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Secretome of apoptotic cells causes cardioprotection and inhibits ventricular remodeling after acute myocardial infarction

Doctoral viva

Dr.med.univ. Michael Lichtenauer

Supervisor

Univ.Doz. Dr.med.univ. Hendrik Jan Ankersmit

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Vienna – Austria

Medical University Vienna, Department of Thoracic Surgery - Vienna – Austria

Wien, 11.01.2012

Background

Table 1 | Randomized trials in patients with acute myocardial infarction or ischemic heart failure

Trial name	Number of patients	Cell type	Dose	Route of delivery	Timing of delivery	Primary end point	Comments
Acute myocardial infarction							
BOOST	60	nBMC	128 ml	i.c.	Day 6 ± 1	LVEF ↑	Effect diminished after 18 and 61 months
REPAIR-AMI	187	mnBMC	50 ml	i.c.	Day 3–6	LVEF ↑	NA
Leuven-AMI	66	mnBMC	130 ml	i.c.	Day 1	LVEF ↔	Regional contractility ↑ Infarct size ↓
ASTAMI	97	mnBMC	50 ml	i.c.	Day 6 ± 1	LVEF ↔	NA
FINCELL	77	mnBMC	80 ml	i.c.	Day 3	LVEF ↑	NA
REGENT	117	mnBMC (unselected vs $CD34^+/CXCR4^+$)	50–70 ml (unselected) 100–120 ml (selected)	i.c.	Day 3–12	LVEF ↑ with both cell types	NA
HEBE	189	mnBMC vs mnPBC	60 ml (mnBMC) 150 ml (mnPBC)	i.c.	Day 3–8	Regional contractility ↔	NA
Ischemic heart failure							
MAGIC	97	SkM	400 or 800×10^6	i.m.	>Week 4	LVEF ↔	LVEDV ↓ LVESV ↓
TOPCARE-CHD	58	mnBMC vs CPC	50 ml	i.c.	Month 81 ± 72	LVEF ↑ (mnBMC) LVEF ↔ (CPC)	NA

Only patients with complete imaging studies are considered here. Dose refers to the average amount of bone marrow or peripheral blood that was harvested, or the number of transplanted skeletal myoblasts. Abbreviations: ↓, decreased; ↑, increased; ↔, no significant change; CPC, circulating blood-derived progenitor cells; i.c., intracoronary; i.m., intramuscular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; mnBMC, mononucleated bone marrow cells; mnPBC, mononucleated peripheral blood cells; NA, not applicable; nBMC, nucleated bone marrow cells; SkM, skeletal myoblasts.

Can Stem Cells Repair a Damaged Heart? . In *Stem Cell Information*. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services <http://stemcells.nih.gov/info/scireport/chapter9>

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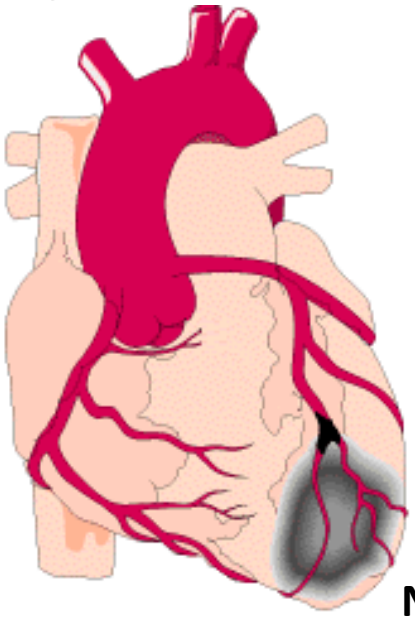
Myocardial Infarction



Necrosis

Attraction of immune cells

Secretion of pro-inflammatory cytokines

IL-1 IL-6 TNF-α

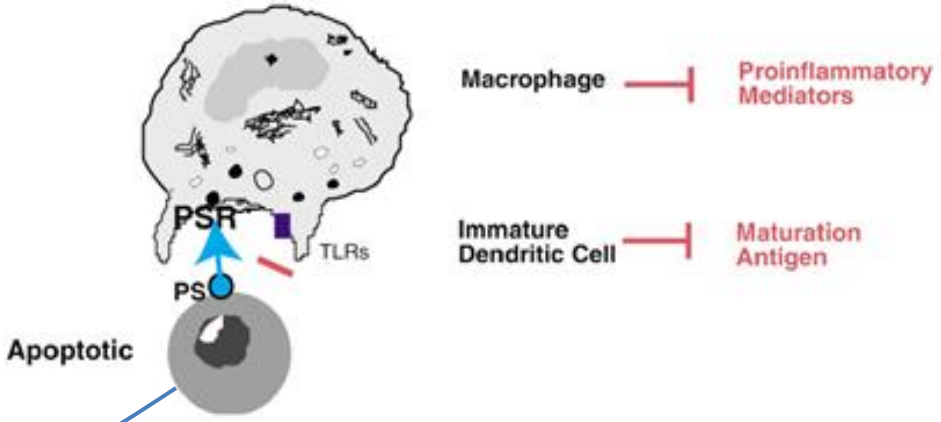
Amplification of inflammation

The Dying Stem Cell Hypothesis

by Anker *et al.*

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

apoptotic cells induce transient immunosuppression



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

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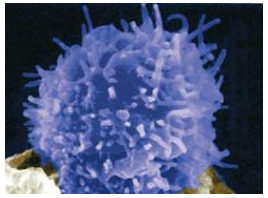
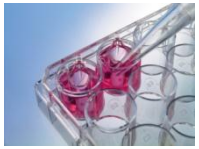
Experimental Design



Peripheral Blood Mononuclear Cells (PBMC)

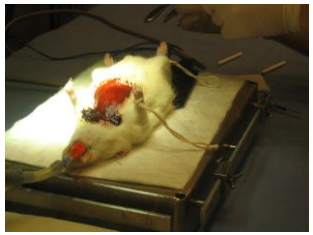
Irradiation & Induction of Apoptosis



Cell Culture for 18-24h

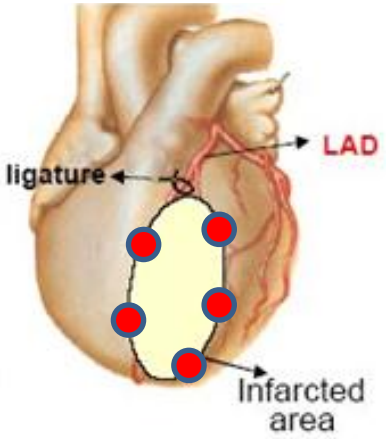
Flow Cytometry
Annexin-positivity >70%

Model of Experimental AMI

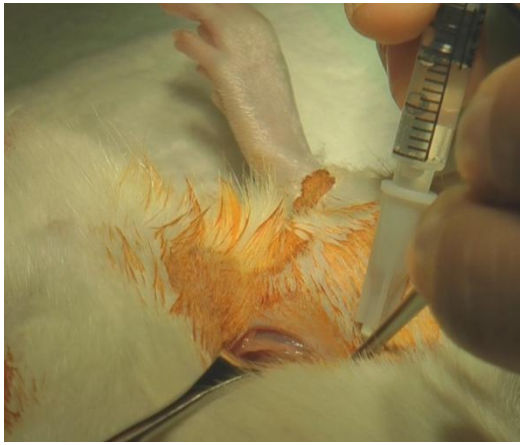


anesthetized and mechanically ventilated rat

Intramyocardial Injection



Intravenous Injection



Controls
↓
Injection of

Non-irradiated viable PBMC

Cell Culture Medium

Sham Operation

intercostal thoracotomy

ligation of the coronary artery

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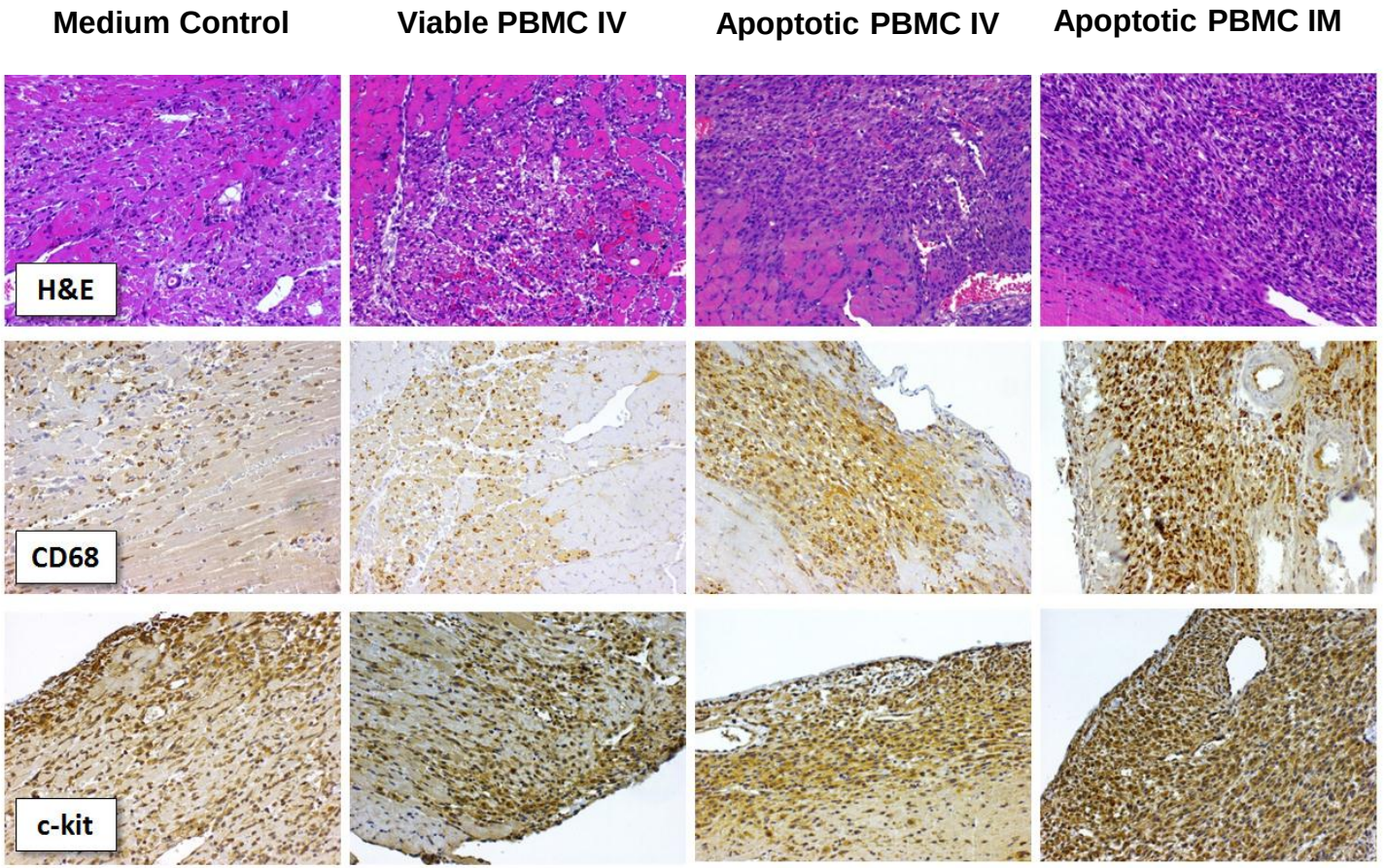
Results

Histology and Immunohistology 3 days after induction of MI



n=5-6 per group

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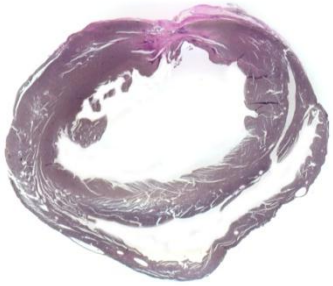
Results

Scar Dimension 6 Weeks after Induction of MI

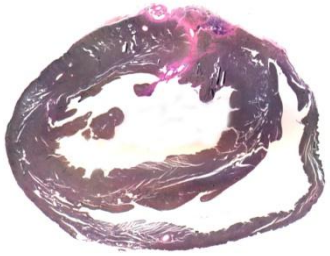
Control

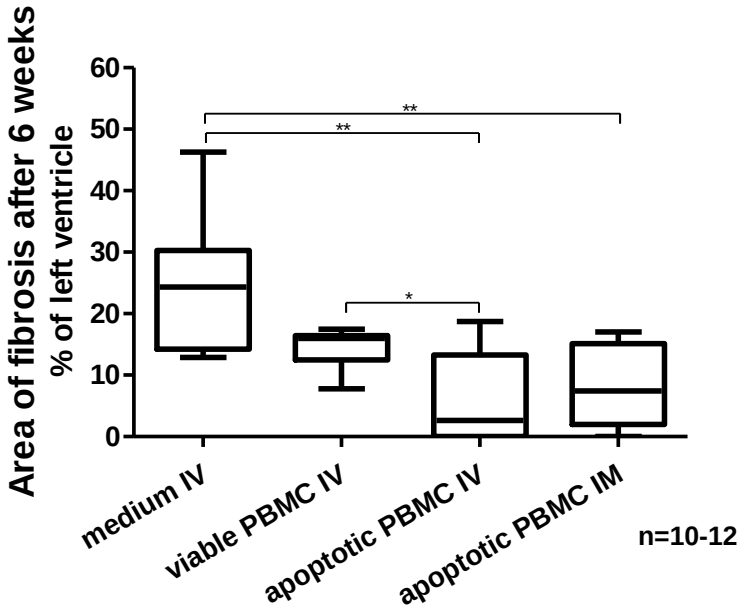
Intravenous Administration of viable PBMC

Intravenous Administration of IA-PBMC



Intramyocardial Injection of IA-PBMC





IA-PBMC suspensions of irradiated apoptotic peripheral blood mononuclear cells

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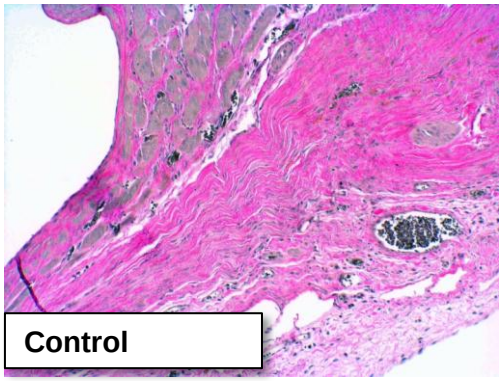
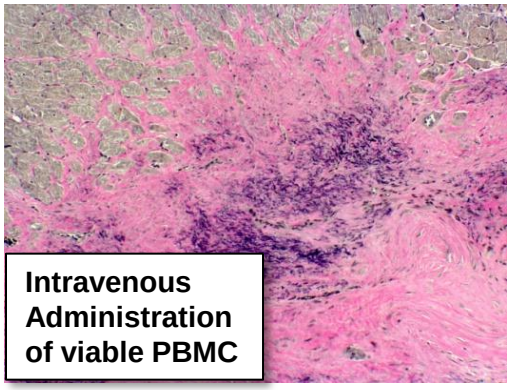
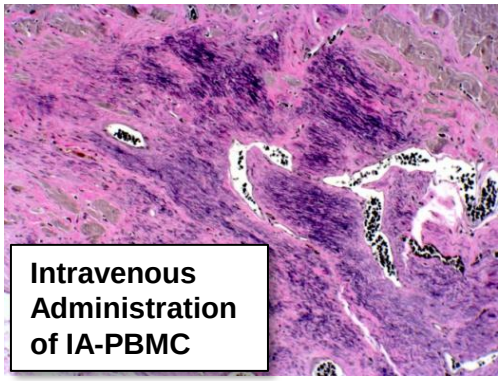
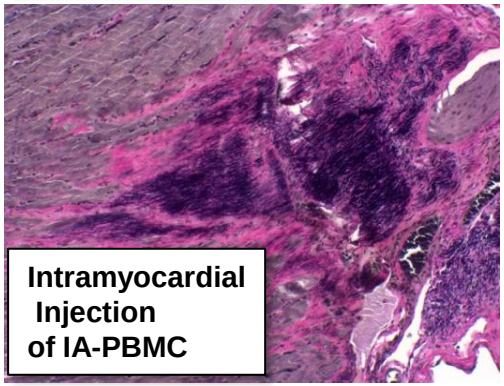
Results

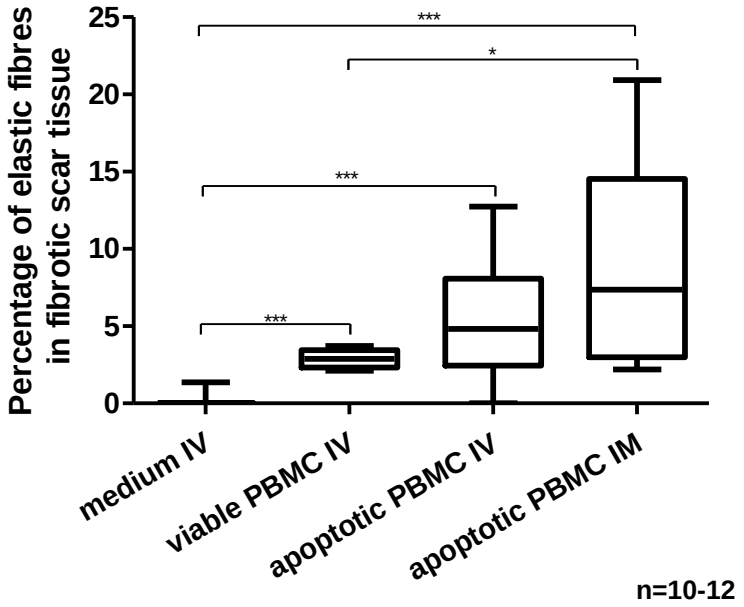
Composition of Scar Tissue











n=10-12

IA-PBMC suspensions of irradiated apoptotic peripheral blood mononuclear cells

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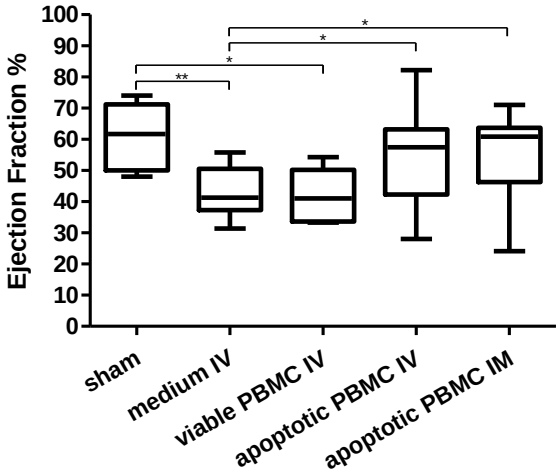
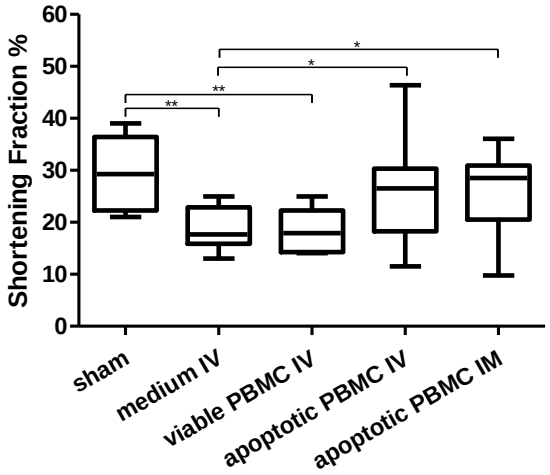
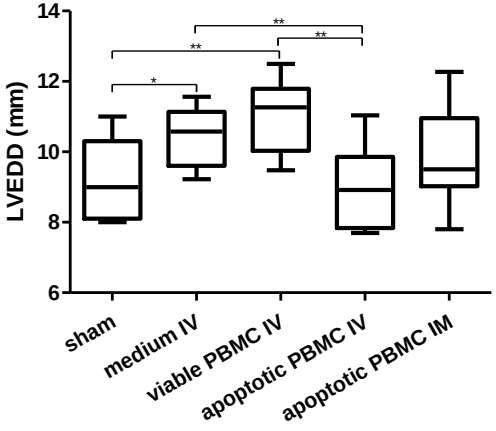
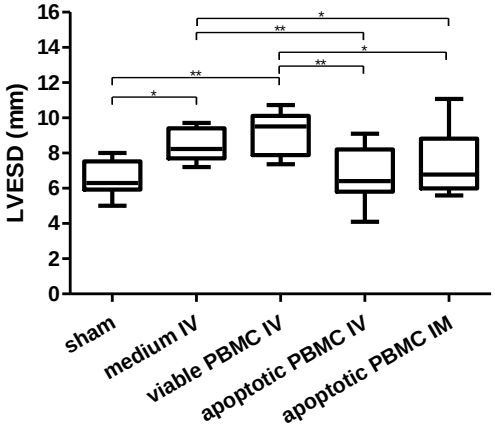
Results

Evaluation of Cardiac Function









n=10-12

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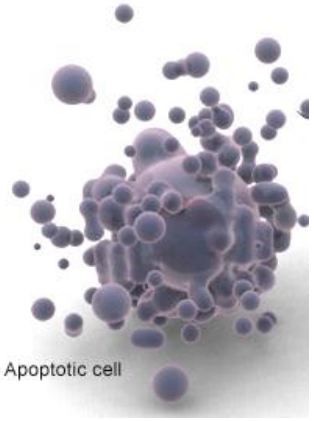
Conclusion

Administration of irradiated apoptotic PBMC after myocardial infarction induces …



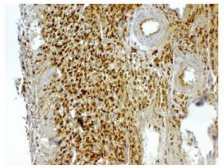
Reduction of Pro-inflammatory Signals

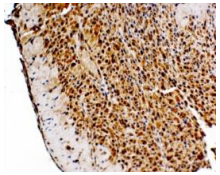
Il-1β ↓
IL-6 ↓

Up-regulation of Pro-angiogenic mediators

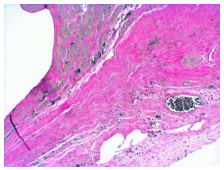
Interleukin-8 ↑
MMPs ↑

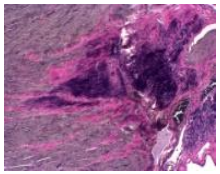
Increased Homing of CD68⁺ and c-kit⁺ Cells





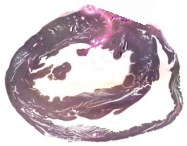
Favorable Elastin/Collagen Ratio





Better Recovery of Cardiac Function

Ejection Fraction ↑
Shortening Fraction ↑
Dilation ↓



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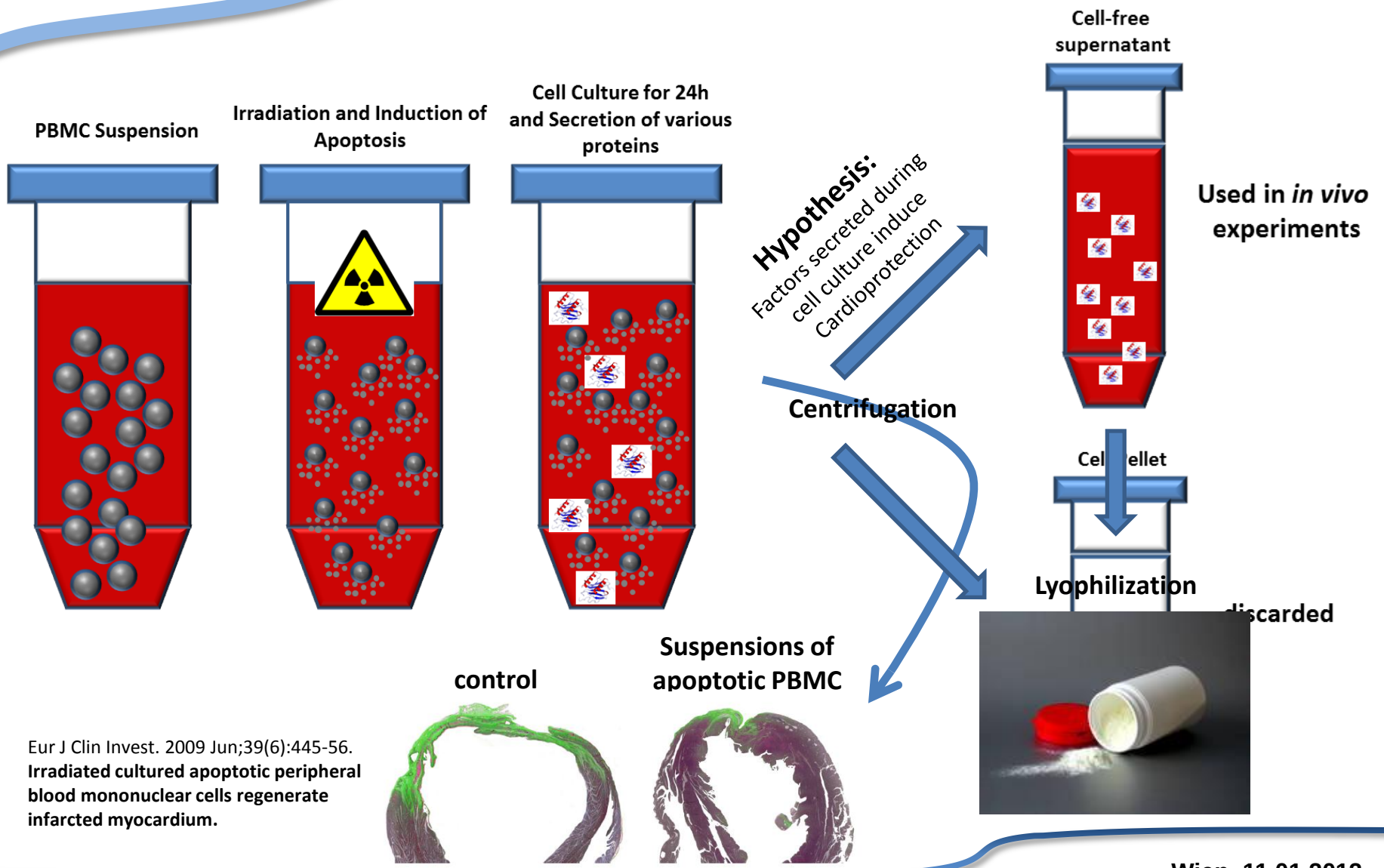
Experimental Set-up



Eur J Clin Invest. 2009 Jun;39(6):445-56.
Irradiated cultured apoptotic peripheral blood mononuclear cells regenerate infarcted myocardium.

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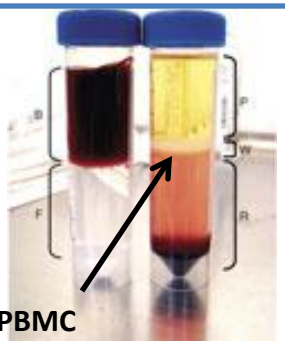
Production of APOSEC

(Cell culture supernatants of apoptotic PBMC)

Venous Blood Withdrawal

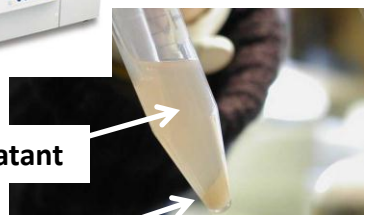
Ficoll Cell Separation



Irradiation

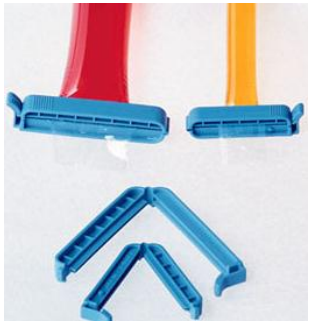
Incubation for 24h

Centrifugation



Cell Pellet (is discarded)

Dialysis



Lyophilization

Lyophilized Cell Culture Supernatant - Aposec -

PBMC peripheral blood mononuclear cells

Wien, 11.01.2012

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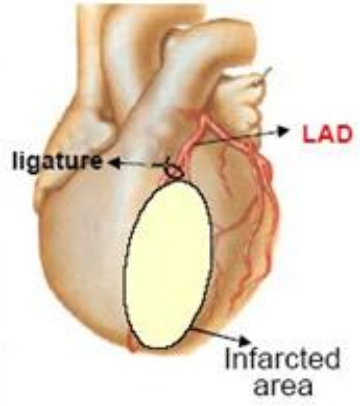
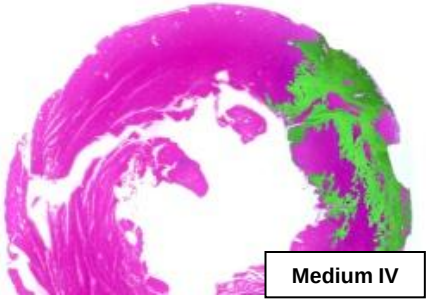
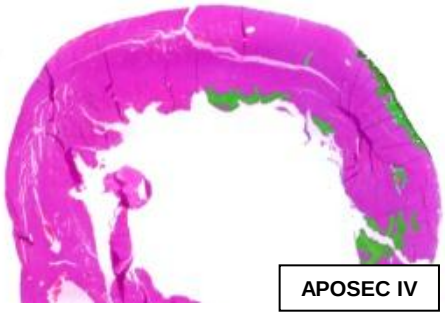
APOSEC

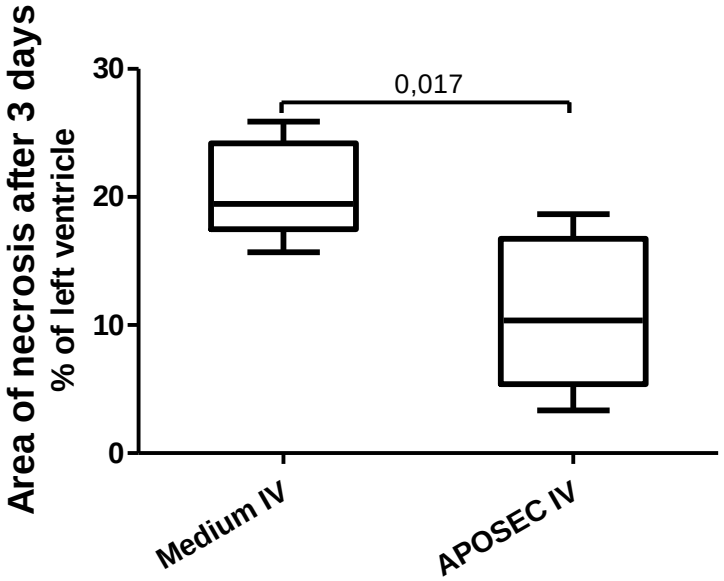
AMI – Small Animal Model



Results after 72h







n=5-6 per group

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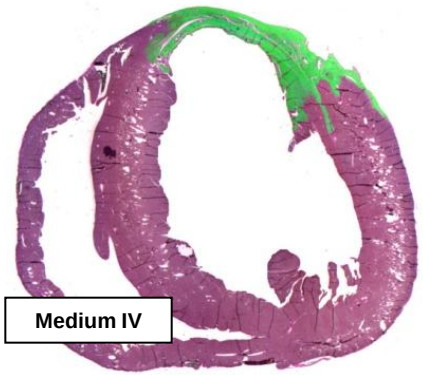
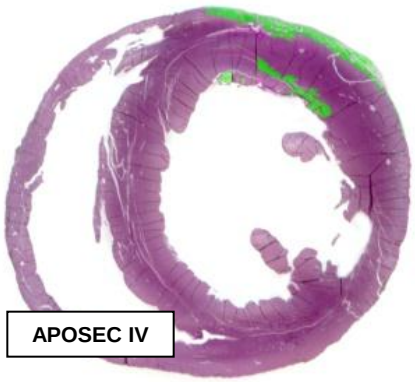
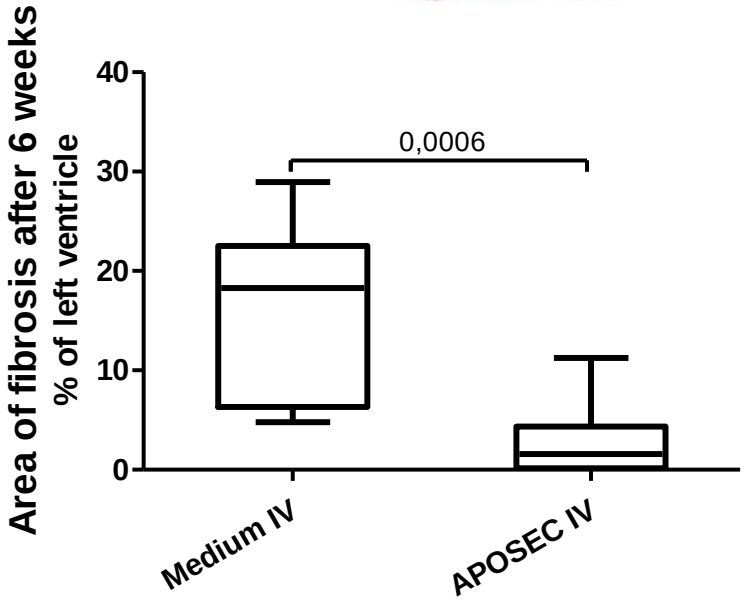
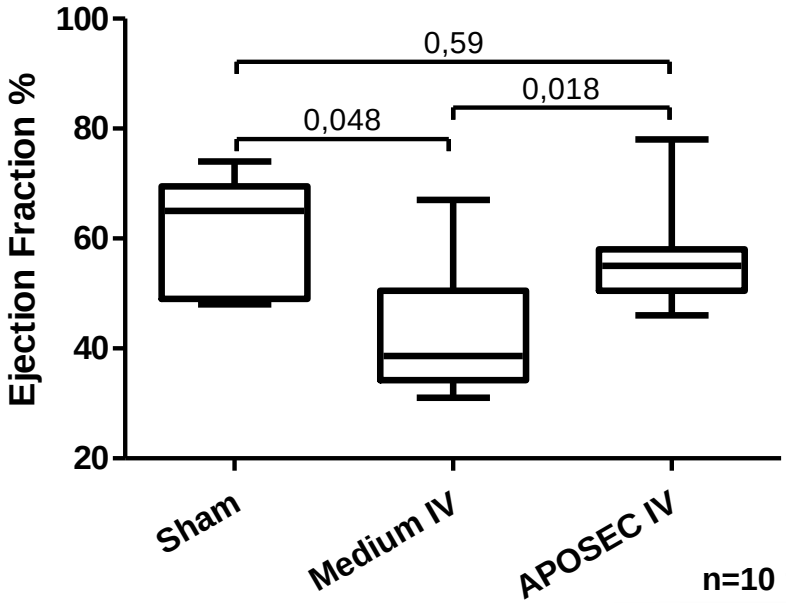
AMI – Small Animal Model

Results after 6 Weeks









n=10 per group

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APOSEC

AMI – Large Animal Model

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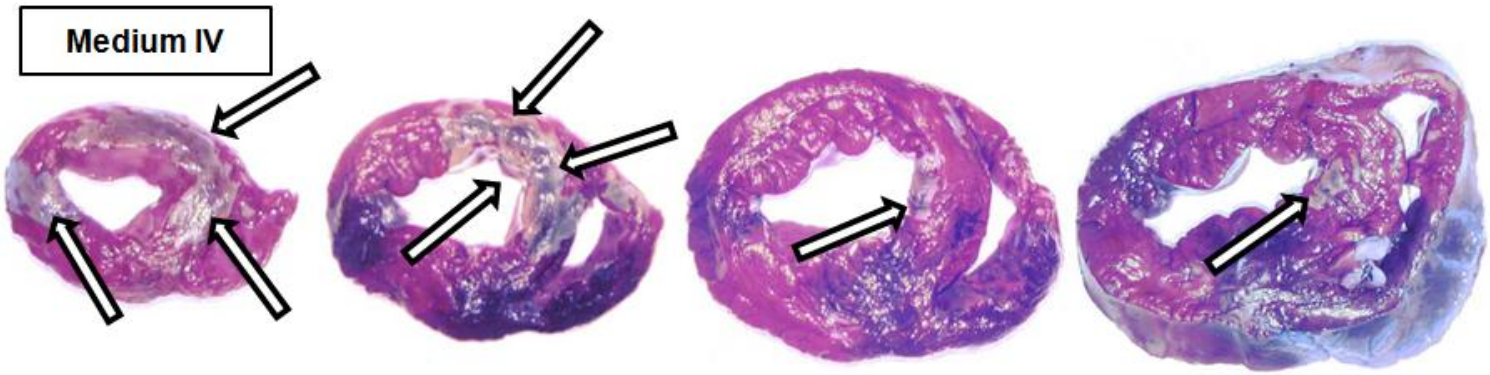
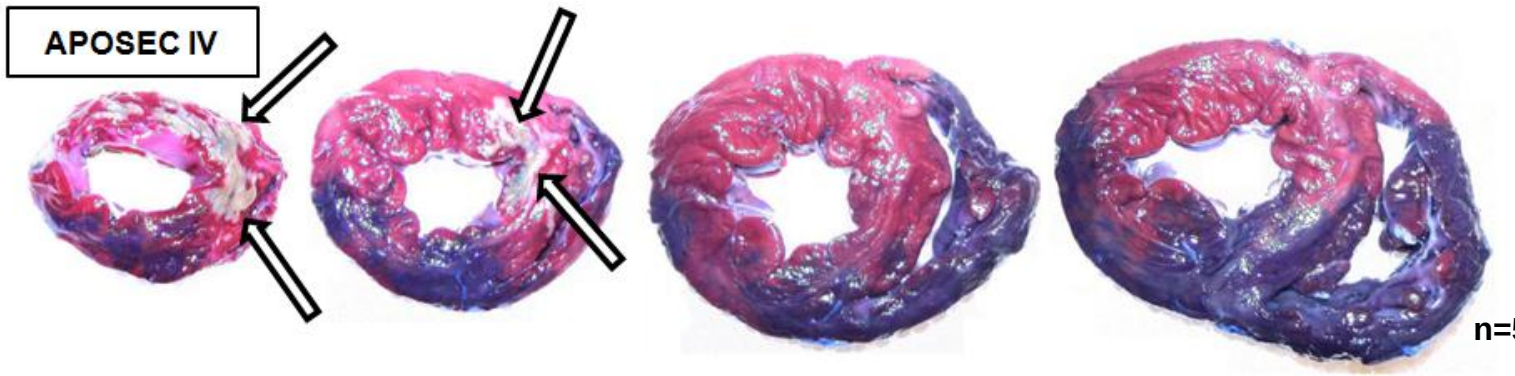
APOSEC

Reperfused AMI
Large Animal Model

Results after 24 Hours





n=5

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APOSEC

Reperfused AMI
Large Animal Model

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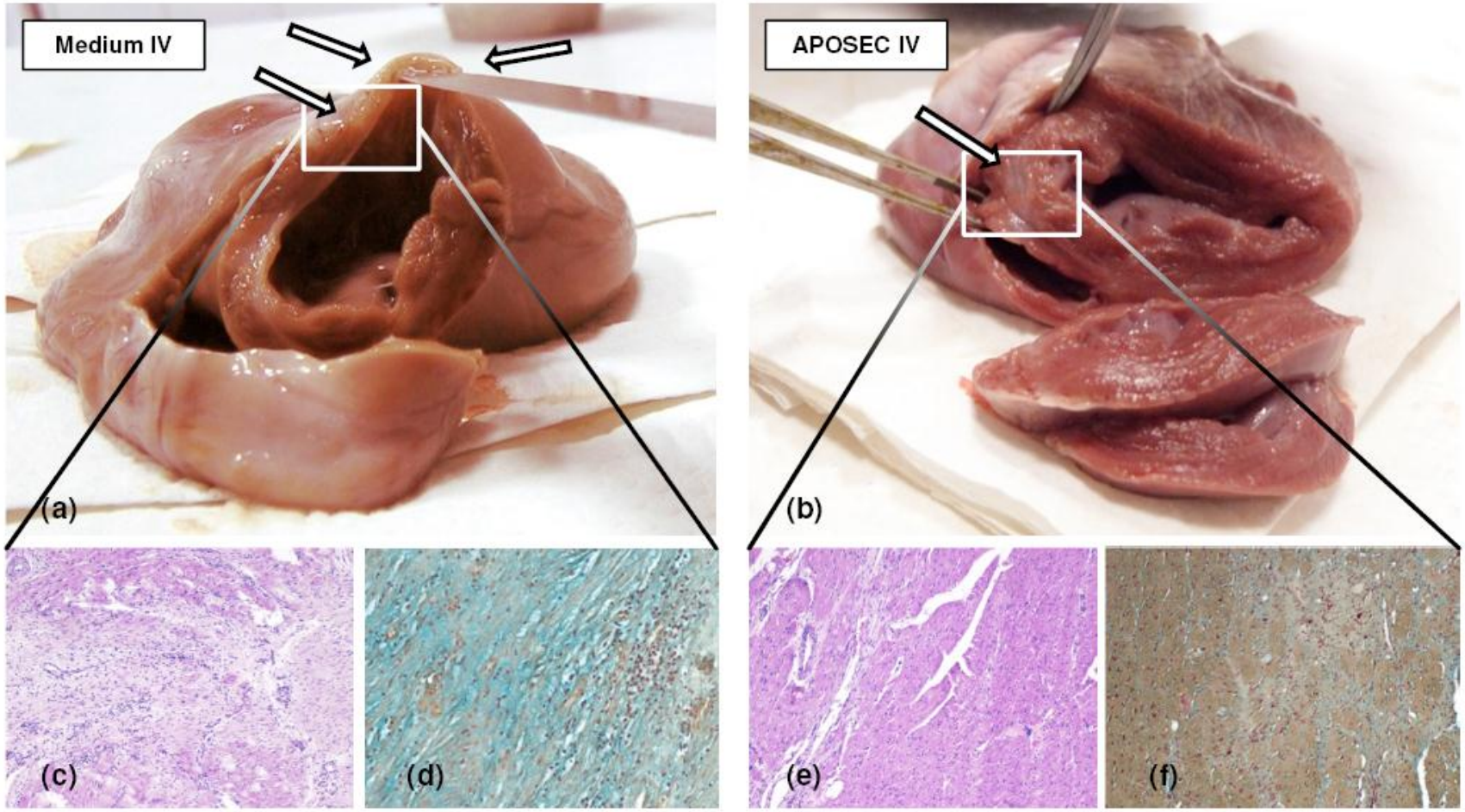
Results after 30 Days



n=7-9 per group

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APOSEC

Reperfused AMI
Large Animal Model

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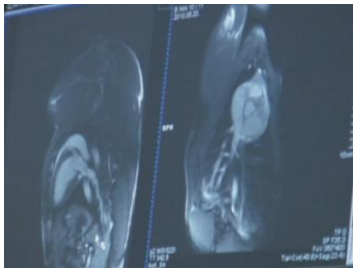
Results MRI Analysis



Cardiac MRI evaluation 3 and 30 days after Ischemia/Reperfusion Injury

	Parameters	Medium control (n=8)	$250 \cdot 10^6$ apoptotic PBMC (low dose APOSEC, n=7)	$1 \cdot 10^9$ apoptotic PBMC (high dose APOSEC, n=9)
after 3 days	age (days)	90 ±0	90 ±0 *ns*	90 ±0 *ns*
	LVEDV (ml)	67·59 ±2·7	64·19 ±5·4 *ns*	63·73 ±1·6 *ns*
	LVESV(ml)	38·42 ±2·5	35·96 ±3·0 *ns*	33·93 ±2·1 *ns*
	LVSV (ml)	29·17 ±1·3	28·23 ±3·2 *ns*	29·77 ±1·8 *ns*
	LVEF (%)	43·38 ±1·9	43·63 ±2·8 *ns*	46·65 ±2·9 *ns*
	HR/min.	111 ±6	109 ±5 *ns*	111 ±13 *ns*
	CO (l/min.)	3·24 ±0·1	3·03 ±0·3 *ns*	3·28 ±0·3 *ns*
	CI (l/min/m^2)	3·64 ±0·14	3·59 ±0·4 *ns*	3·82 ±0·37 *ns*
	Infarct %	18·17 ±1·7	14·01 ±1·9 *ns*	8·66 ±1·5 **
after 30 days	age (days)	120 ±0	120 ±0 *ns*	120 ±0 *ns*
	LVEDV (ml)	54·74 ±4·1	53·43 ±3·5 *ns*	65·99 ±3·5 *ns*
	LVESV(ml)	32·93 ±4·0	31·89 ±3·2 *ns*	28·71 ±3·5 *ns*
	LVSV (ml)	21·84 ±1·8	21·54 ±2·0 *ns*	37·29 ±1·7 ***
	LVEF (%)	40·54 ±3·6	40·64 ±3·5 *ns*	57·05 ±3·3 **
	HR/min.	114 ±7	108 ±8 *ns*	107 ±5 *ns*
	CO (l/min.)	2·44 ±0·1	2·28 ±0·1 *ns*	3·98 ±0·2 ***
	CI (l/min/m^2)	2·46 ±0·12	2·40 ±0·15 *ns*	3·51 ±0·15 ***
	Infarct %	12·60 ±1·3	11·50 ±1·6 *ns*	6·92 ±1·4 *

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Analysis of Protein Content of APOSEC

(Cell culture supernatants of apoptotic PBMC)

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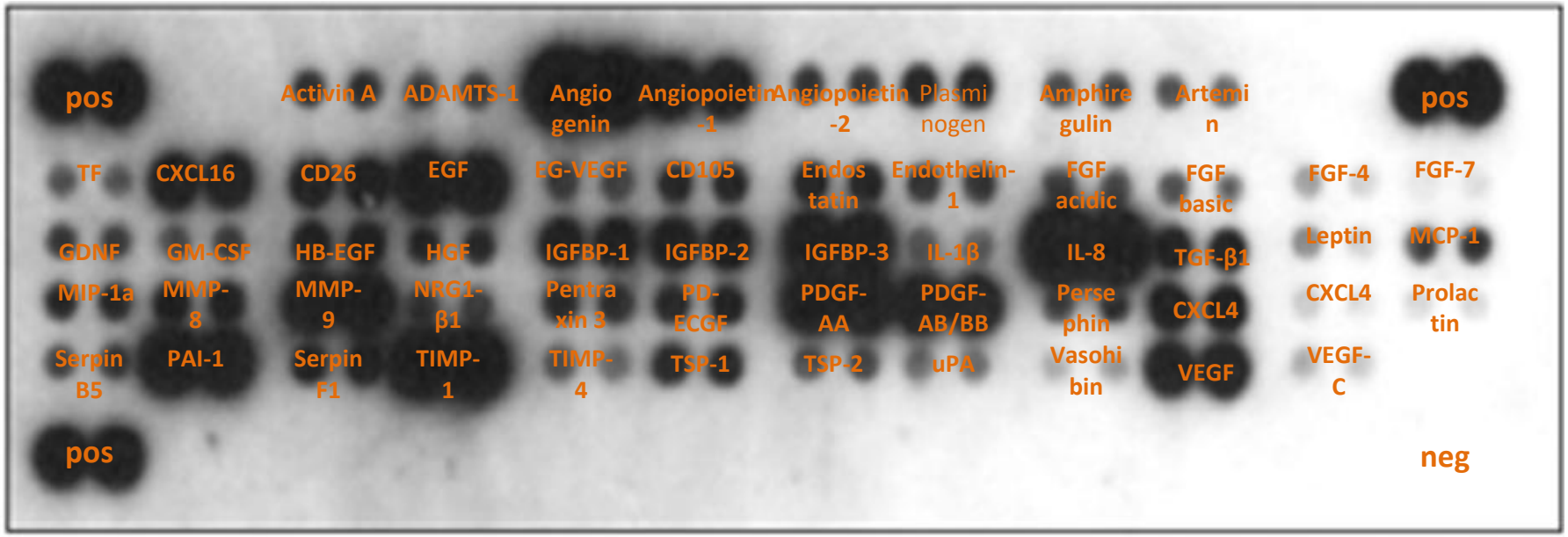
Membran Array – Angiogenic Factors



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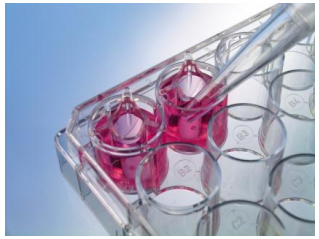
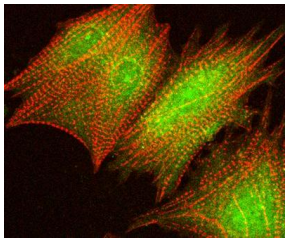
APOSEC

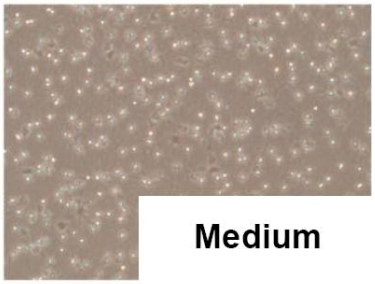
Mechanism of Action

Cell Culture of human Cardiomyocytes





Cell Starvation Assay



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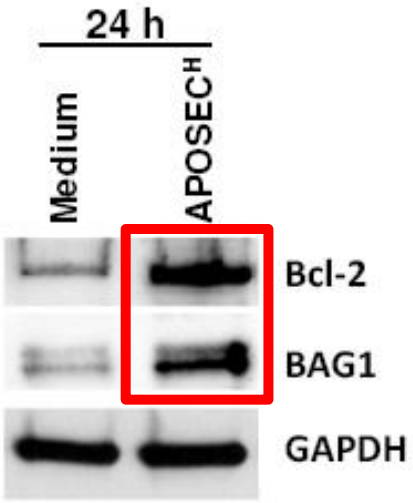

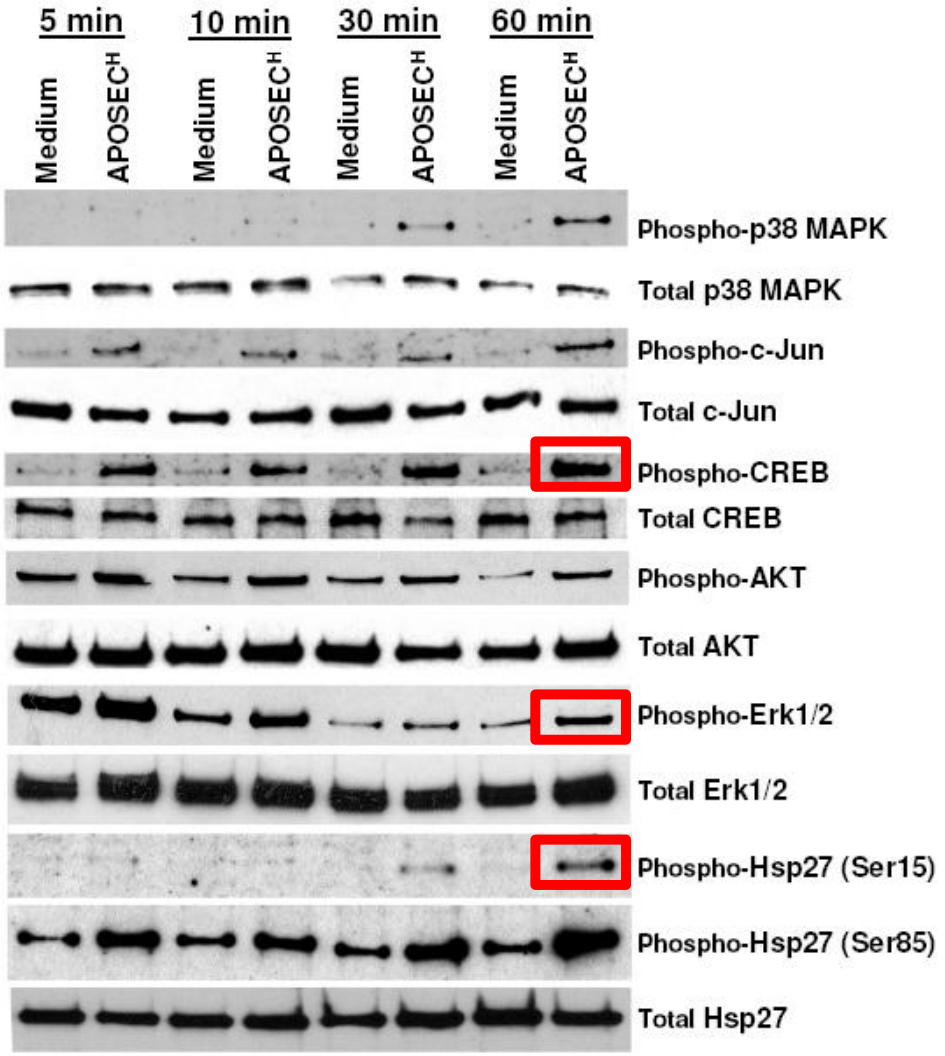

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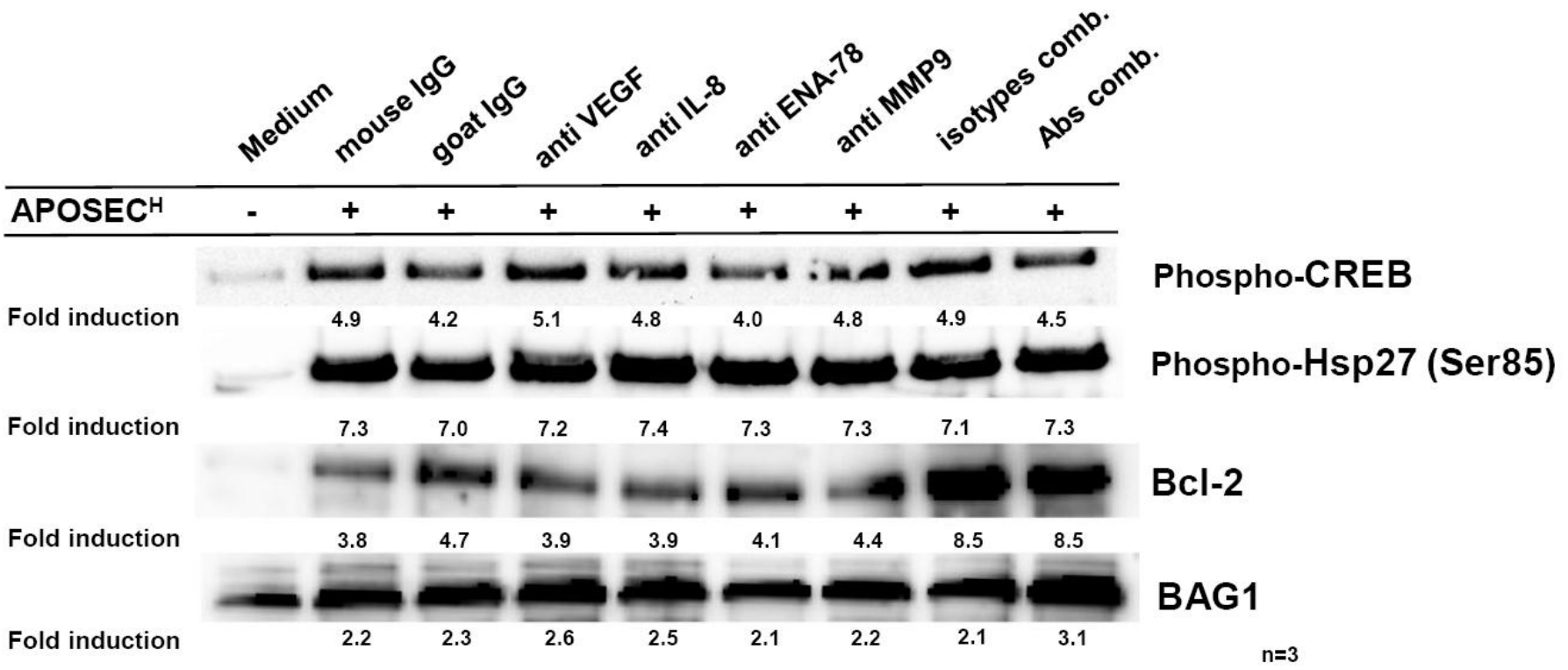
Mechanism of Action

Cell Culture of human Cardiomyocytes – Factor Inhibtion Assay



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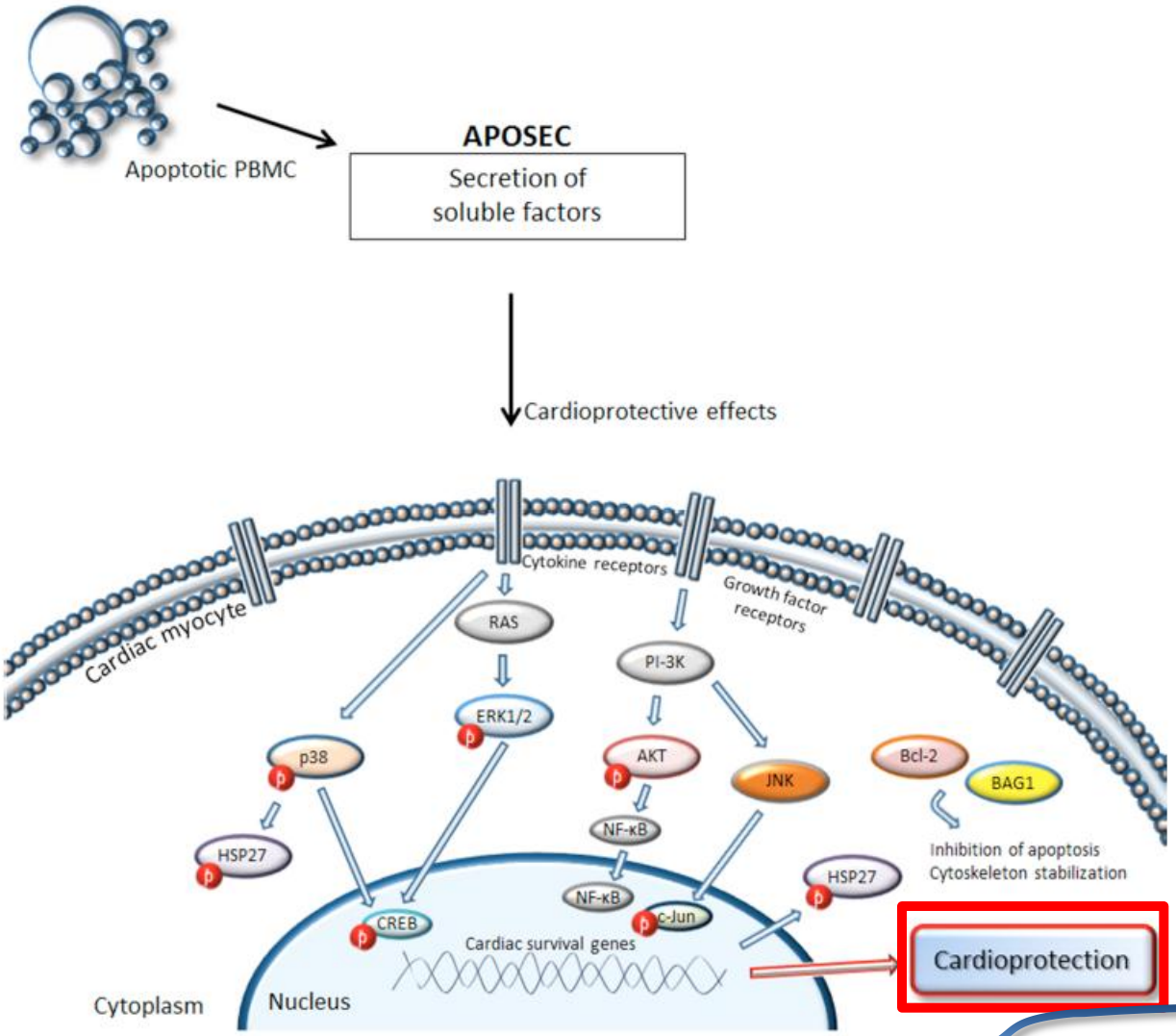
Mechanism of Action



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Christian Doppler Laboratory
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