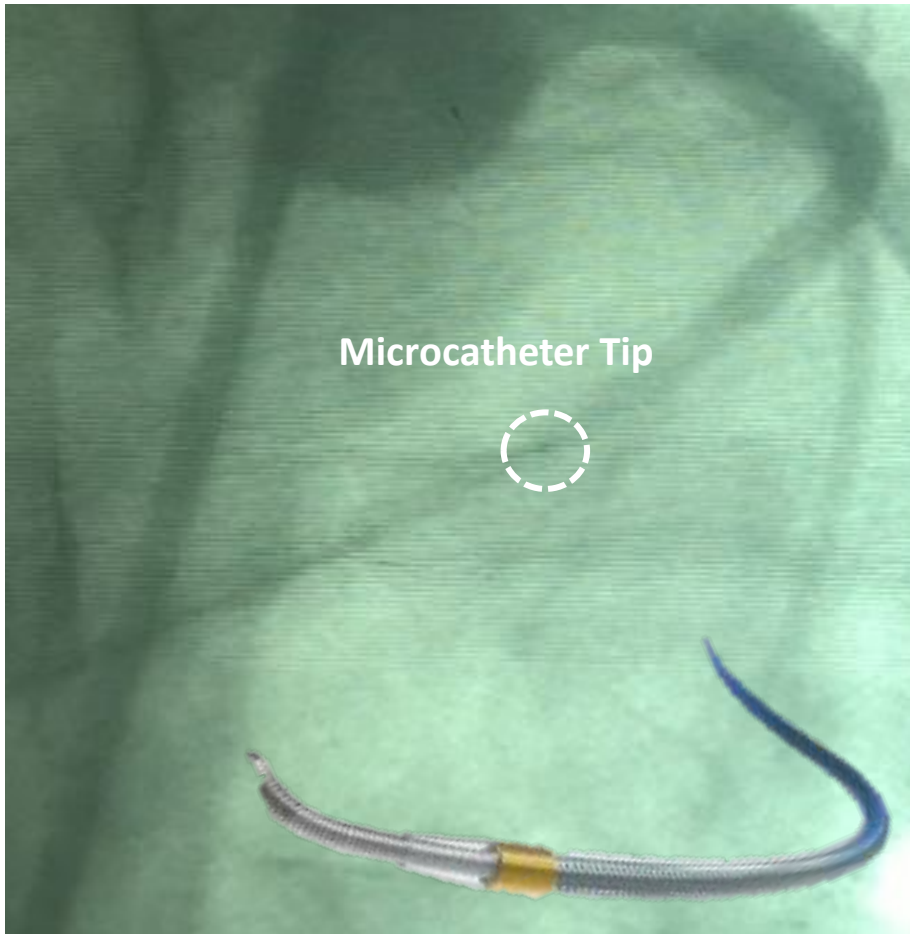
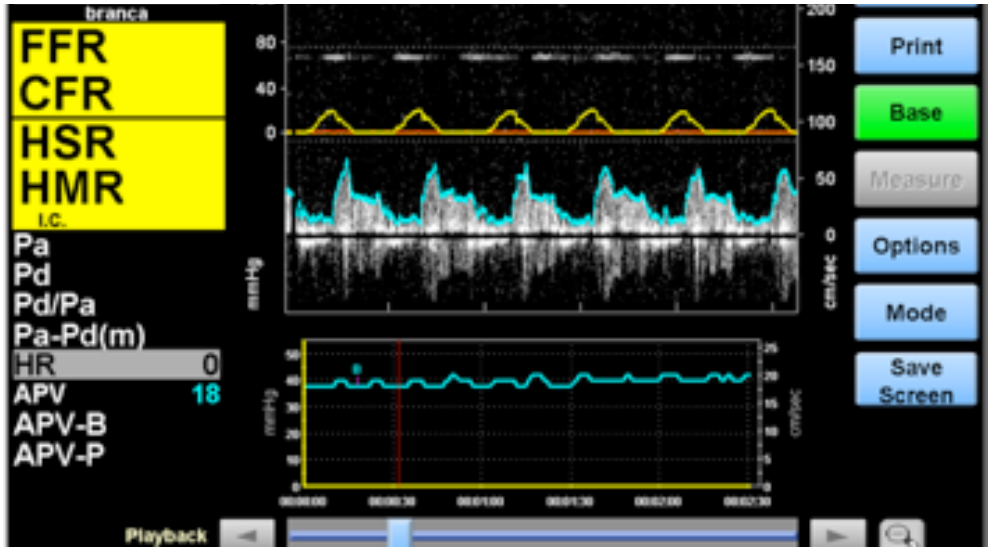
Study Procedures

Intracoronary delivery of hUCM-MSC & coronary flow





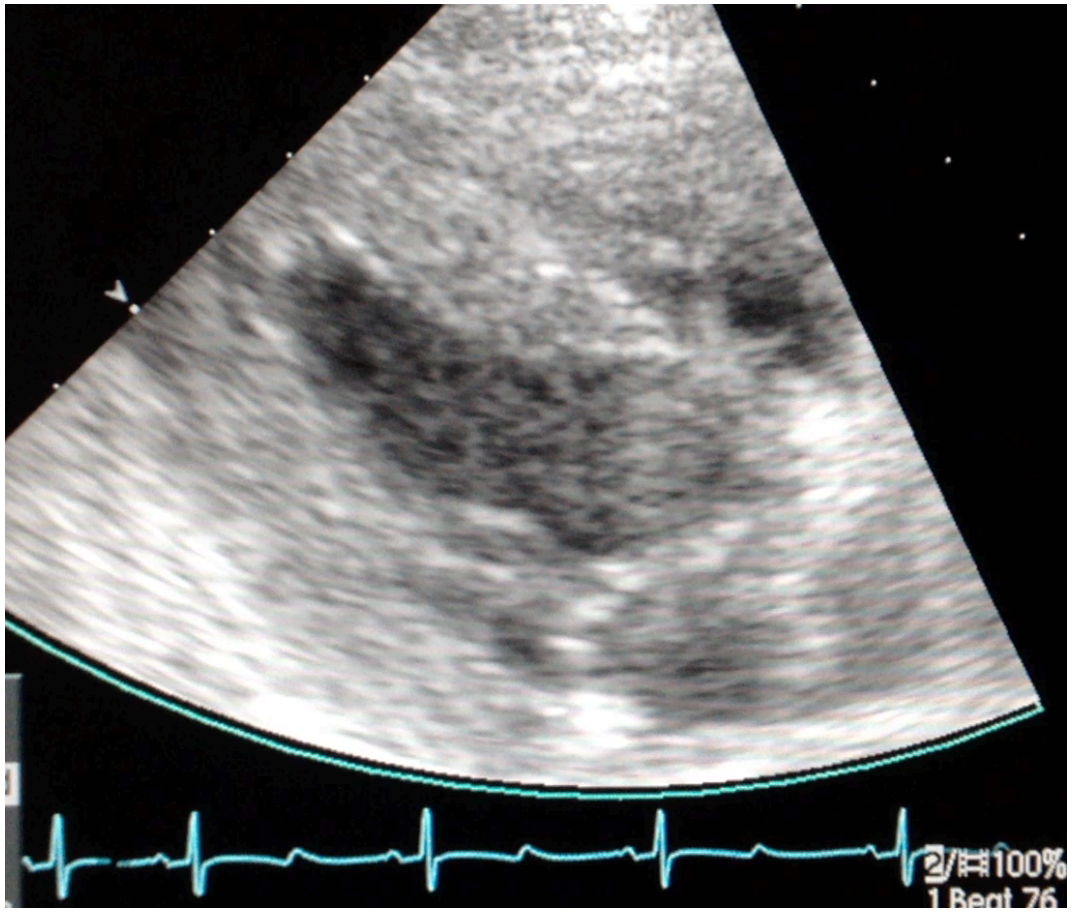
Intracoronary infusion of $0.5x10^6$ hUCM-MSC/Kg/min (or matching placebo) through a microcatheter in the infarct related artery



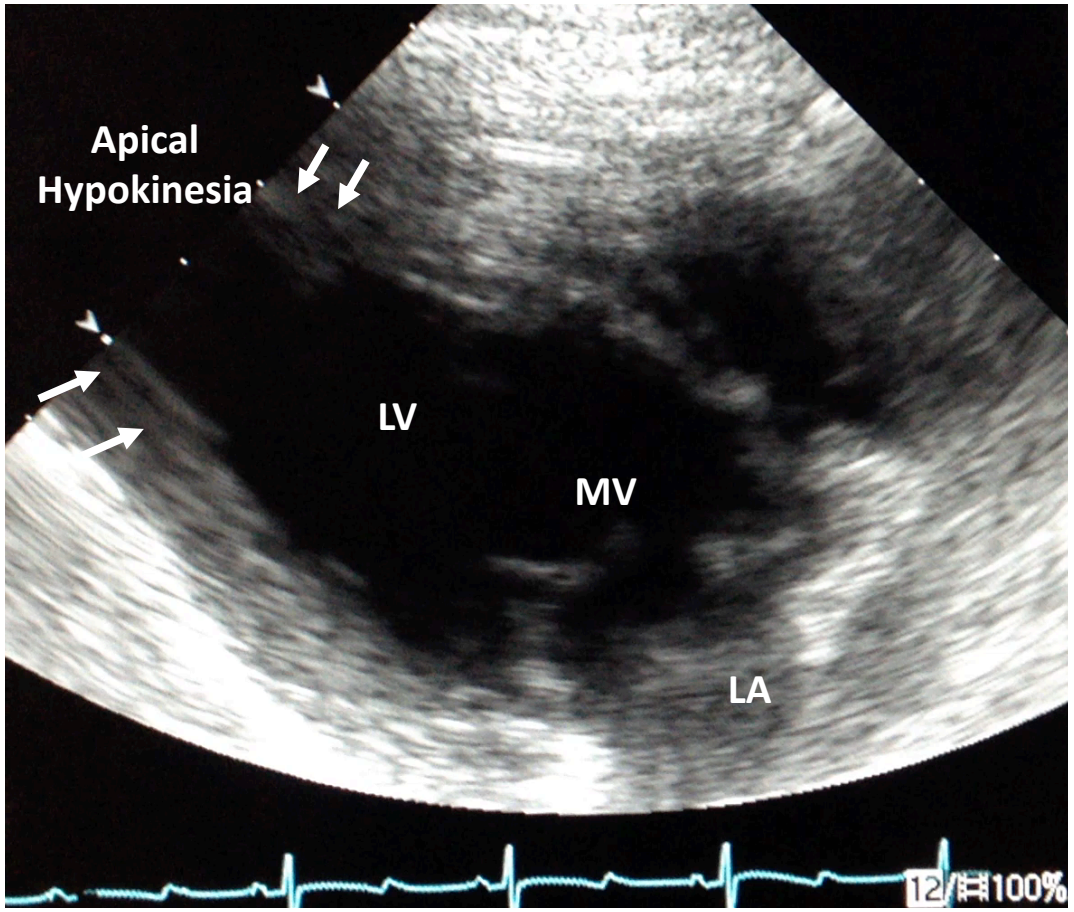
Real time flow velocity measurement using a doppler sensor-tipped guidewire

Study Procedures
Transthoracic Echocardiogram

Sham (normal LV)

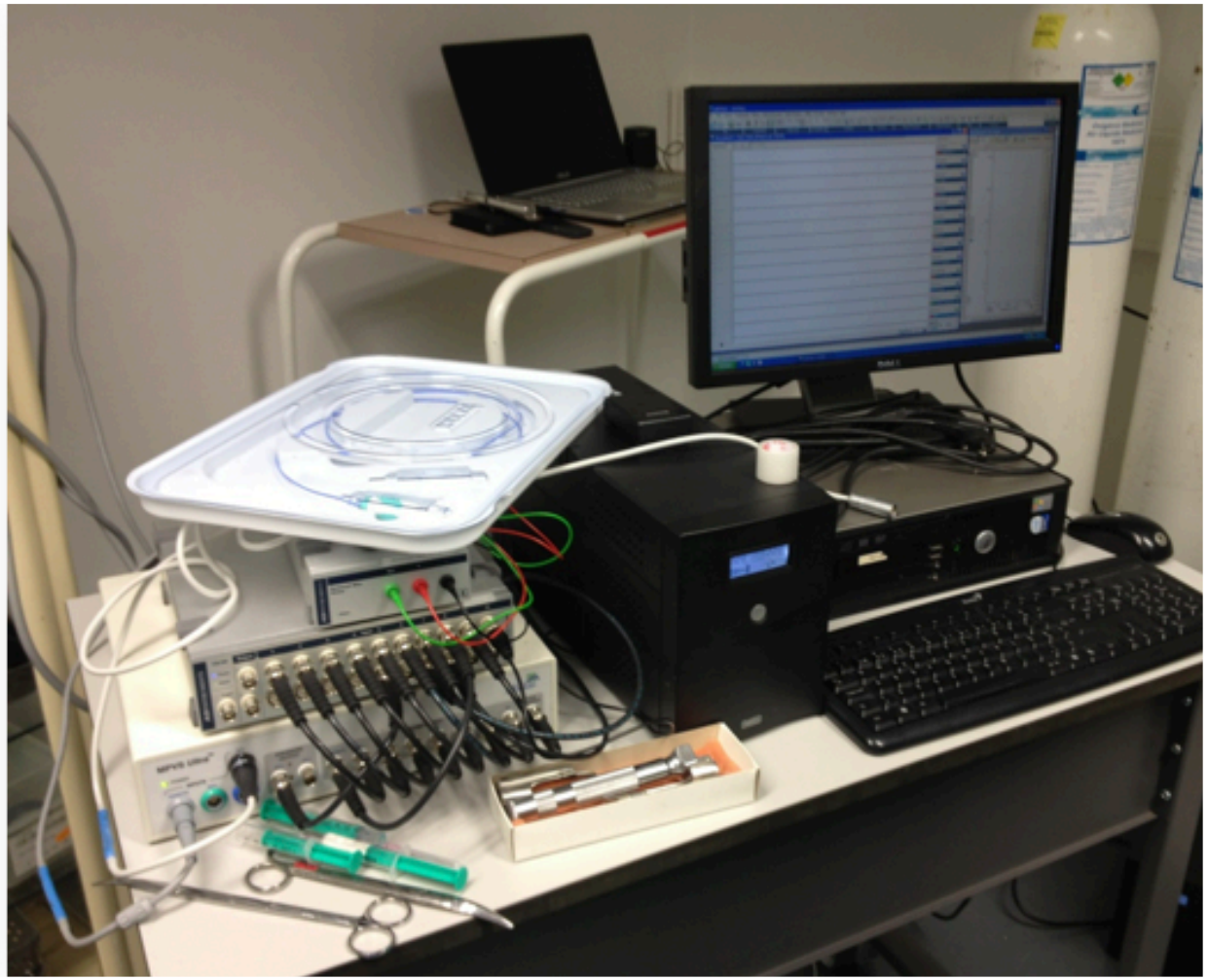


Post-AMI TTE (PLAX)

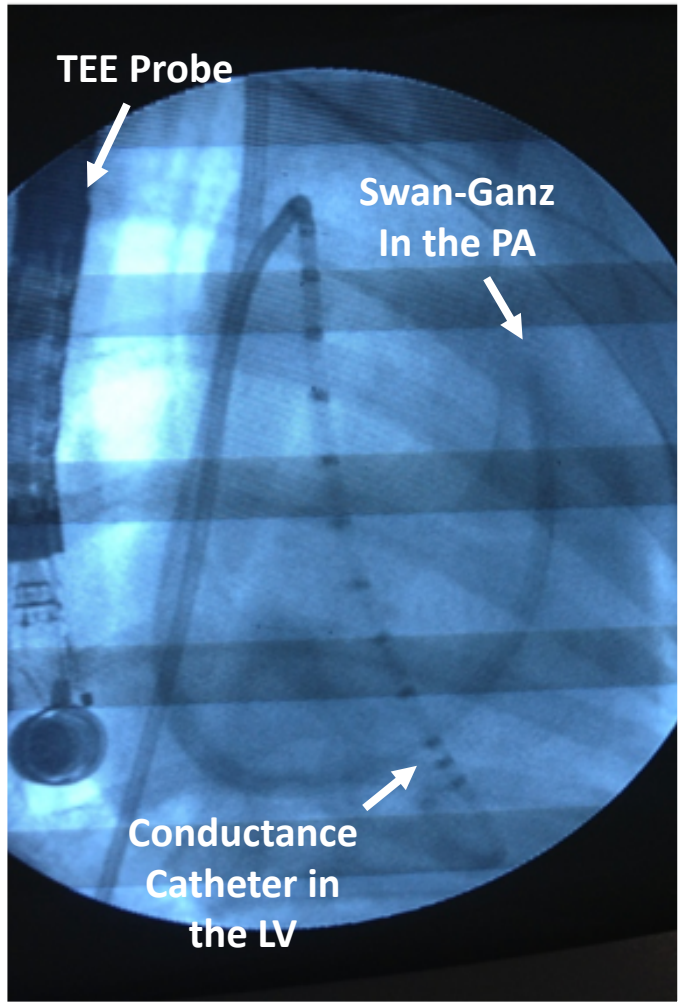


Study Procedures

Invasive Hemodynamic assessment (P-V loop)



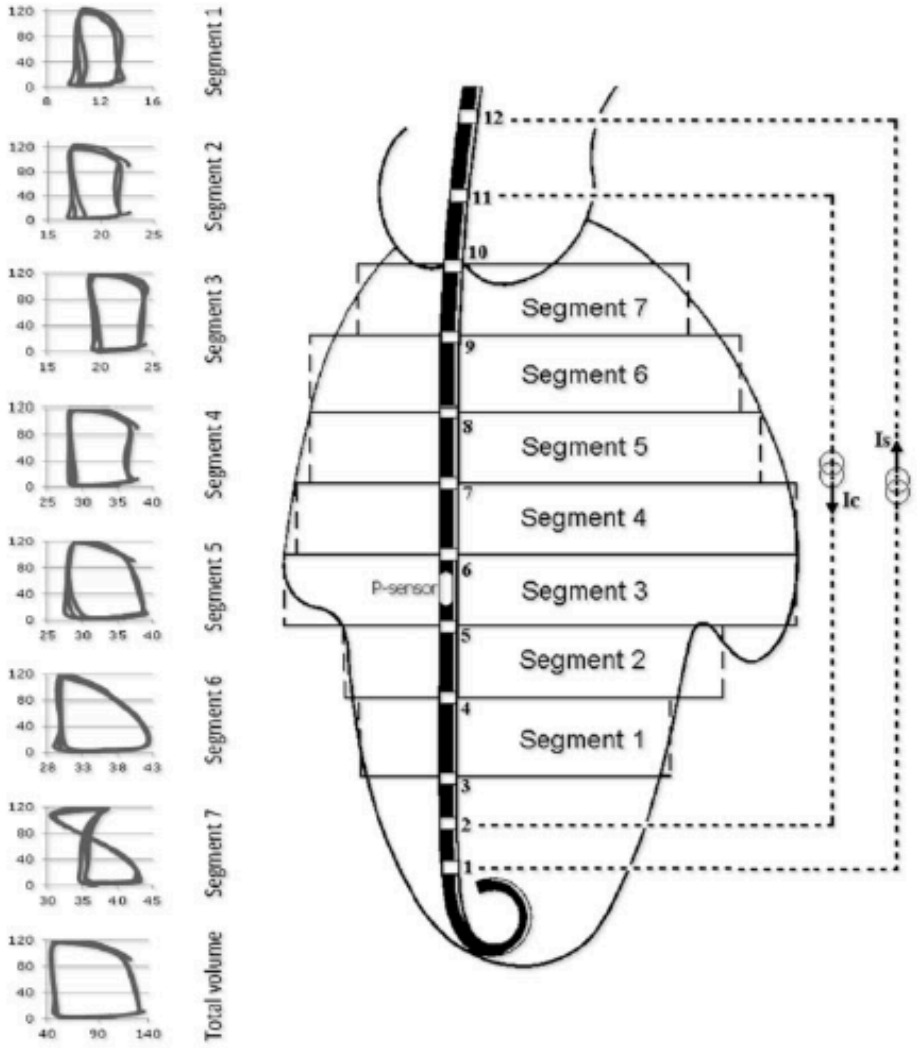
Power Lab 16-Chanel acquisition system (ADInstruments™)



5F 12-eletrode catheter
and solid-state pressuer sensor (Millar™)

Study Procedures

Invasive Hemodynamic assessment (P-V loop)



- **Volume calibration**
  real-time thermodilution-derived CO measurement
  saline injection in the right atrium for parallel conductance determination

- **Load-independent measurements**
  inferior vena cava occlusion (25 mm balloon)
  ventilation suspended at end-expiration

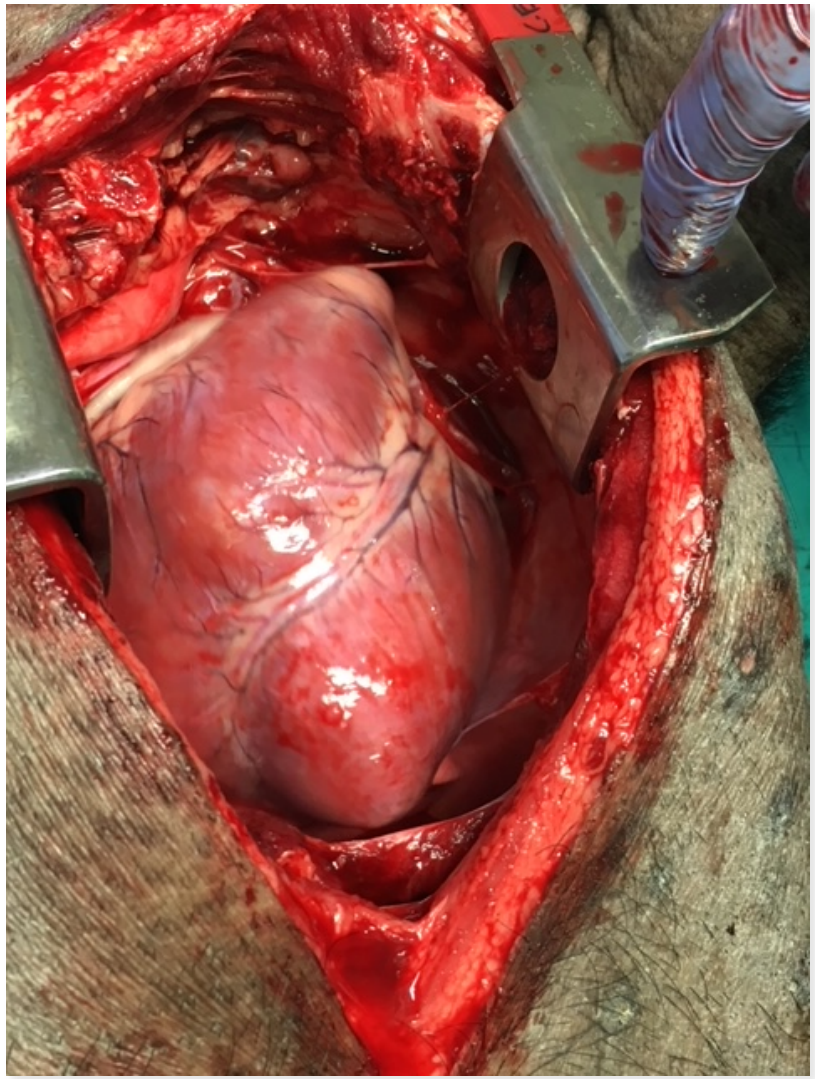
- Volume data calibrated for swine BSA according to corrections for mini-pig strains.

- **Pressure measurement**
  Solid state transducer (built in)

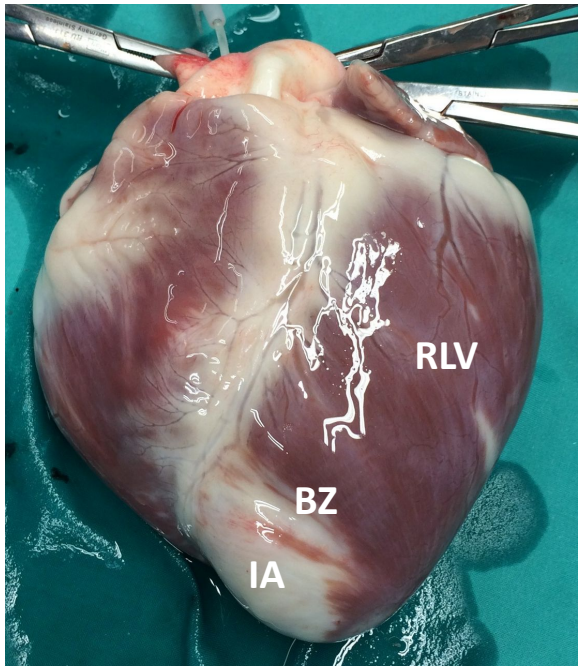
- Evaluations at rest and under steady-state inotropic stimulation with dobutamine 0.5 µgr/Kg/min.

Study Procedures

Euthanasia and harvest of biological specimens



- Blood collection (20 ml) for biomarker determination (NTproBNP and galectine-3).
- Beating hearts collected by median sternotomy and euthanasia by exsanguination under deep anaesthesia.
- Antegrade perfusion with hiperK+ cardioplegic solution
- Tissue biopsies collected in the infarct area, border-zone and remote left ventricle.
- Cryopreservation for molecular biology studies



Results of the randomized controled study

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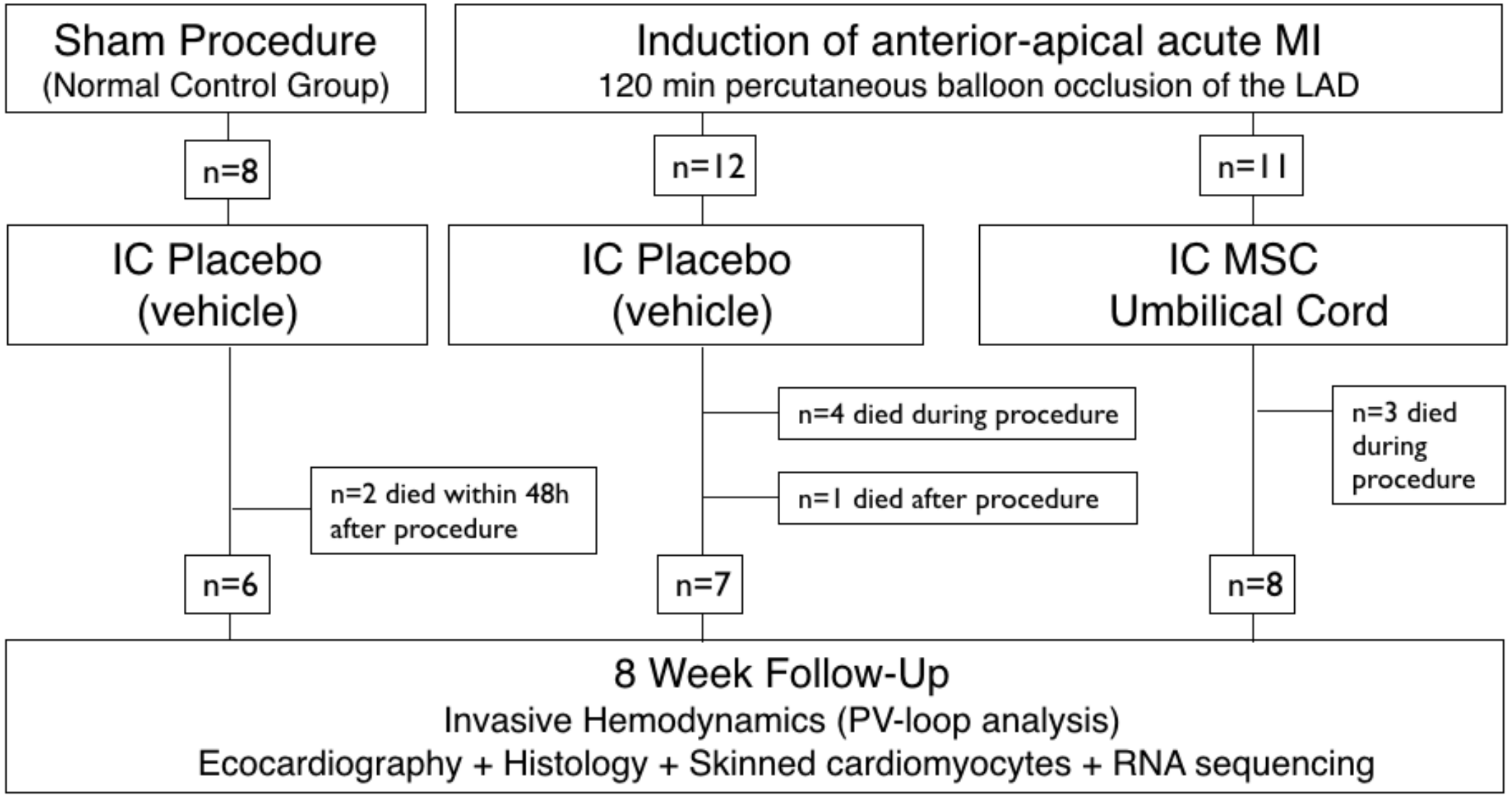
Baseline Characteristics of study animals

	Sham (n=8)	AMI+Vehicle (n=12)	AMI+hUCM-MSC (n=11)	p value*
Weight (Kg)	29.1±4.0	31.6±9.7	38.8±8.6	0.258
SBP (mmHg)	111.6±14	113.5±12.3	104.2±10.2	0.302
DPB (mmHg)	68.8±11.7	64.9±14.7	63.4±8.9	0.333
MBP (mmHg)	87.0±13.4	84.4±13.3	79.8±9.8	0.479
CVP (mmHg)	11.0±2.3	10.9±2.4	11.6±3-0	0.614

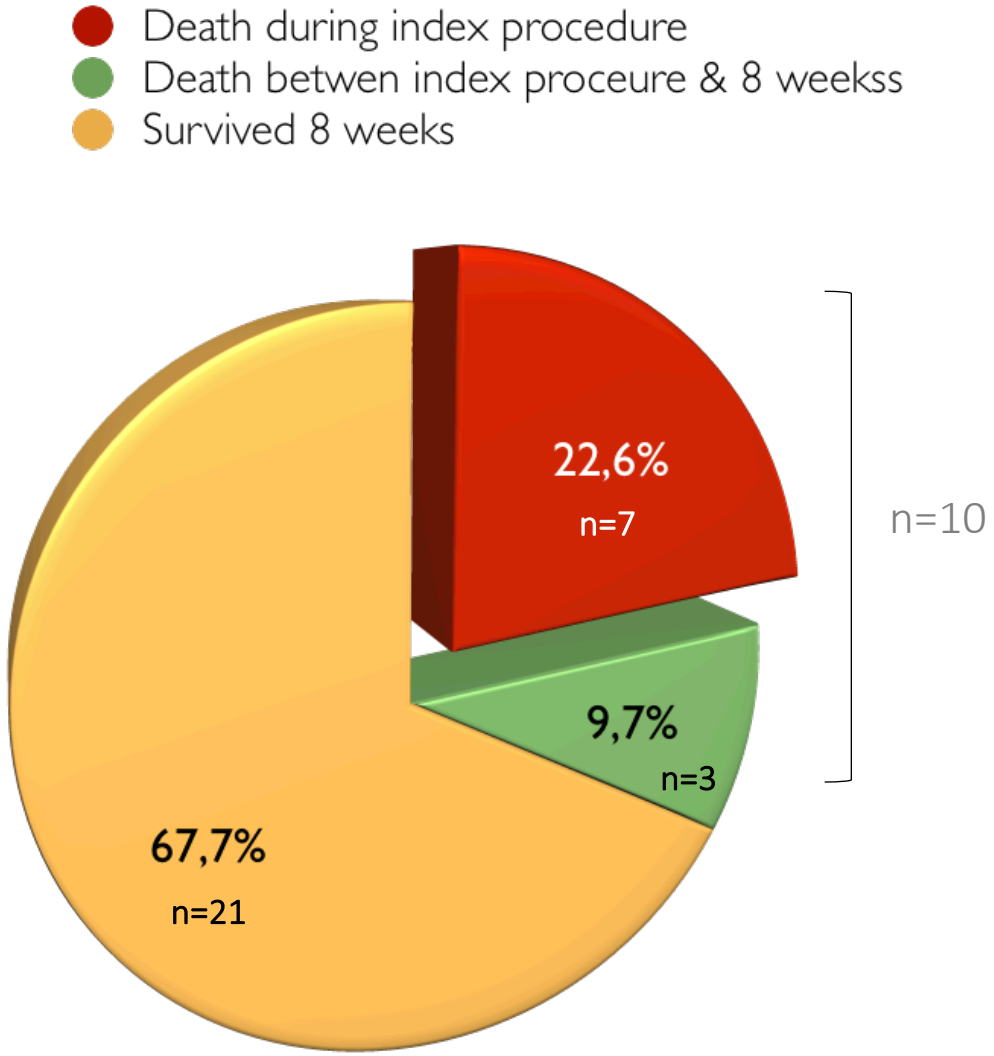
SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MBP, Mean Blood Pressure; CVP, Central Venous Pressure; MI, Myocardial Infarction; hUCM-MSC, Human Umbilical Cord Matrix Mesenchymal Stromal Cells

*Analysis of Variance (ANOVA)

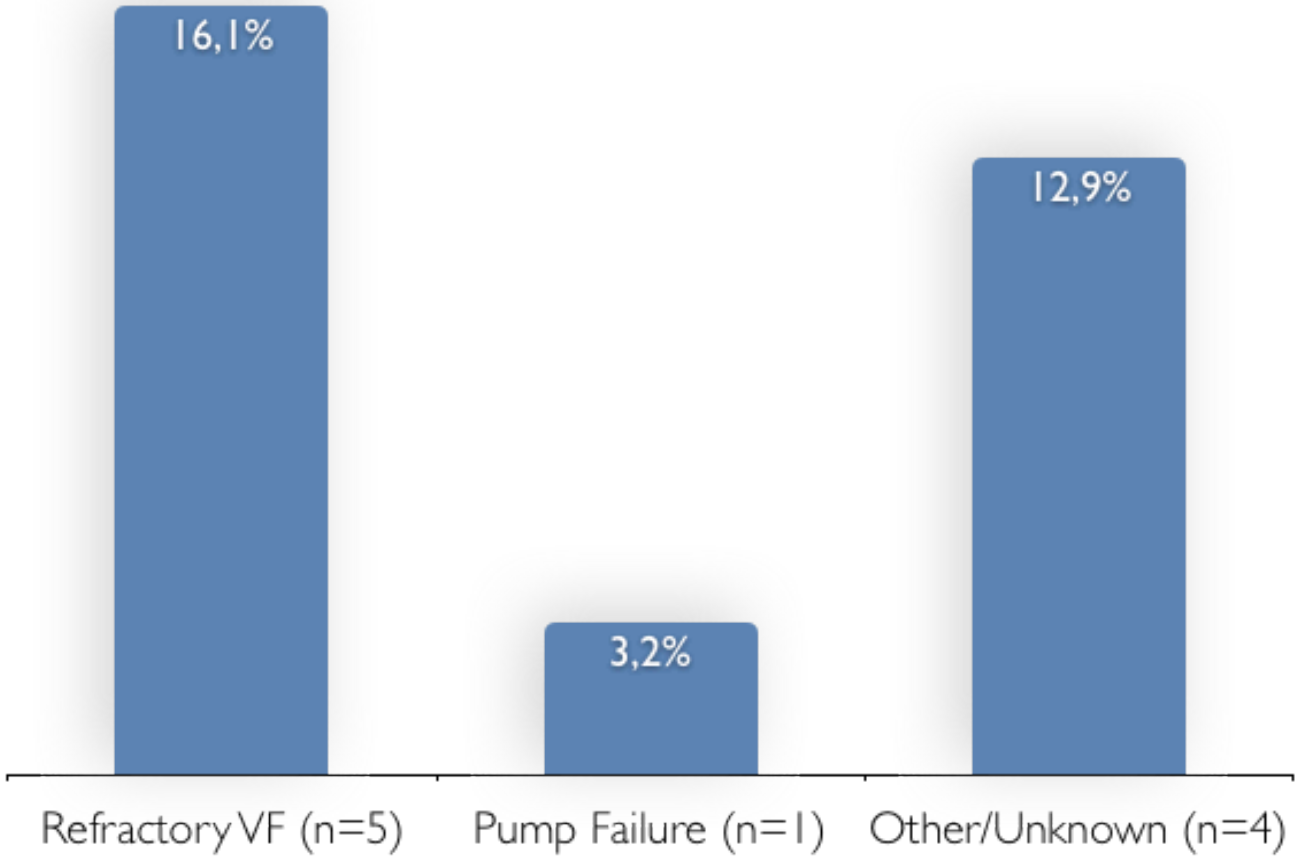
Study flowchart



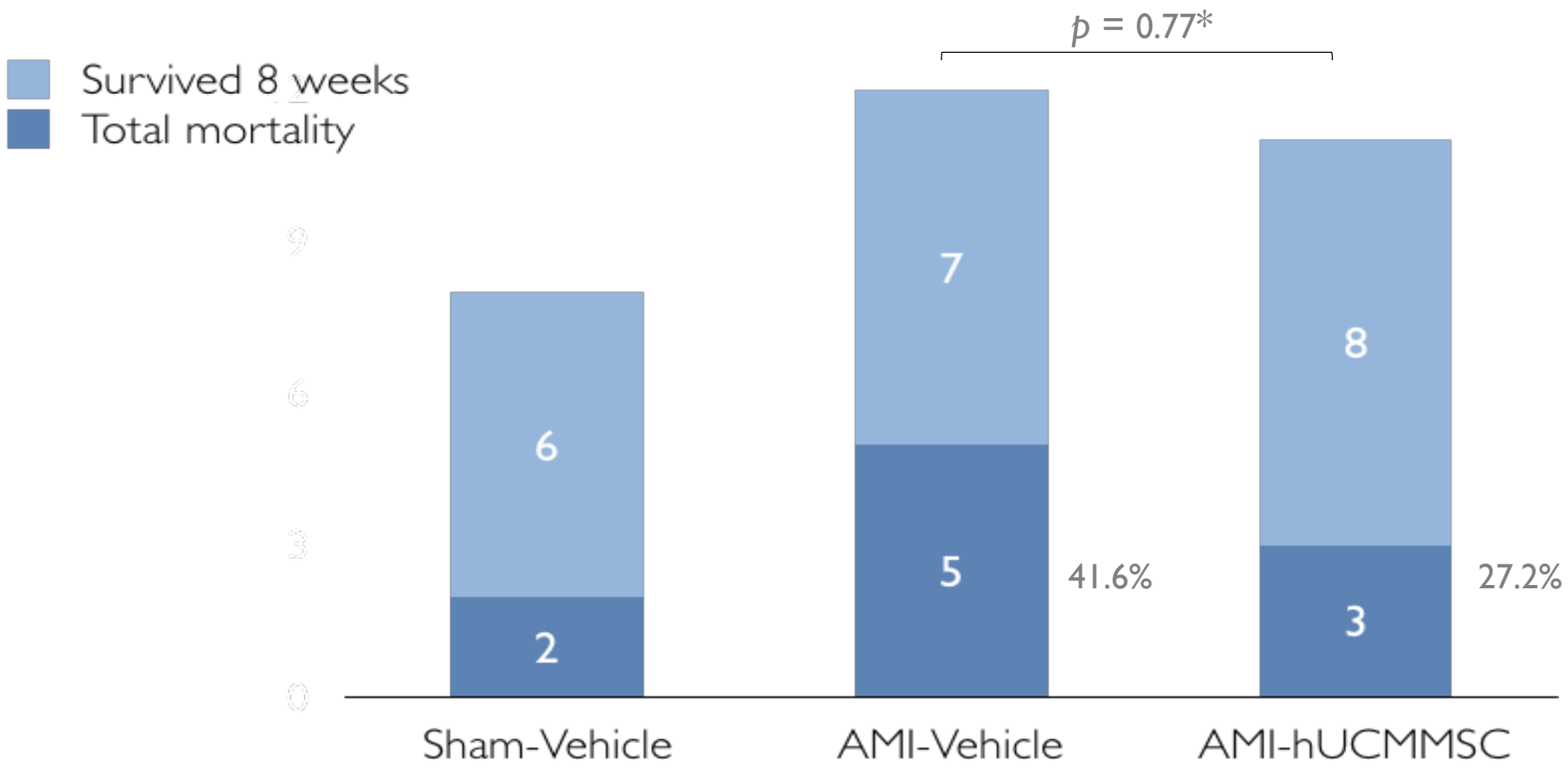
Randomized study
Mortality and causes of death



50% of total mortality due to fatal arrhythmia

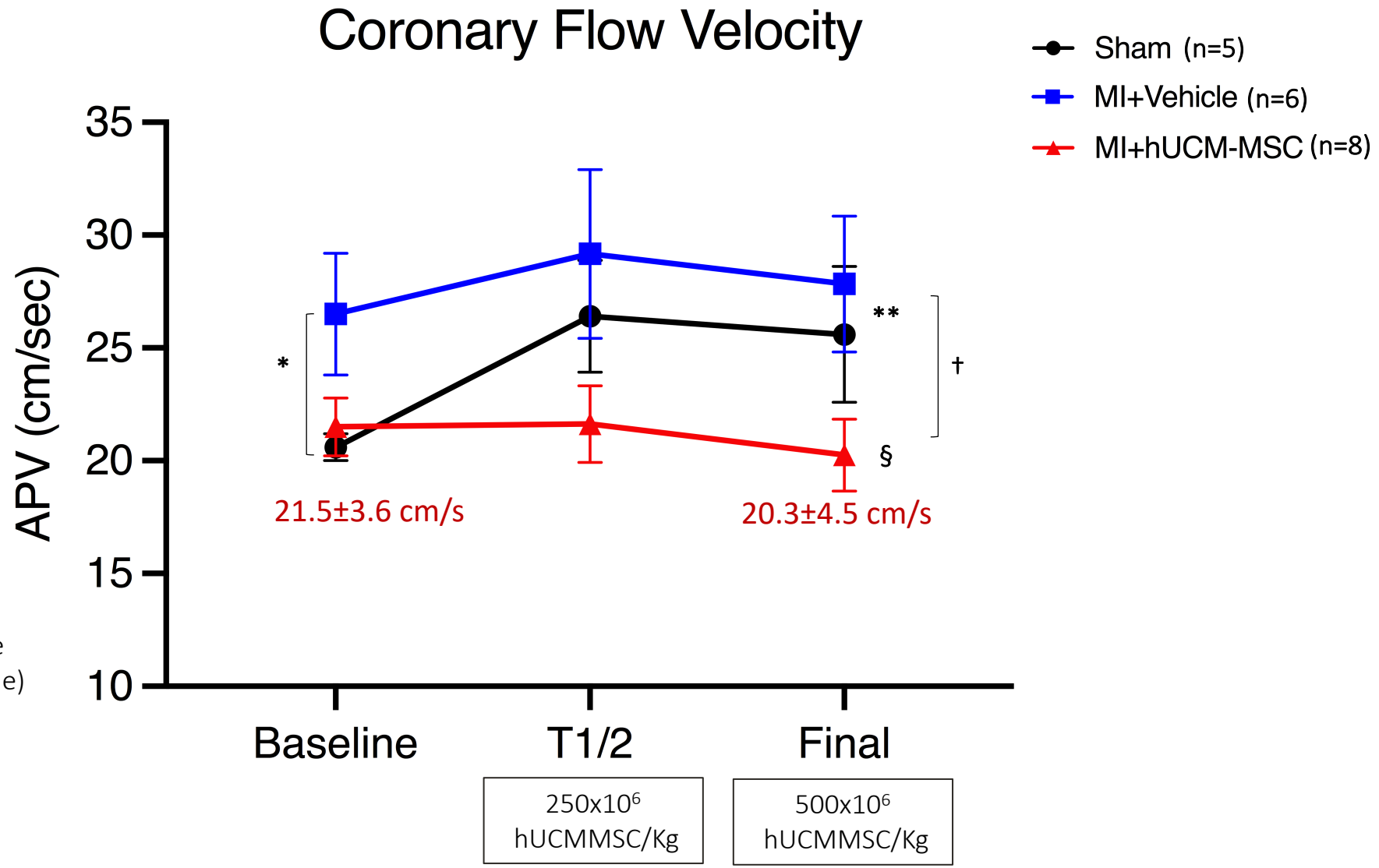


Randomized study
Mortality and causes of death



*Chi^2 with Yate's correction

Safety of hUCM-MSC intracoronary delivery



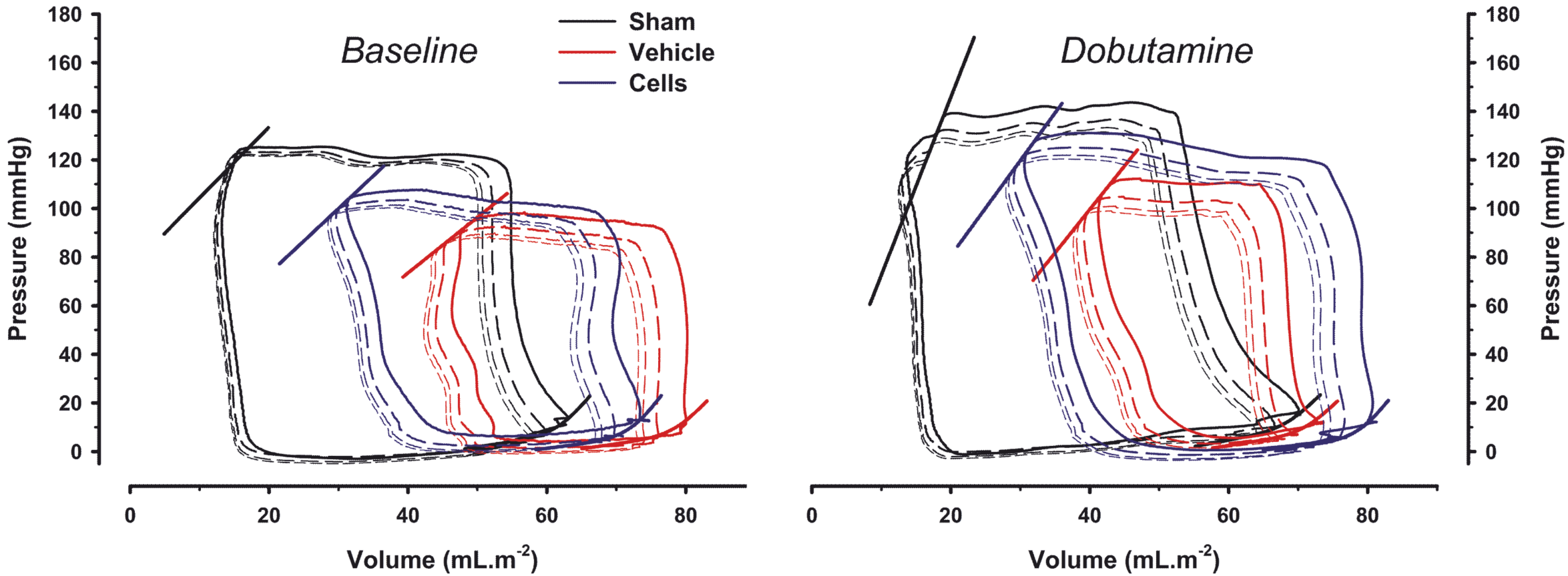
*p=0.074 for baseline AMI vs Sham

**p=0.03 for final vs baseline in vehicle injected animals (Sham and AMI-Vehicle)

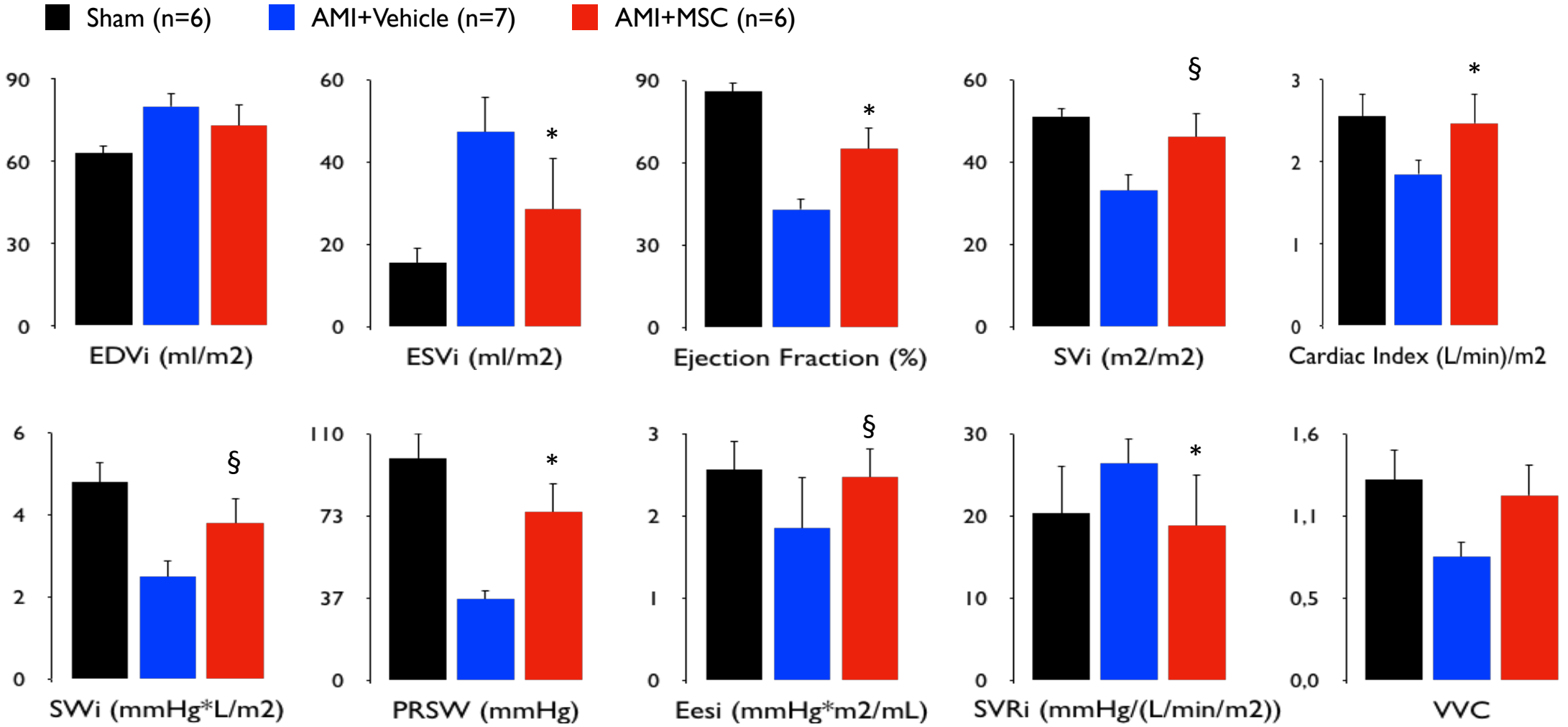
§p=0.25 for final vs baseline in hUCMMSC injected animals

†p=0.03 for final hUCMMSC vs Vehicle (Sham and AMI-Vehicle)

Pressure-volume relationships

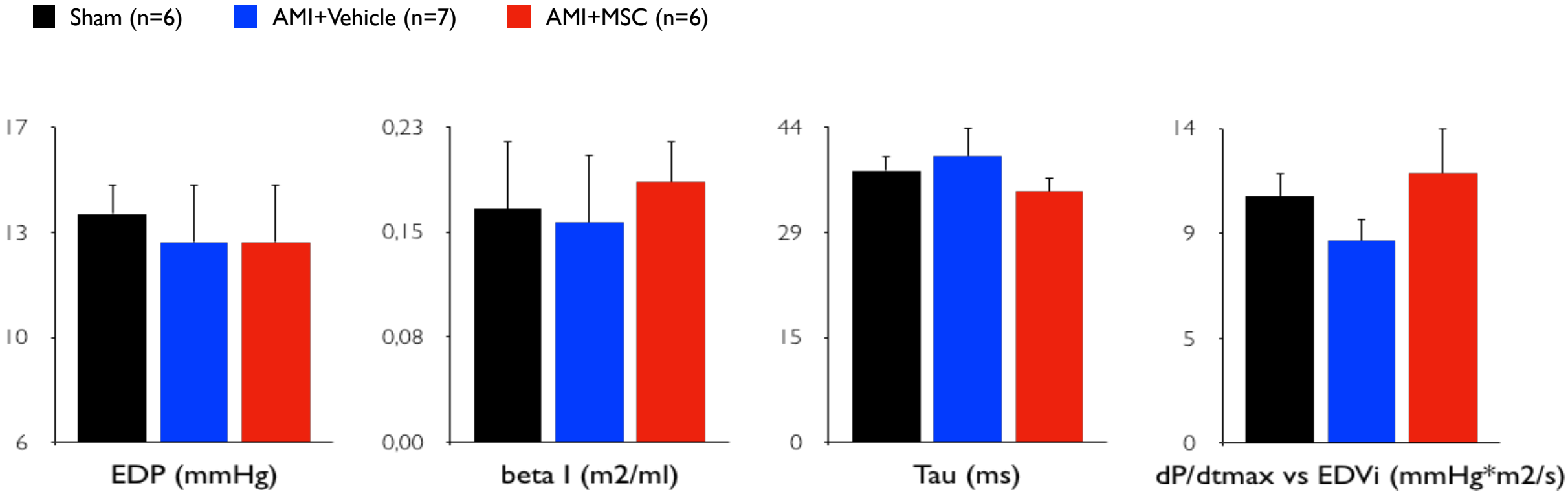


Pressure-Volume Relationships
Remodelling and LV systolic function parameters



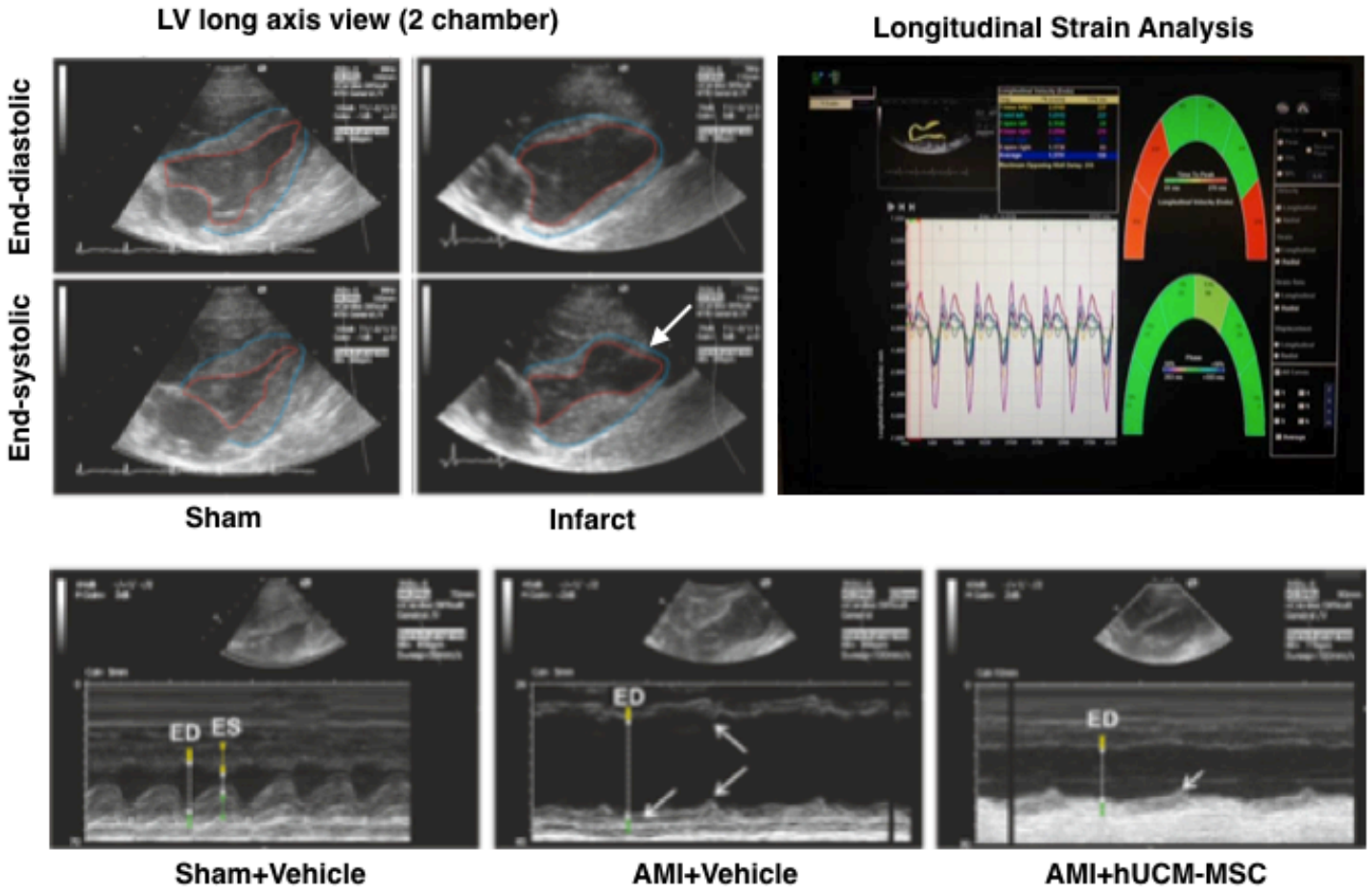
*p<0.05 vs AMI-Vehicle §p<0.1 vs AMI-Vehicle

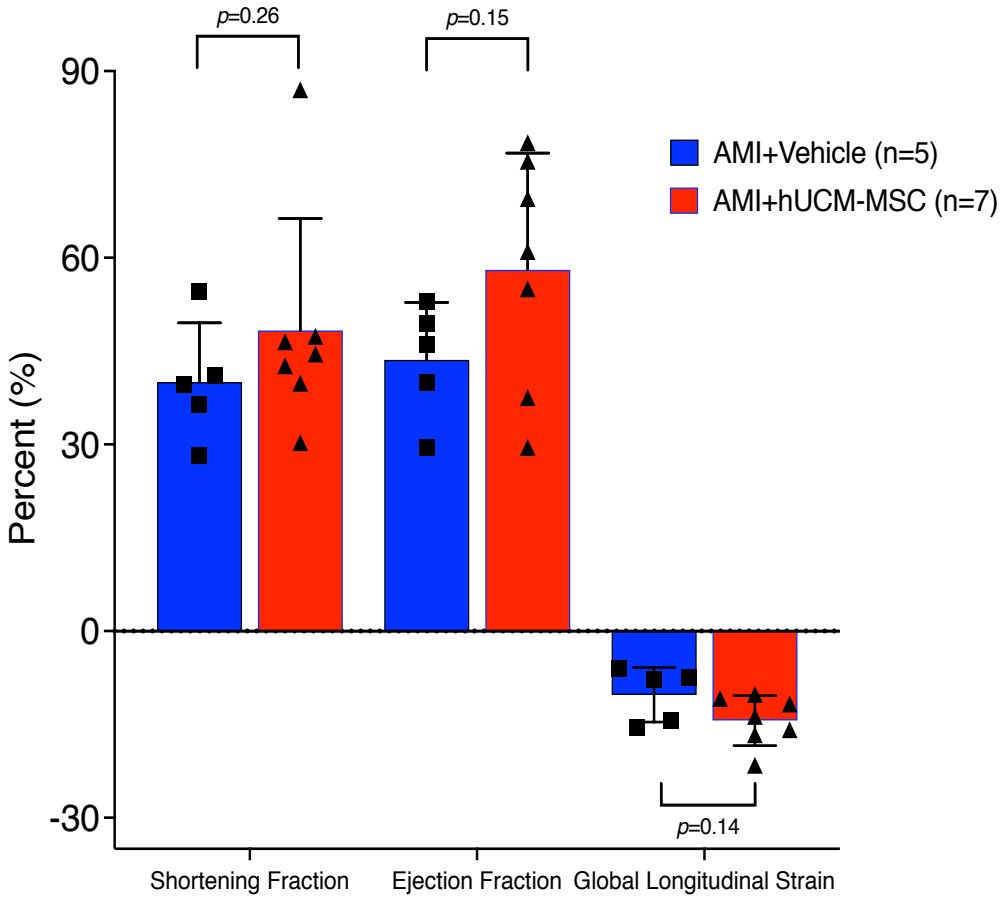
Pressure-Volume Relationships
Diastolic Function Parameters



p>0.1 for all comparisons

Transthoracic Echocardiogram
Morphometric and functional analysis





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Transthoracic Echocardiogram
Morphometric and functional analysis

	EDD (mm)	ESD (mm)	Shortening Fraction (%)	EF (%)	Wall thickness (mm)		GLS (%)	Longitudinal T2P Global
					Mid-Cavity	Apical*		
Sham-vehicle (n=3)	33.8±3.6	18.5±5.7	45.6±8.3	47.0+6.2	9.4±2.5	9.3±2.5	-14.6±2.4	400±53.7
AMI-vehicle (n=5)	38.8±7.3	23.1±7.2	40.0±4.2	43.7±4.1	9.1±1.9	5.4±1.9	-10.2±1.9	374.0±50.5
AMI-MSC (n=7)	35.5±8.5	19.1±8.2	48.6±6.8	58.0±7.1	10.7±2.7	6.7±2.7	-14.3±1.5	407.0±30.2

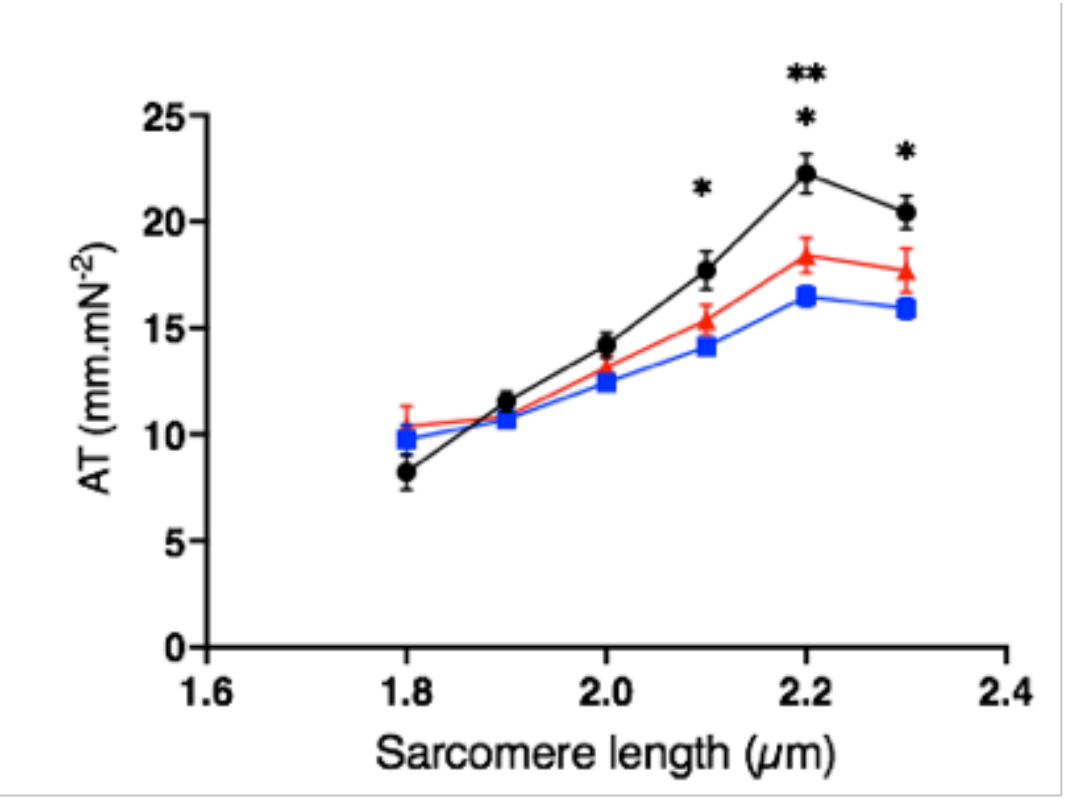
EF, ejection fraction; EDD, end-diastolic diameter; ESD, end-systolic diameter; GLS, Global Longitudinal Strain; T2P, Time to Peak.

*p=0.068 (ANOVA)

Skinned Cardiomyocytes

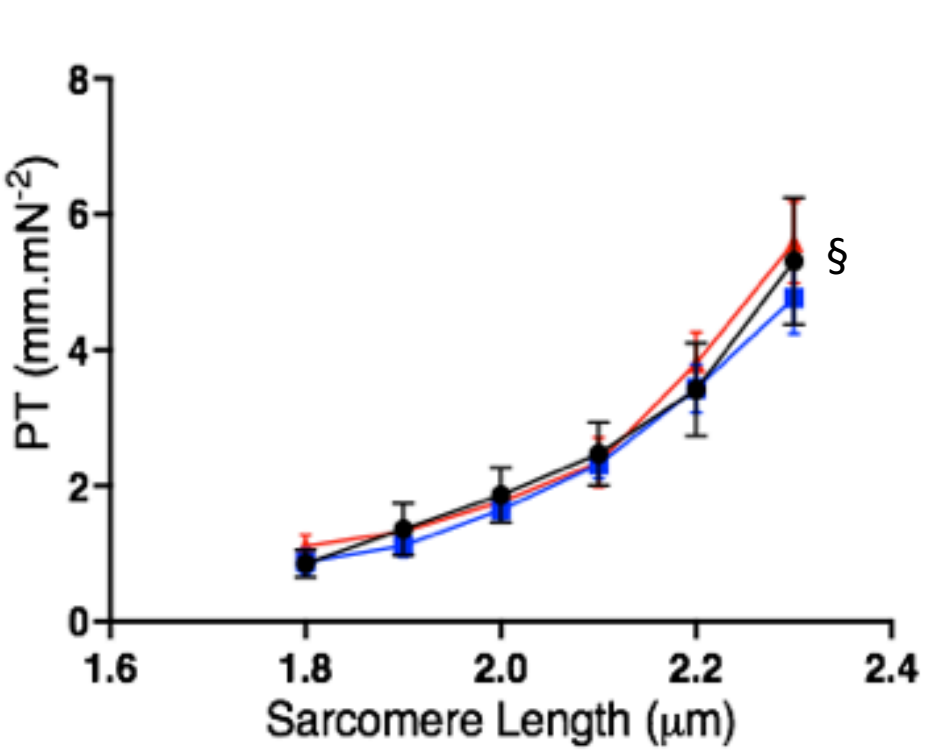
Active and Passive Tension in isolated Cardiomyocytes

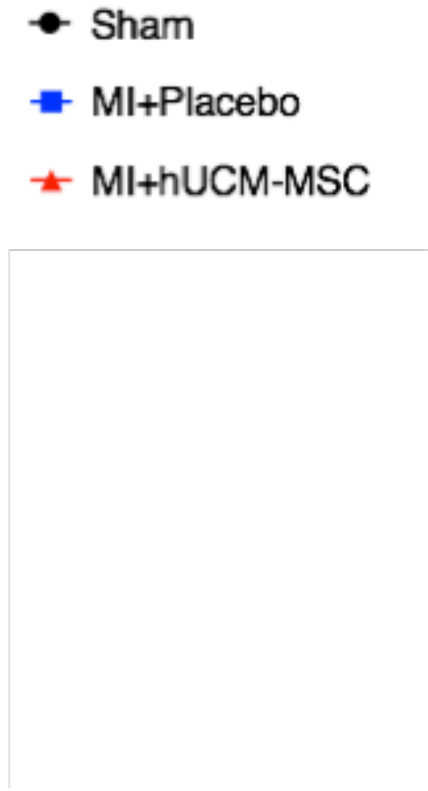
Active Tension



*p<0.05 for Sham vs AMI-Vehicle
**p<0,05 for Sham vs Cells

Passive Tension

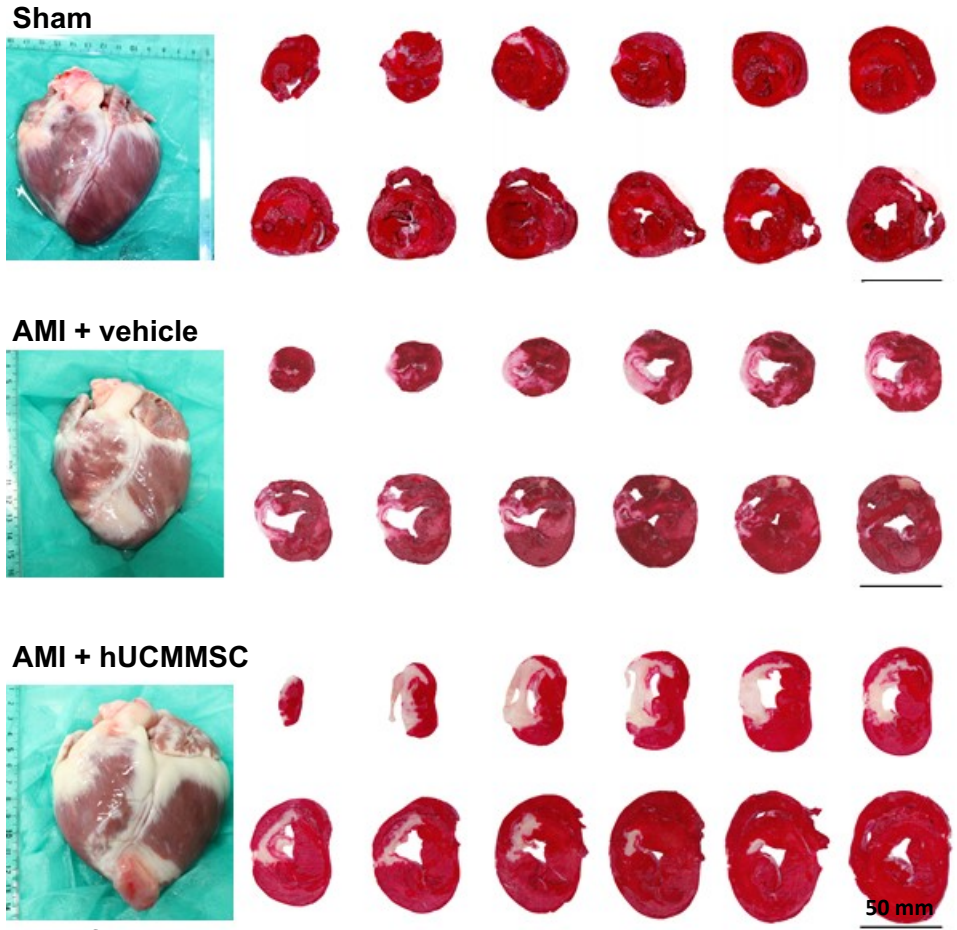


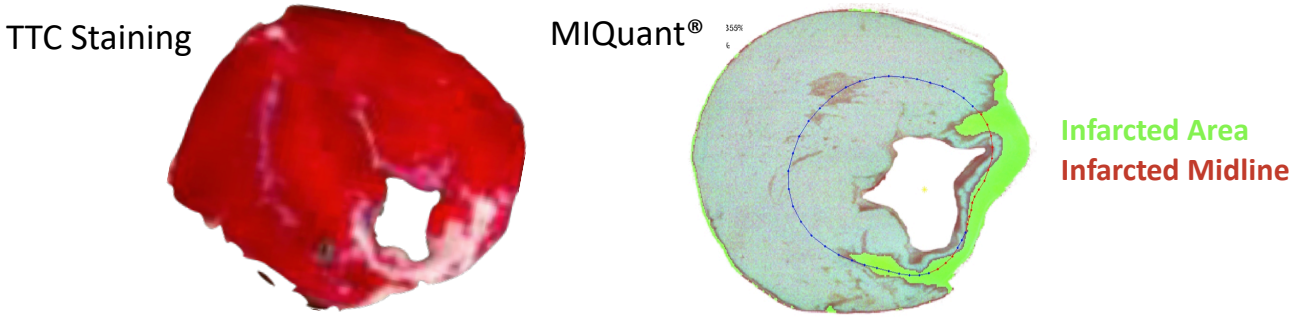


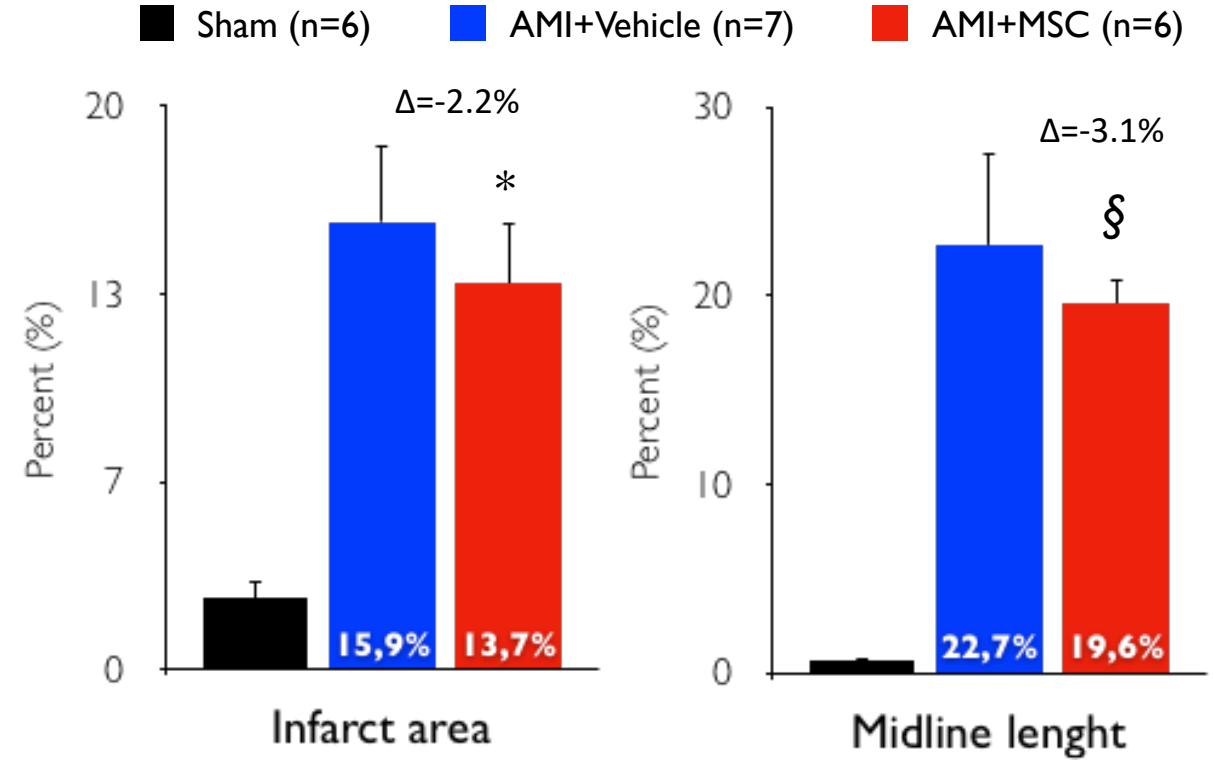
§p values ranged from 0.57 to 0.99

Histopathological examination

Histological Infarct Size





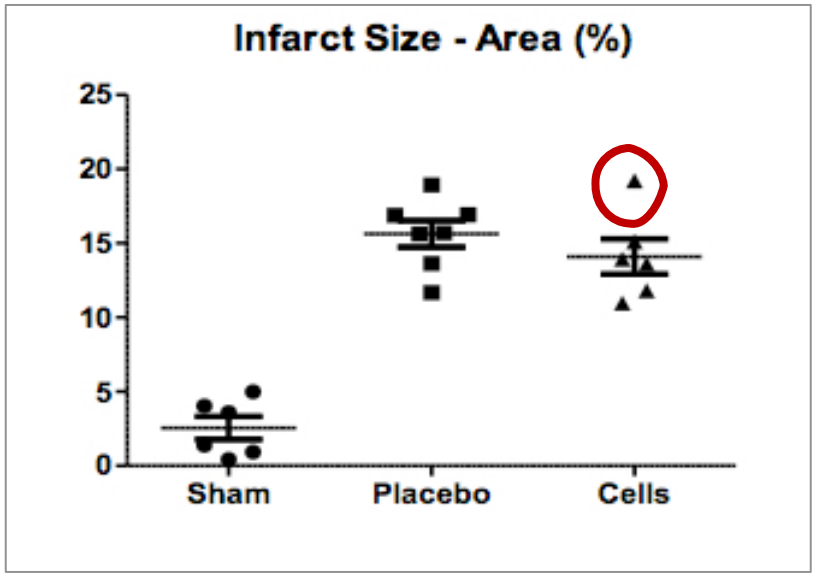


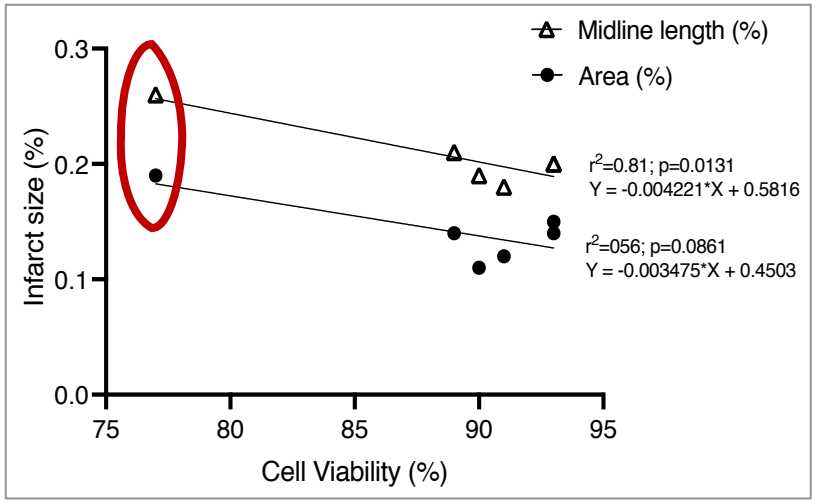
*p=0.23 vs AMI-Vehicle

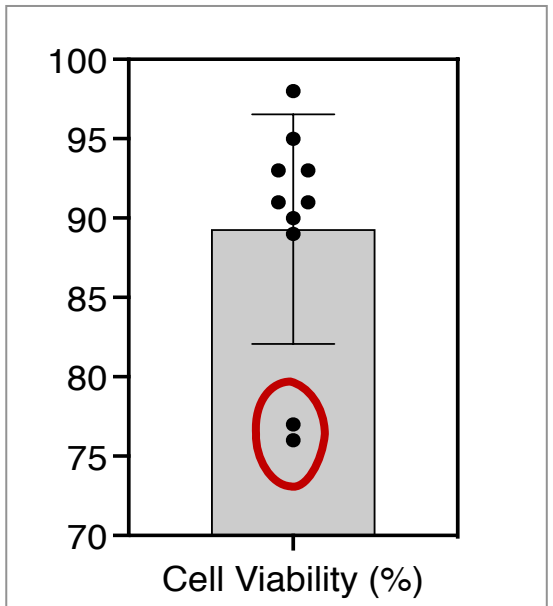
§p=0.16 vs AMI-Vehicle

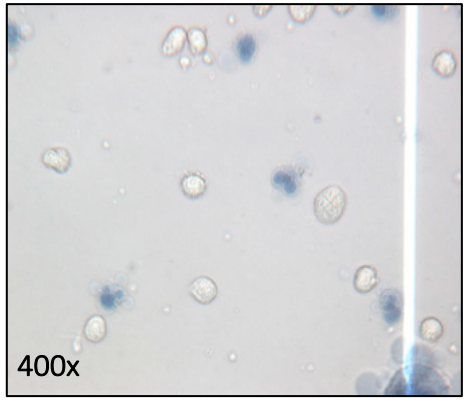
Histopathological examination

Relationship between infarct size and cell viability

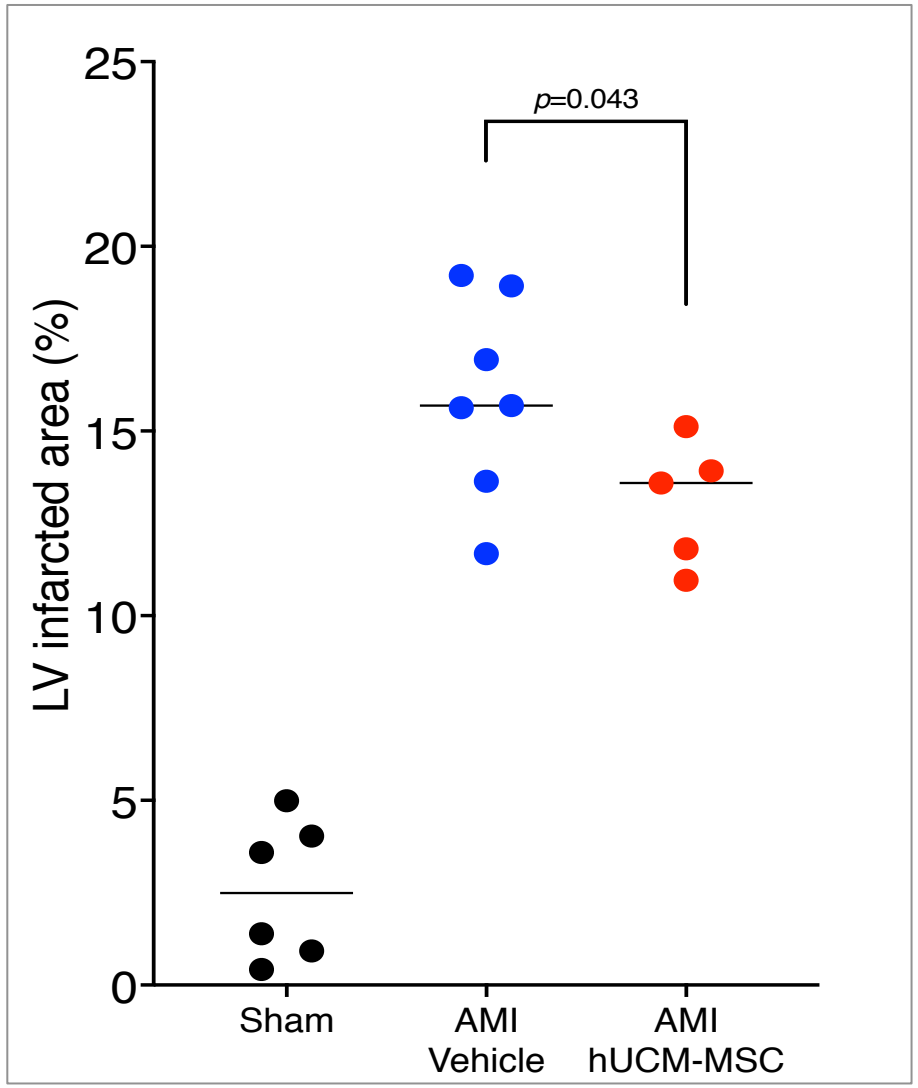






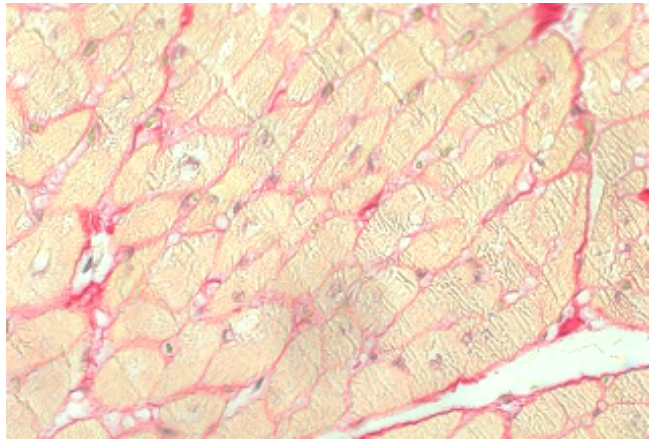


Trypan-Blue Staining

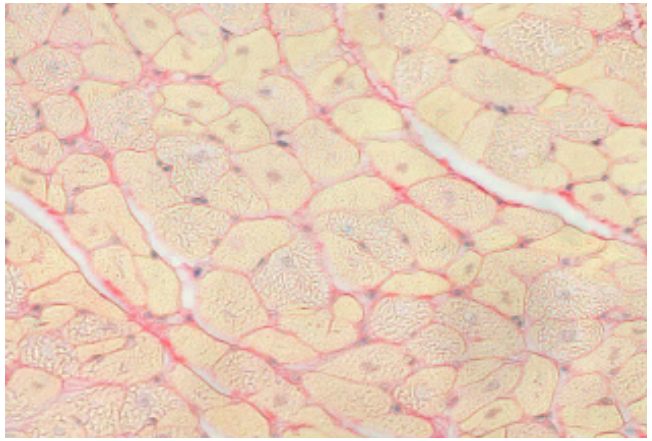


Histopathological examination
Fibrosis and hypertrophy in remote myocardium

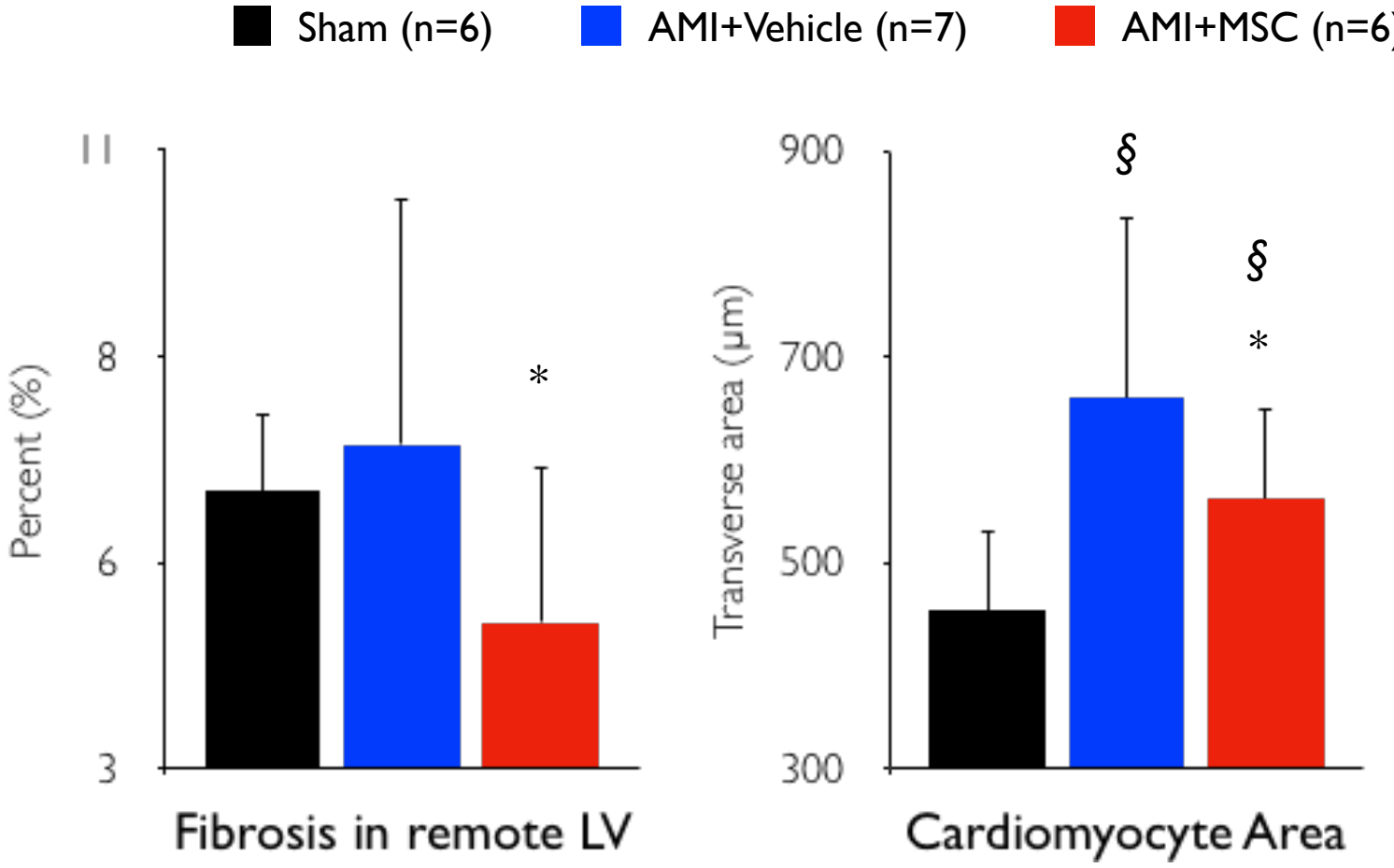
AMI-Vehicle



AMI-hUCMMSC



Picro-Sirius Red Staining

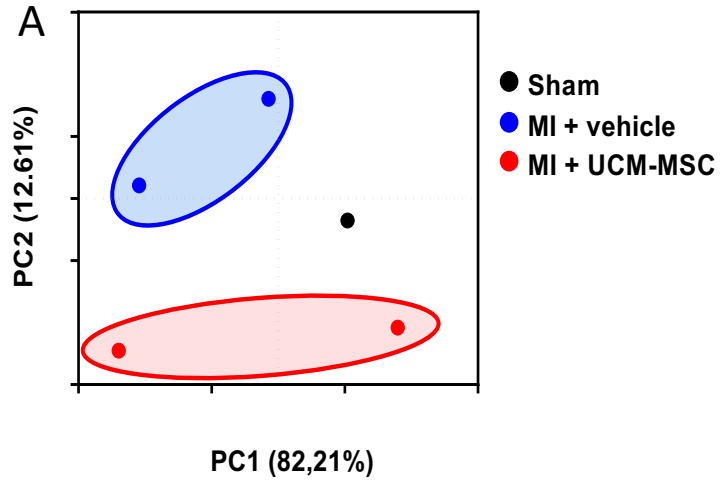


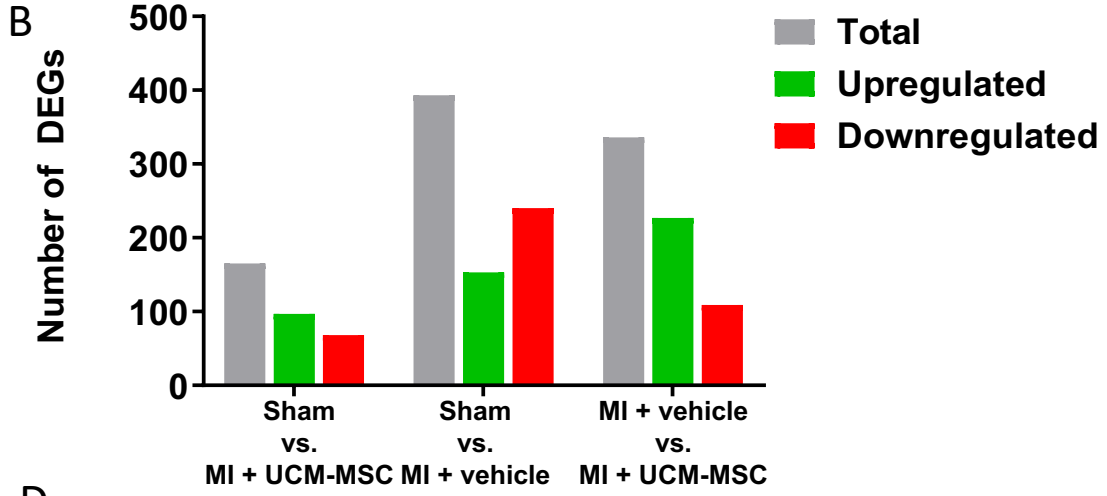
*p=0.14 vs AMI-Vehicle

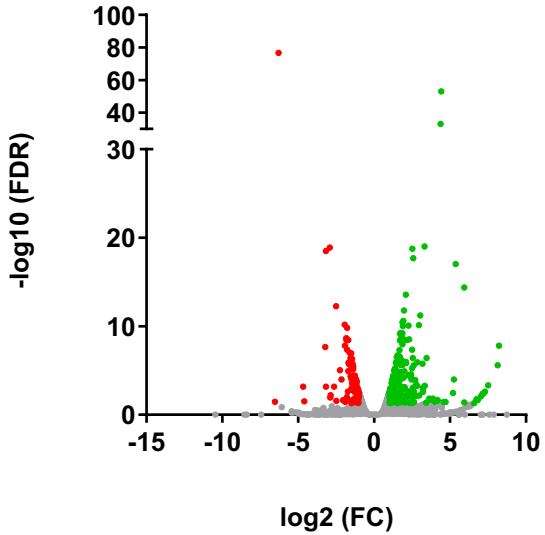
*p=0.187 vs AMI-Vehicle

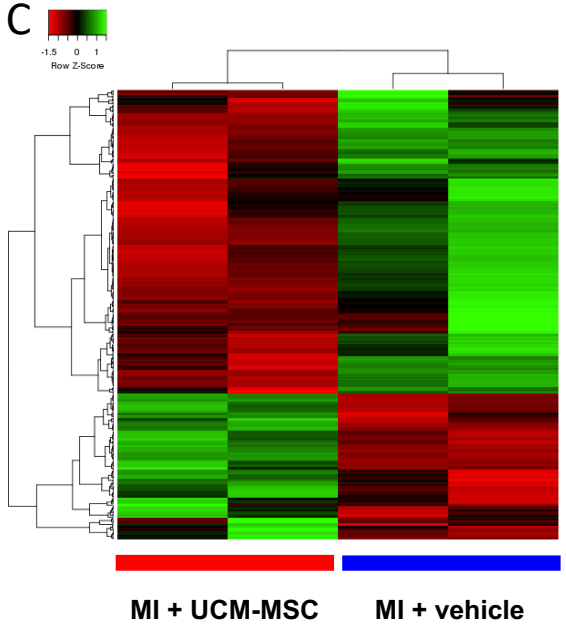
§p<0.05 vs Sham

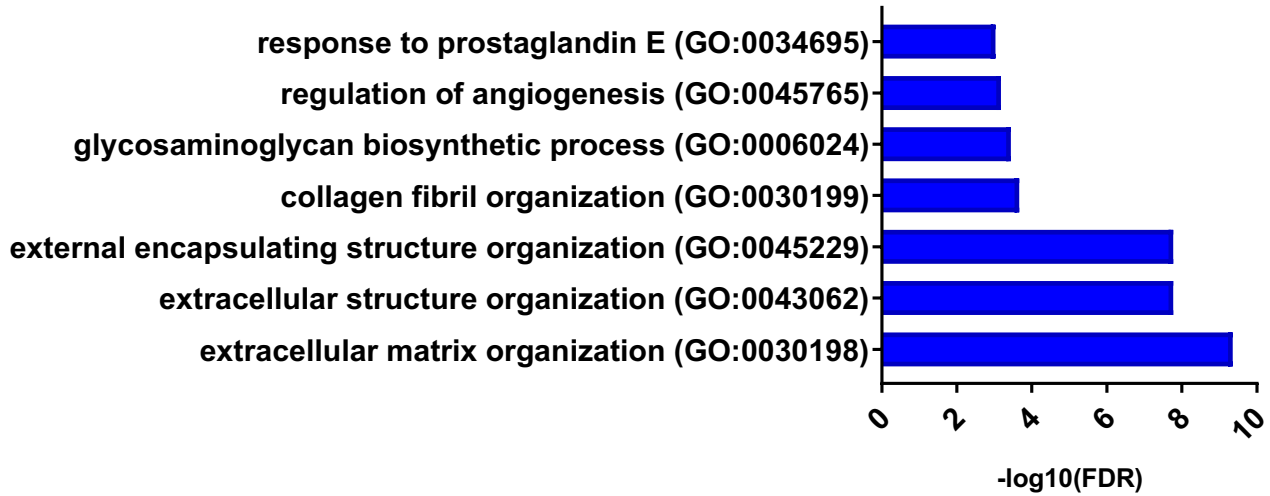
Differential gene expression
Whole genome analysis (RNA-seq)





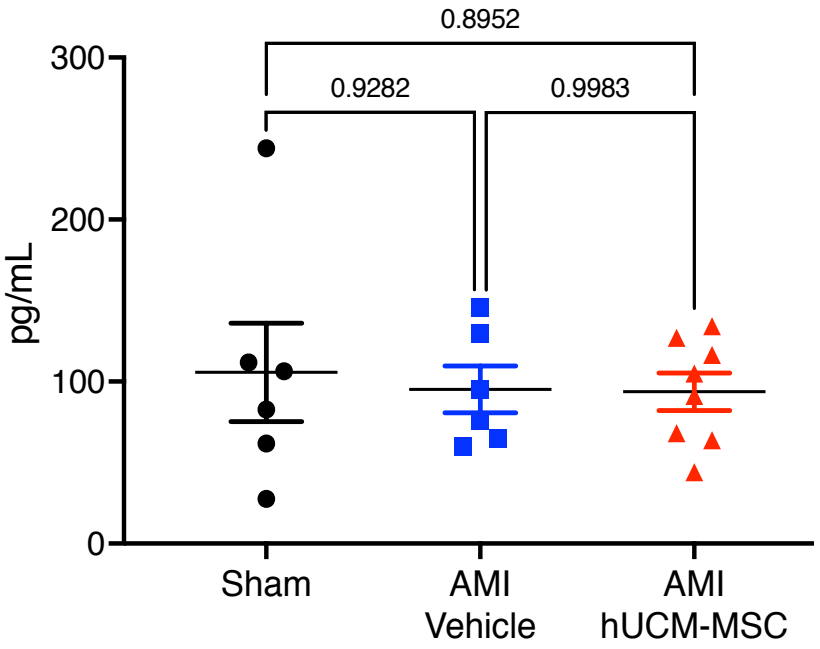




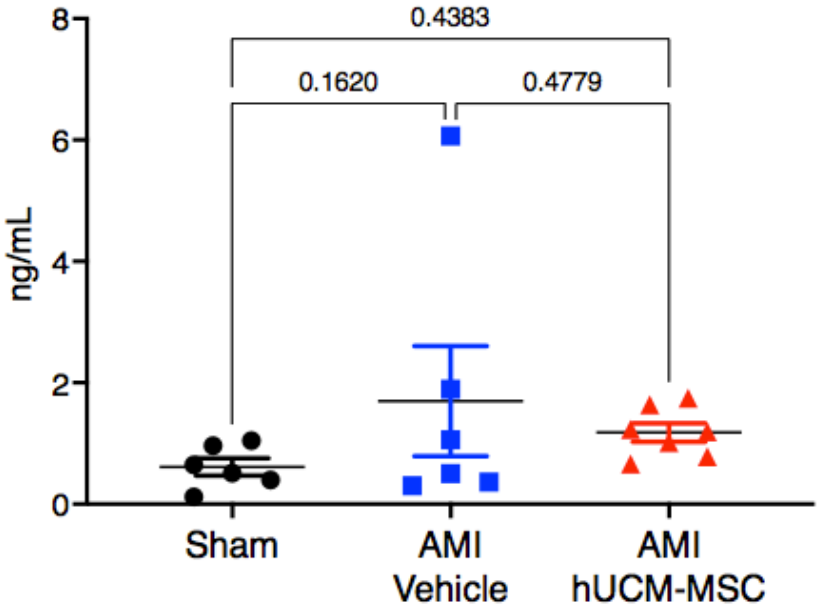


Serum Biomarkers

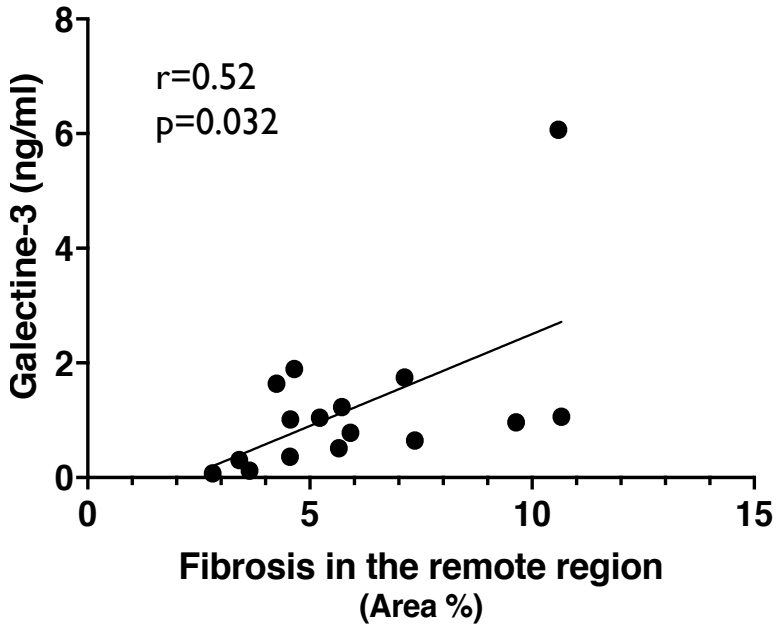
NT-proBNP



Galectine-3



Galectine-3 vs Fibrosis in remote area



Limitations

Main study Limitations

- Small sample size and type II error for exploratory endpoints.
- Lack of functional evaluation at intermediate time points and short follow-up.
- No comparison with other cell dose and IC infusion rates.
- No comparison with injection of hUCM-MSC at a later time point after MI
- Lack of advanced imaging modalities to assess *in vivo* infarct size
- Lack of the thrombotic milieu of STEMI

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Conclusions

Conclusions

- In the selected dose and infusion rates, **intracoronary delivery of a clinical grade GMP-compliant allogeneic hUCM-MSCs cell product was safe** early after reperfused AMI, with a neglectable effect on coronary flow (as assessed by real time APV).
- As compared with placebo, hUCM-MSC **improved left ventricular systolic function** at 8-weeks post-AMI.
- Effect was **not explained by reduction of infarct size** alone.
- Improved **intrinsic cardiomyocyte contractility, lower SVR and favourable modifications of tissue remodelling of the remote myocardium** were observed, providing clues for the biological effects, which deserve further clarification.
- Translational studies are warranted and have a solid background.

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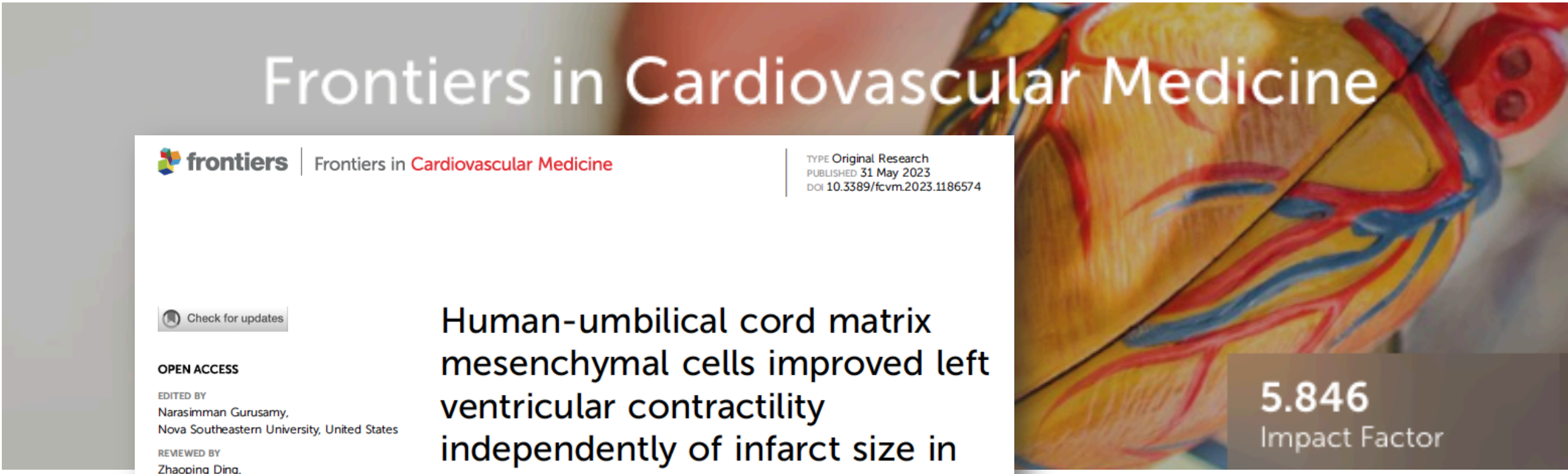

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Human-umbilical cord matrix mesenchymal cells improved left ventricular contractility independently of infarct size in swine myocardial infarction with reperfusion

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