



TURIN, 20<sup>TH</sup>—21<sup>ST</sup> NOVEMBER 2008

# GREAT INNOVATIONS IN CARDIOLOGY

4<sup>TH</sup> JOINT MEETING WITH MAYO CLINIC

4<sup>TH</sup> TURIN CARDIOVASCULAR NURSING CONVENTION



ABSTRACT SESSION (PART I)

Chairmen

**F. Bertello (Torino), M. Sicuro (Aosta)**



# PURIFIED AND EXPANDED BMC FOR CARDIAC REPAIR AFTER AMI: PRELIMINARY PRECLINICAL RESULTS

Dott. Monica Gunetti, PhD

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Struttura Complessa Oncoematologia Pediatrica, Centro Trapianti Cellule  
Staminali  
e Terapia Cellulare**

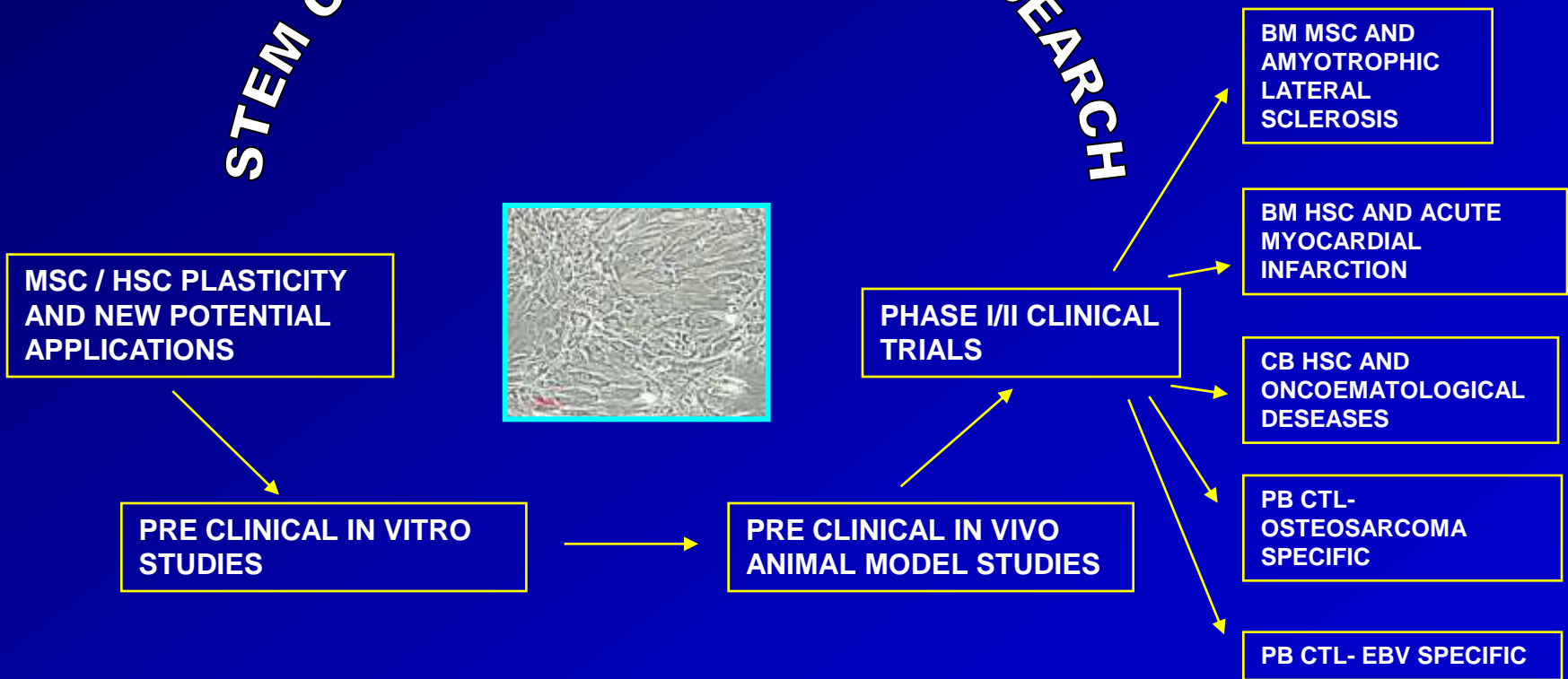


Direttore: Dr. Franca Fagioli



# STEM CELL TRANSPLANTATION AND CELLULAR THERAPY LABORATORY EXPERTIES

## STEM CELL TRANSLATIONAL RESEARCH



# HSC EX-VIVO EXPANSION

*Blood. 89: 2644, 1997*

*Leukemia. 12: 718, 1998*

*Blood. 93: 3736, 1999*

*Blood. 100: 4391, 2002*

*Bone Marrow Transp. 29: 443, 2002*

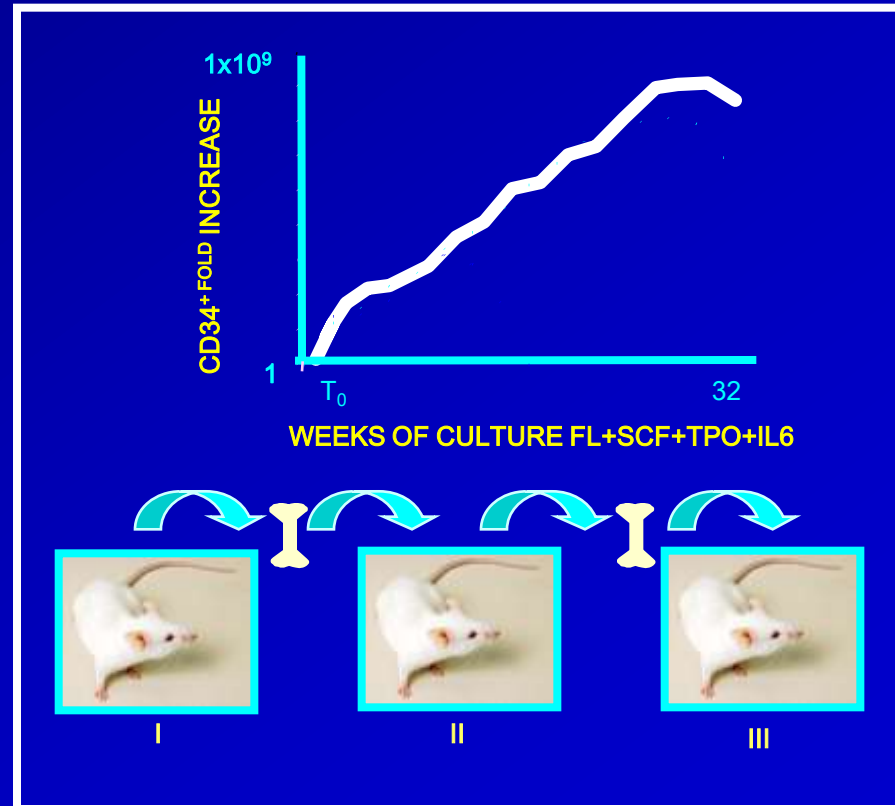
*Exp. Hem. 31: 261, 2003*

*Blood. 103: 4440, 2004*

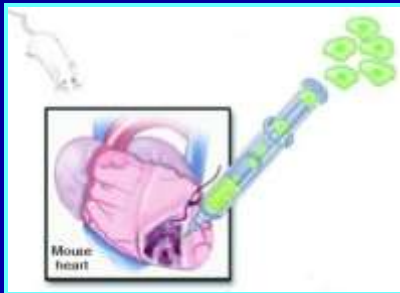
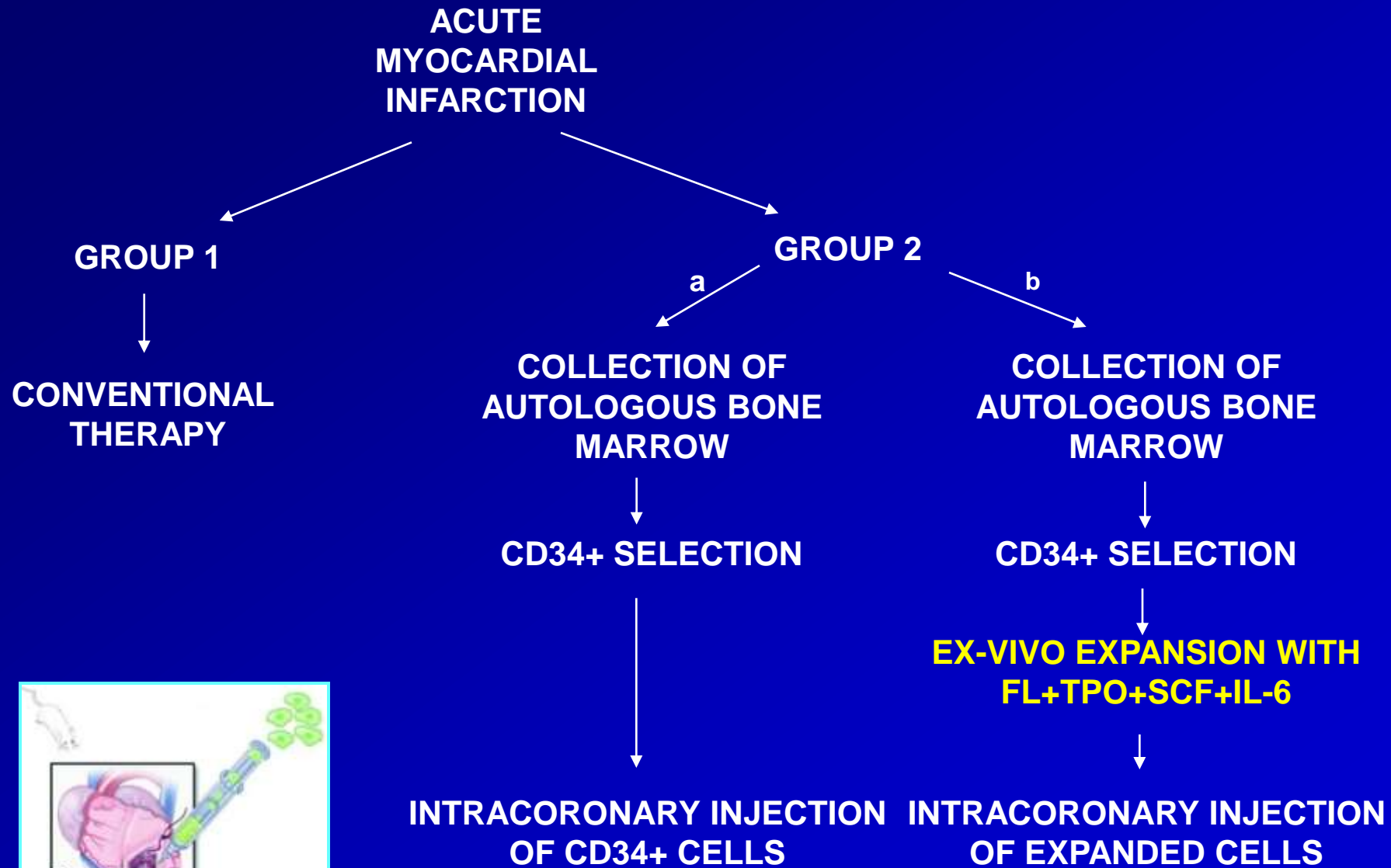
*Stem Cells. 24: 1201, 2006*

*Exp Hematol. 36: 235, 2008*

*Stem Cells. 26: 1620, 2008*



# EX-VIVO EXPANSION CELL THERAPY DESIGN



# CTPs IMMUNOPHENOTYPE ANALYSIS

## BONE MARROW SAMPLES OF HEALTHY DONORS

	CD34+	CD133+	CD14+	CD31+
<b>BASAL</b> <i>n° tot cell</i>	2.2x10 <sup>6</sup>	1.4x10 <sup>6</sup>	0.2 x10 <sup>6</sup>	3.4 x10 <sup>6</sup>
<b>+1 WEEK</b> <i>n° tot cell</i>	3.9 x10 <sup>6</sup>	3 x10 <sup>6</sup>	1.7 x10 <sup>6</sup>	10.1 x10 <sup>6</sup>

**A. Basal CD34+ bone marrow cell immunophenotype and after 1 week of expansion**

CD34+CD133+	CD34+KDR+
1.4 x10 <sup>6</sup>	0.01 x10 <sup>6</sup>
5.4 x10 <sup>6</sup>	0.02 x10 <sup>6</sup>

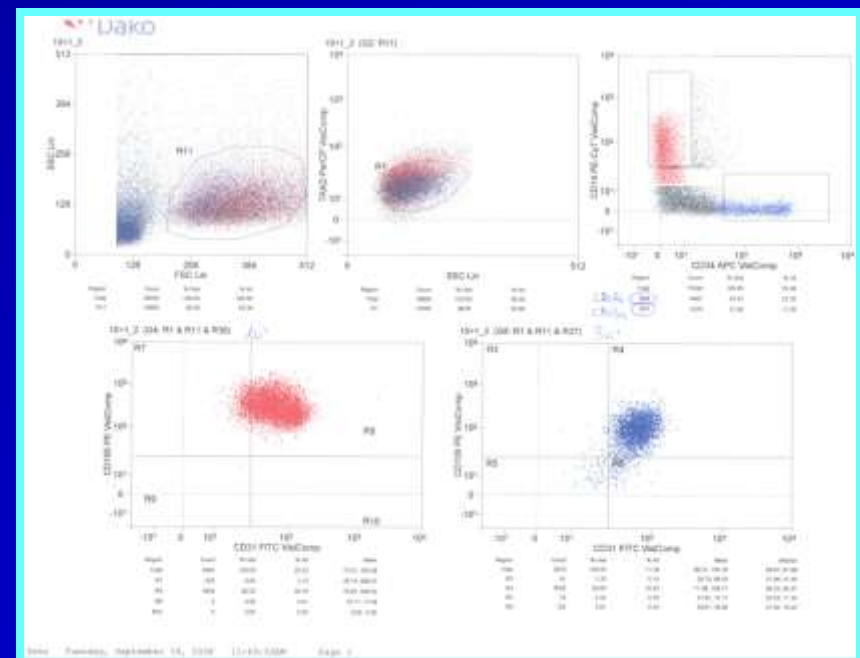
**B. Immunophenotype of CD34 bone marrow cell subpopulations**

	CD14+CD105+	CD14+KDR+
<b>BASAL</b> <i>n° tot cell</i>	0.7 x10 <sup>6</sup>	0.03 x10 <sup>6</sup>
<b>+1 WEEK</b> <i>n° tot cell</i>	5.3 x10 <sup>6</sup>	0.2 x10 <sup>6</sup>

**C. Immunophenotype of CD14 bone marrow cell subpopulations**

	CD31+CD105+	CD31+KDR+
<b>BASAL</b> <i>n° tot cell</i>	0.1 x10 <sup>6</sup>	0.04 x10 <sup>6</sup>
<b>+1 WEEK</b> <i>n° tot cell</i>	2.7 x10 <sup>6</sup>	0.07 x10 <sup>6</sup>

**D. Immunophenotype of bone marrow cell subpopulations during expansion**



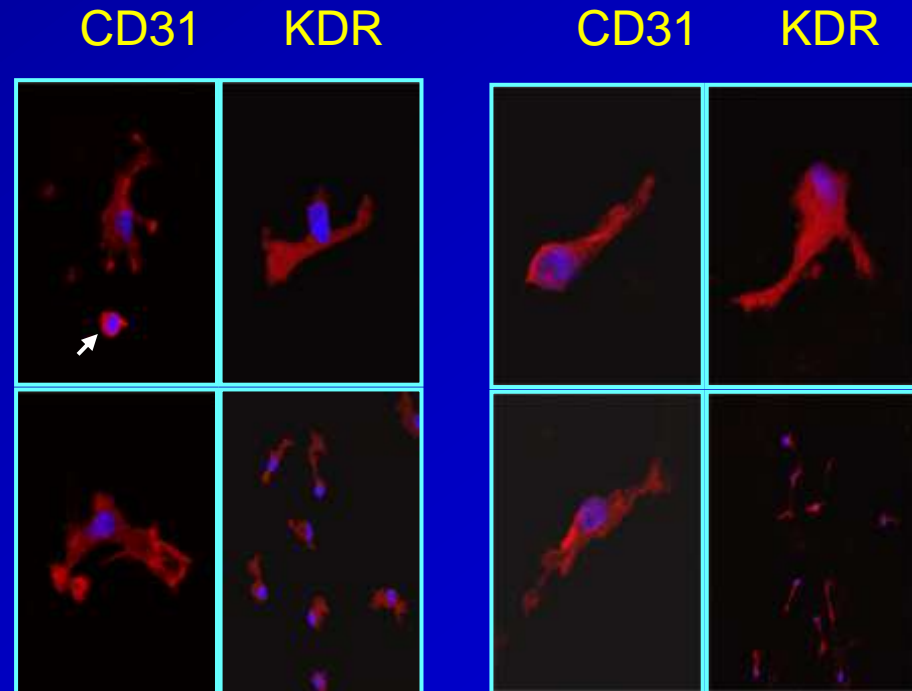
UP reg	DOWN reg	NOt reg	NOt expr
CD 14	VE-Cadherin	MHC 11	CNN1
Endoglin	NG 2	CD 34	FABP
NANOG	CXCR4	GALC	GAP43
NPN-1	GATA1	GATA2	SOX10
S100B	KDR	cKIT	
TUBB3	Mel-CAM	Nestin	
	NEF L	eNOS	
	PDGFRB	PECAM	
	SYN 1	CD133	
	SYP	CD45	
	TEK	RGS5	
		SEMA3A	
		GLAST	
		wWF	

## GENE EXPRESSION PROFILE

## IN VITRO DIFFERENTIATION

CD34+

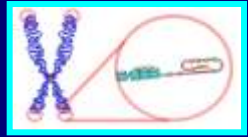
CD34 -



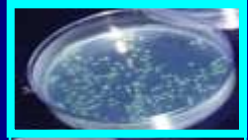
# CTPs QUALITY CONTROLS



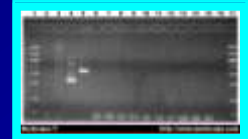
VIABILITY ASSAY: **MORE THEN 98% VIABLE CELLS**



CELL SENEESCENCE : **NO TELOMERE SHORTENING**



STERILITY ASSAY: **NEGATIVE**  
ENDOTOXIN ASSAY: **NEGATIVE**



MYCOPLASMA CONTAMINATION : **NEGATIVE**



CYTOGENETIC ASSAY : **CARIOTYPE NORMAL**



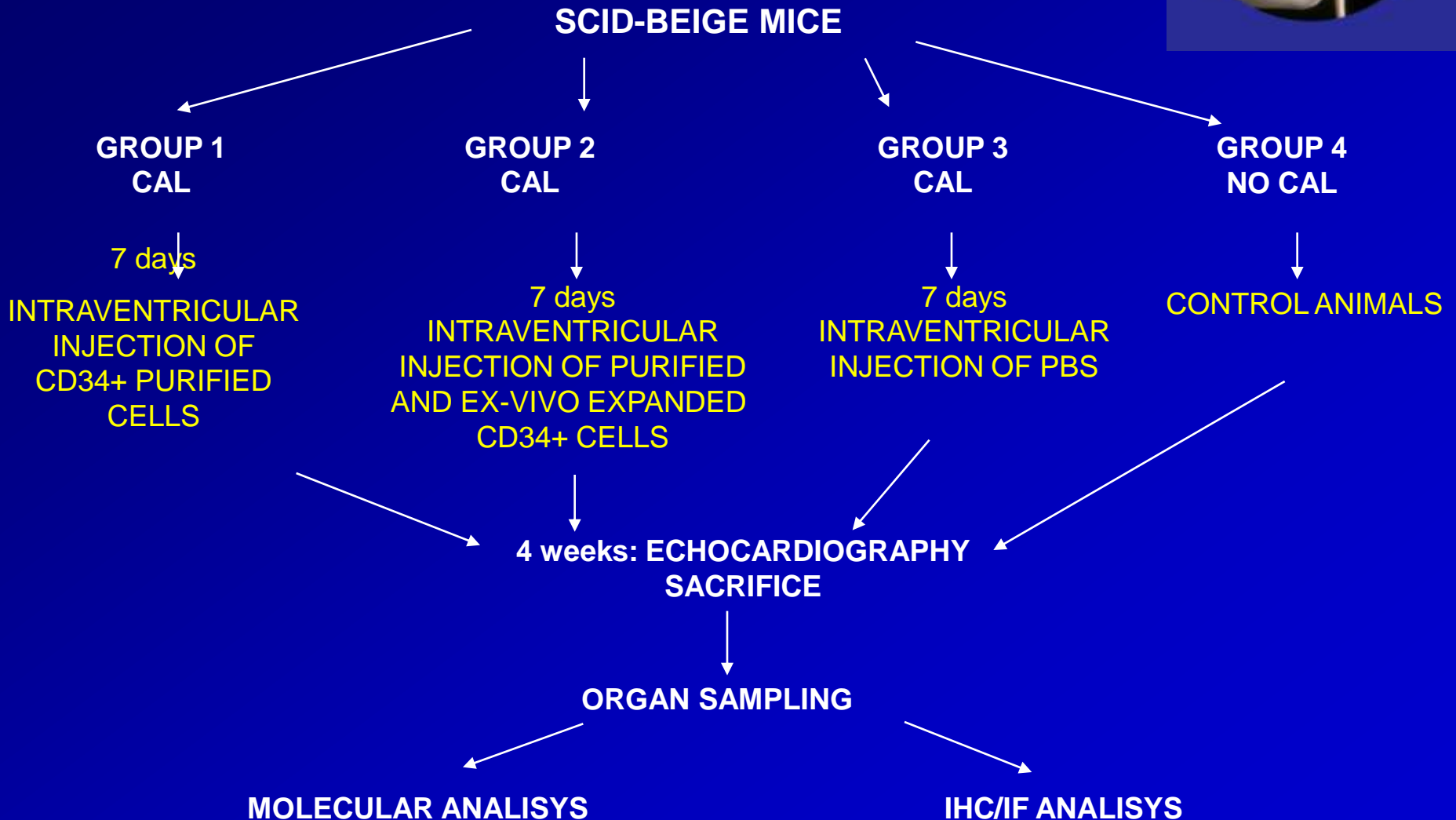
# 1st PRE CLINICAL IN-VIVO STUDIES

LABORATORIO DI FARMACOLOGIA CLINICA CARDIOVASCOLARE, Director Dr. Roberto Latini

Istituto di Ricerche Farmacologiche "Mario Negri", Milano



## EXPERIMENTAL DESIGN :



# MOLECULAR ANALISYS

	n°7 Not Oper.	n° 3360 Not Oper.	n°3398 Not Oper.	n°3359 Not Oper.	n° 3400 PBS	n° 3389 PBS	n° 3383 PBS	n°3393 Basal Cells	n°3388 Basal Cells	n°3323 Exp. Cells	n°3315 Exp. Cells	n°3321 Exp. Cells	n°3324 Exp. Cells	n°3325 Exp. Cells
Left Ventricle	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Right Heart	-	-	-	-	-	-	-	-	-	+	+	-	+	-
Septum	-	-	-	-	-	-	-	-	+	-	-	-	+	-
Lung	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-	-	-	+	-	+	-
Spleen	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	+	-	-	-	-	-	-



PCR images

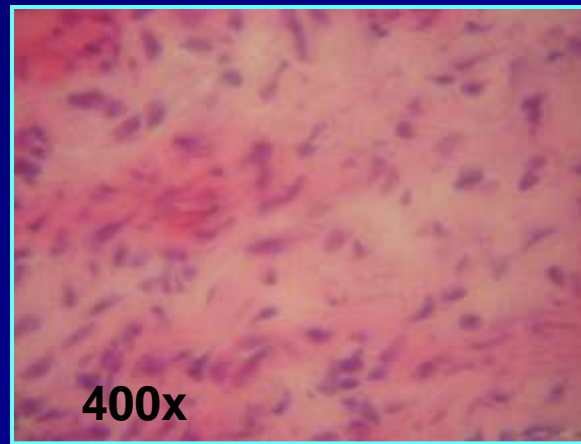
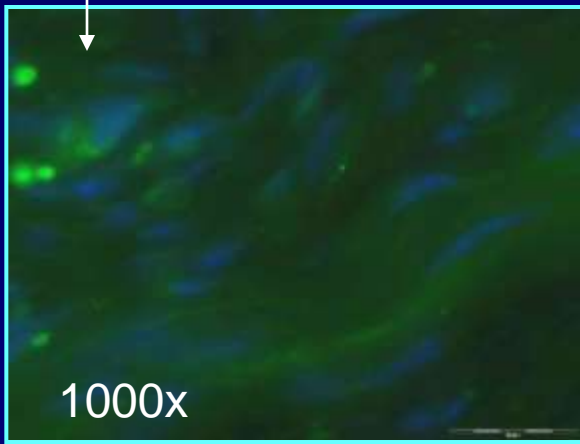
**100% OF THE MICE INJECTED WITH THE EXPANDED CELLS ARE POSITIVE FOR THE HUMAN CHROMOSOME 17  $\alpha$ -SAT IN THE LEFT VENTRICLE**

- 60% OF THE MICE INJECTED WITH THE EXPANDED CELLS ARE POSITIVE FOR THE HUMAN CHROMOSOME 17  $\alpha$ -SAT IN THE RIGHT HEART

- 50% OF THE MICE INJECTED WITH THE BASAL CELLS AND 20% OF THE MICE INJECTED WITH EXPANDED CELLS ARE POSITIVE FOR THE HUMAN CHROMOSOME 17  $\alpha$ -SAT IN THE SEPTUM

- 100% OF THE MICE ARE NEGATIVE FOR THE HUMAN CHROMOSOME 17  $\alpha$ -SAT IN THE LUNG AND SPLEEN

- 50% OF THE MICE INJECTED WITH THE BASAL CELLS ARE POSITIVE FOR THE HUMAN CHROMOSOME 17  $\alpha$ -SAT IN THE LIVER



Cell in scar tissue

## Echocardiography

	CAL+ expanded CD34+ cells N=7	CAL+ no expanded CD34+ cells N=2	CAL+PBS N=3	NO CAL N=6
HR (bmp)	541±23*	550	635±18	638±25
BW (g)	18.89±1.13*	15.5	19.63±0.30	18.15±0.24
LVIDd (mm)	4.36±0.30	4.25	3.97±0.33	2.35±0.02
LVIDs (mm)	3.66±0.21	3.65	3.23±0.47	0.57±0.11
AWThd (mm)	0.44±0.05	0.40	0.53±5.55	0.82±0.03
SF (%)	16.03±2.70	13.5	18.93±5.26	74.78±5.55
EF (%)	22.27±4.23	13.80	33.33±7.39	91.28±2.58

\*mean±SEM

# 2nd PRE CLINICAL IN-VIVO STUDIES



## EXPERIMENTAL DESIGN :

**SCID-BEIGE MICE**

**CAL**



**4 hours: INTRAVENTRICULAR  
INJECTION OF  
CD34+ PURIFIED CELLS**



**7 days: INTRAVENTRICULAR  
INJECTION OF PURIFIED  
AND EX-VIVO EXPANDED  
CD34+ CELLS**



**4 weeks: ECHOCARDIOGRAPHY  
SACRIFICE**



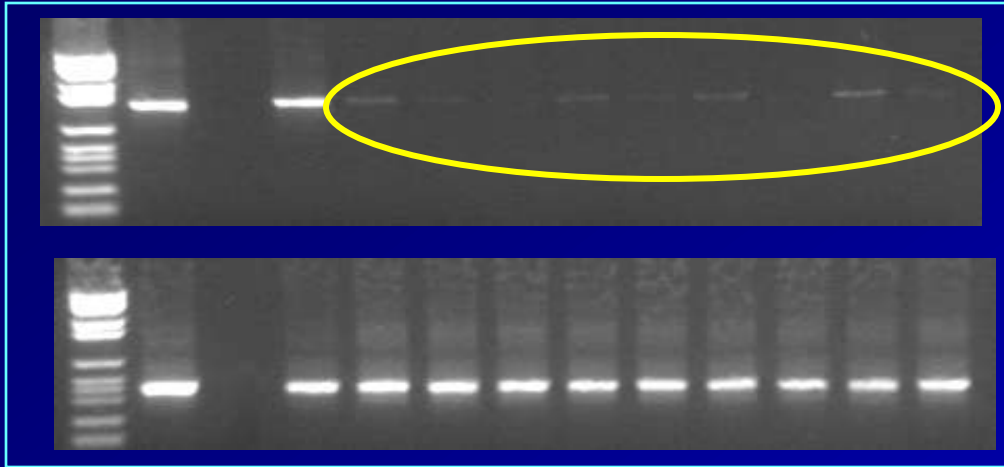
**ORGAN SAMPLING**



**MOLECULAR ANALISYS**

# MOLECULAR ANALISYS

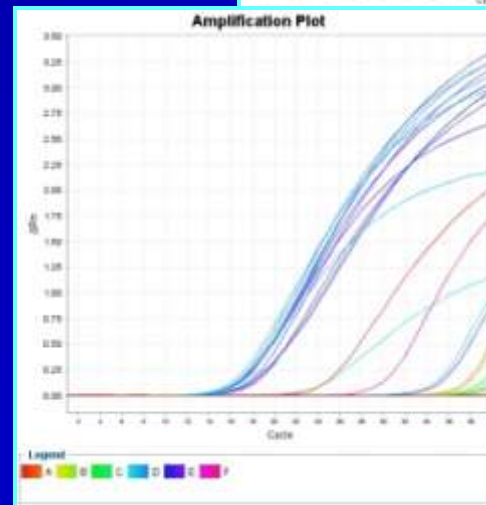
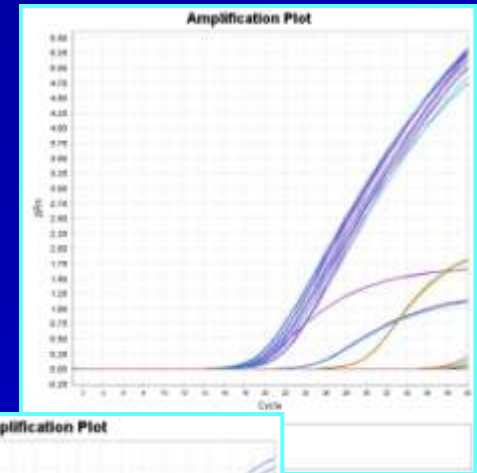
MW C+ C- C+\* T3952 T3953 T3954 T3960 T3961 T3963 T3965 T3967 T3969



h  $\alpha$ -sat cr17

GAPDH

## LEFT VENTRICLE



Quantitative  
Real Time  
PCR  
in progress

liver

kidney



h  $\alpha$ -sat cr17

GAPDH

lung

spleen

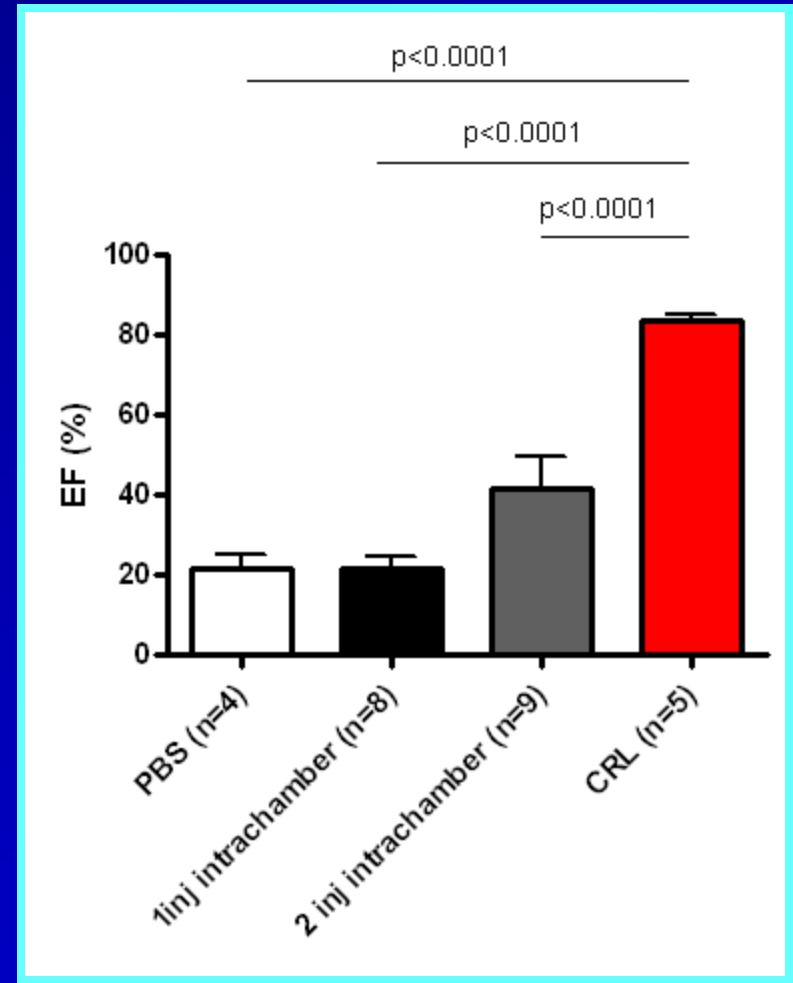
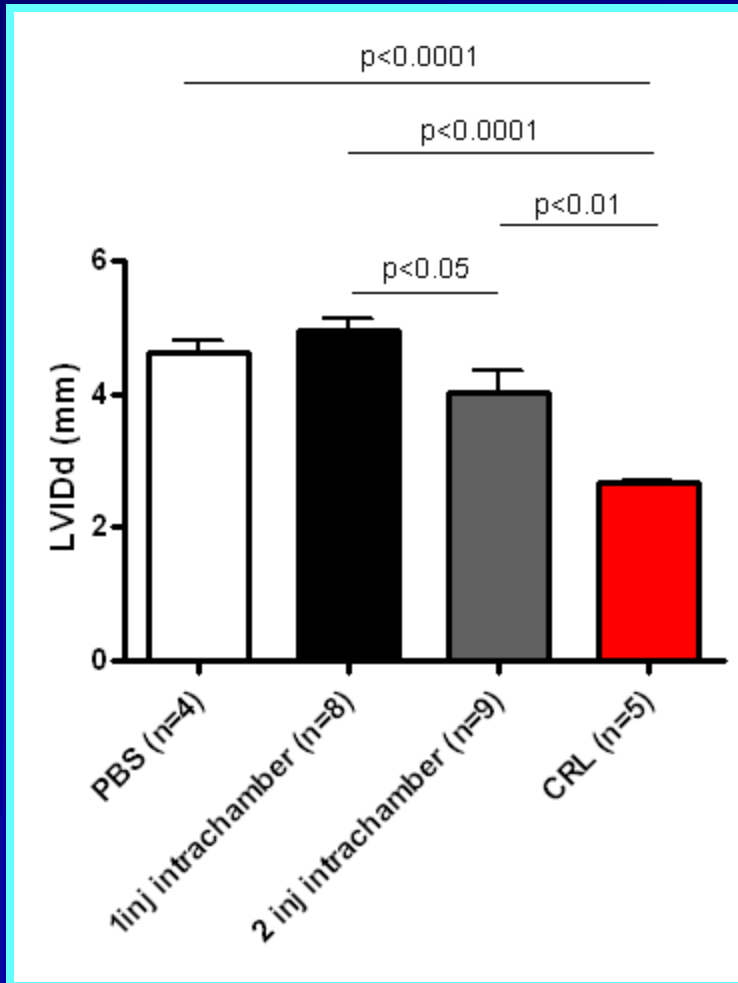


h  $\alpha$ -sat cr17

GAPDH



# Echocardiography



# FUTURE.....

## 1) Timing studies:

- single dose of purified bone marrow CD34+ cells (4 hours after infarct induction)
- single dose of purified bone marrow CD34+ cells (7 days after infarct induction)
- single dose of 1 week *ex-vivo* expanded bone marrow CD34+ cells (7 days after infarct induction)
- double dose of cells: purified CD34+ bone marrow cells (4 hours after infarct induction) + 1 week *ex-vivo* expanded CD34+ cells (7 days after infarct induction)

## 2) Migration and Homing studies

## 3) Differentiation studies of expanded subpopulation

## 4) Large Scale Animal model

## 5) Develop and validate a GMP process to obtain GMP Investigational medicine product to be used in Phase 2 of the trial

## 6) Clinical application of expanded CTP for IMA treatment

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