Thérapie Cellulaire de l'Insuffisance Cardiaque

APPAC

Biarritz, 10 Juin 2015

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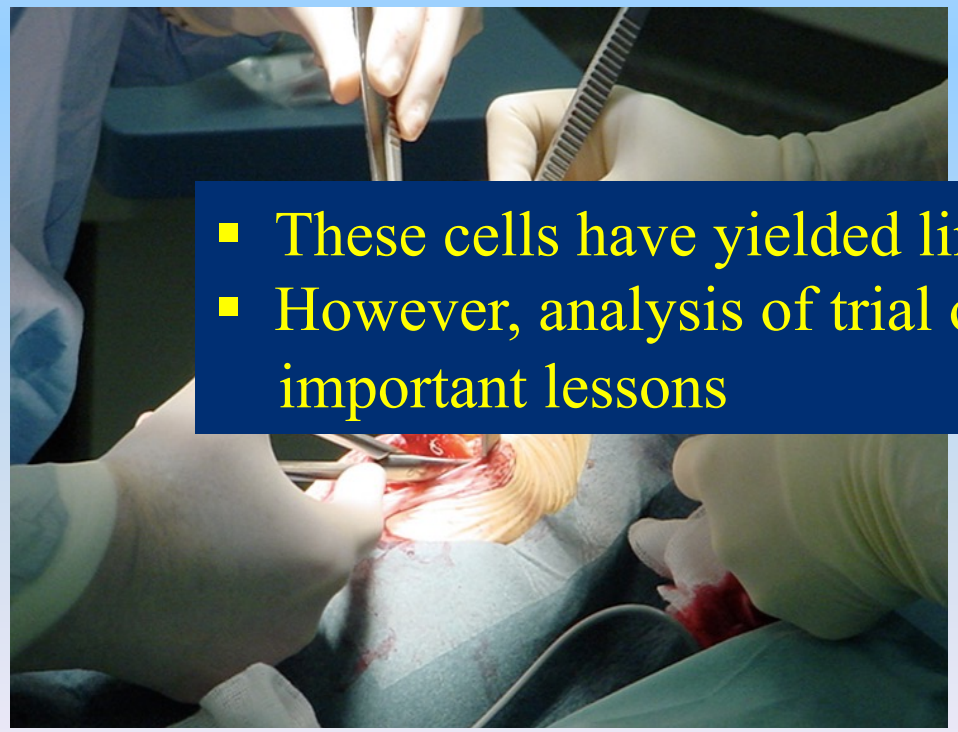
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

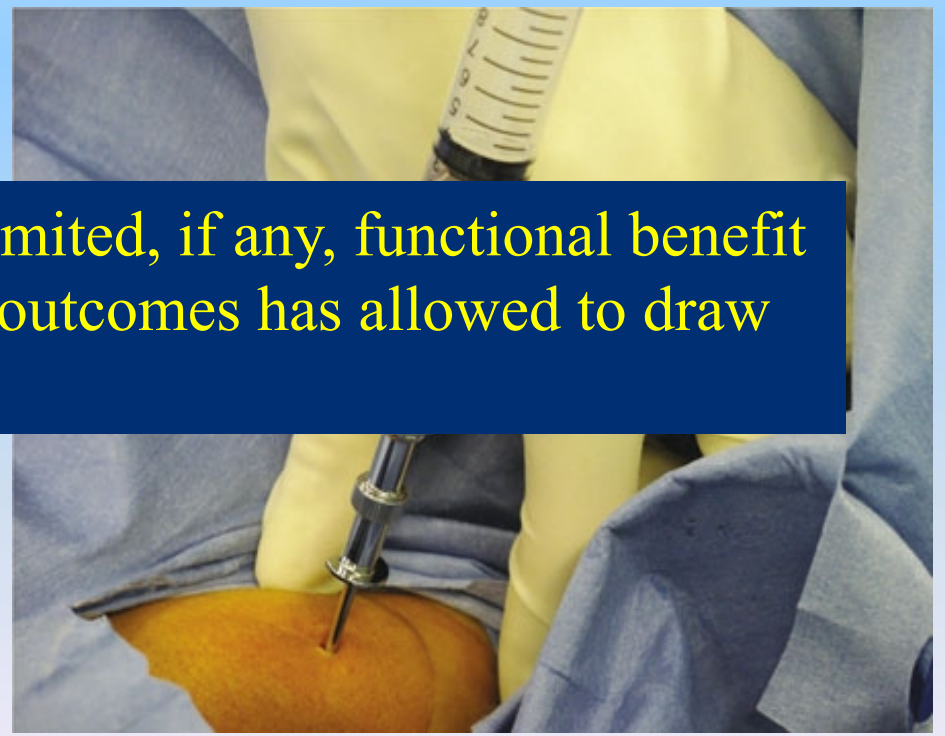
UNIVERSITÉ PARIS DESCARTES

The First Decade of Stem Cell Clinical Trials for Heart Failure

Skeletal Myoblasts



Bone Marrow-Derived Cells



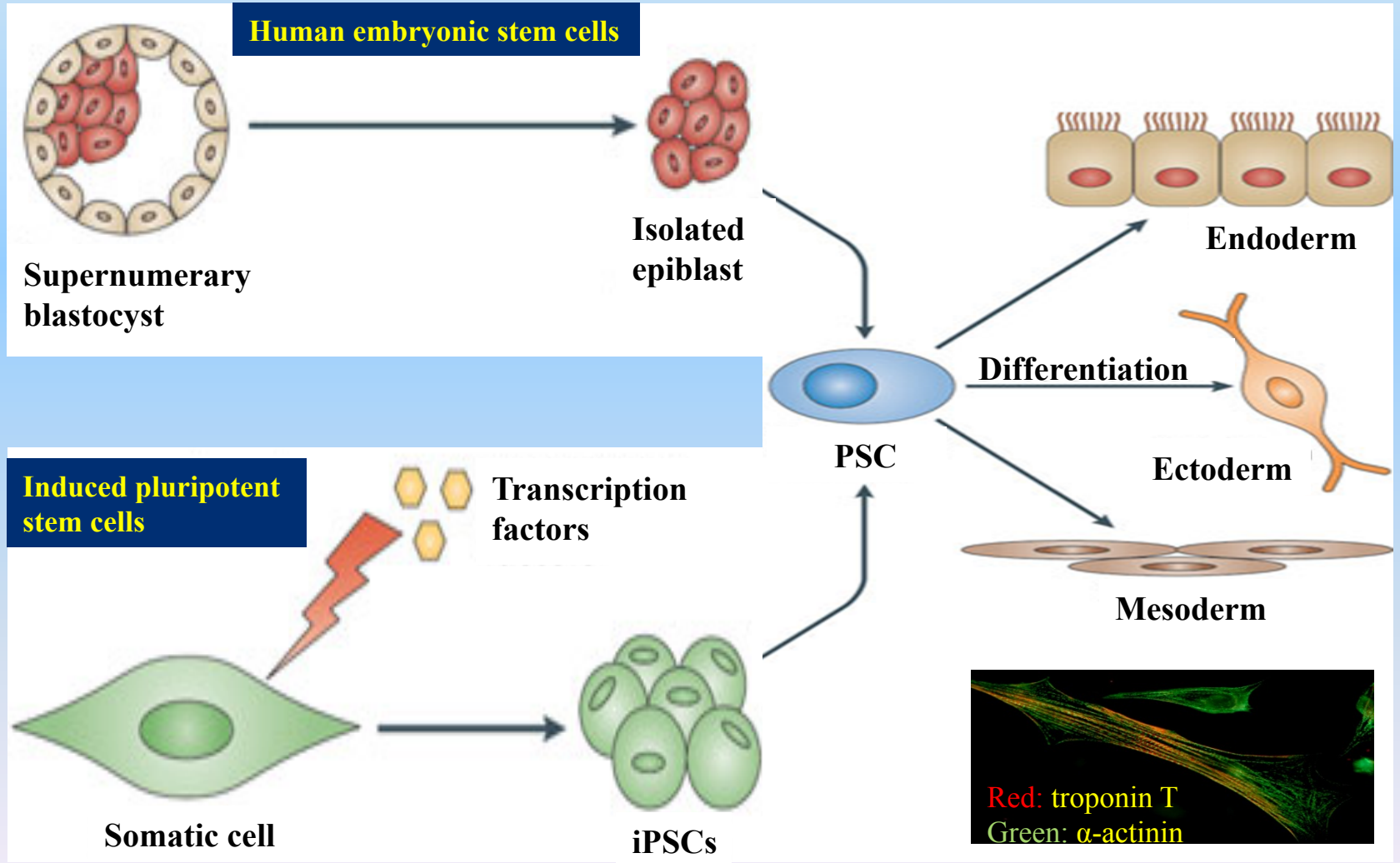
- These cells have yielded limited, if any, functional benefit
- However, analysis of trial outcomes has allowed to draw important lessons

Stem Cells for Heart Failure

Rationale

- Cardiac-commited cells seem to be the most functionally effective
- The epicardial patch-based delivery of cells has distinct advantages over multiple intramyocardial injections
- The common discrepancy between a sustained functional benefit and the lack of permanent cell engraftment suggests a paracrine effect which rationalizes the use of allogeneic cells provided their survival is extended enough to allow them to exert these paracrine effects

Cardiac-Committed Cells : Where Can They Come From ? : Pluripotent Stem Cells



Fairchild Nature Reviews Immunology 2010;10:868-75.

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Cardiac Patches

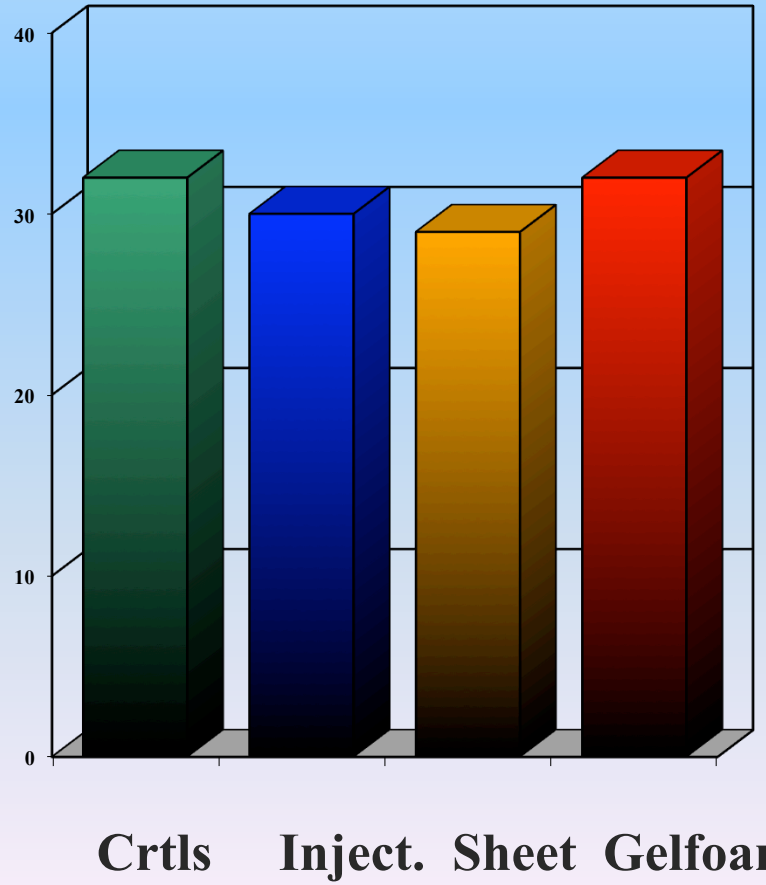
Advantages

- To improve cell retention
- To provide a template enhancing cell survival, proliferation, differentiation and migration
- To strengthen the infarcted myocardium
- To serve as a platform for drug/factor delivery

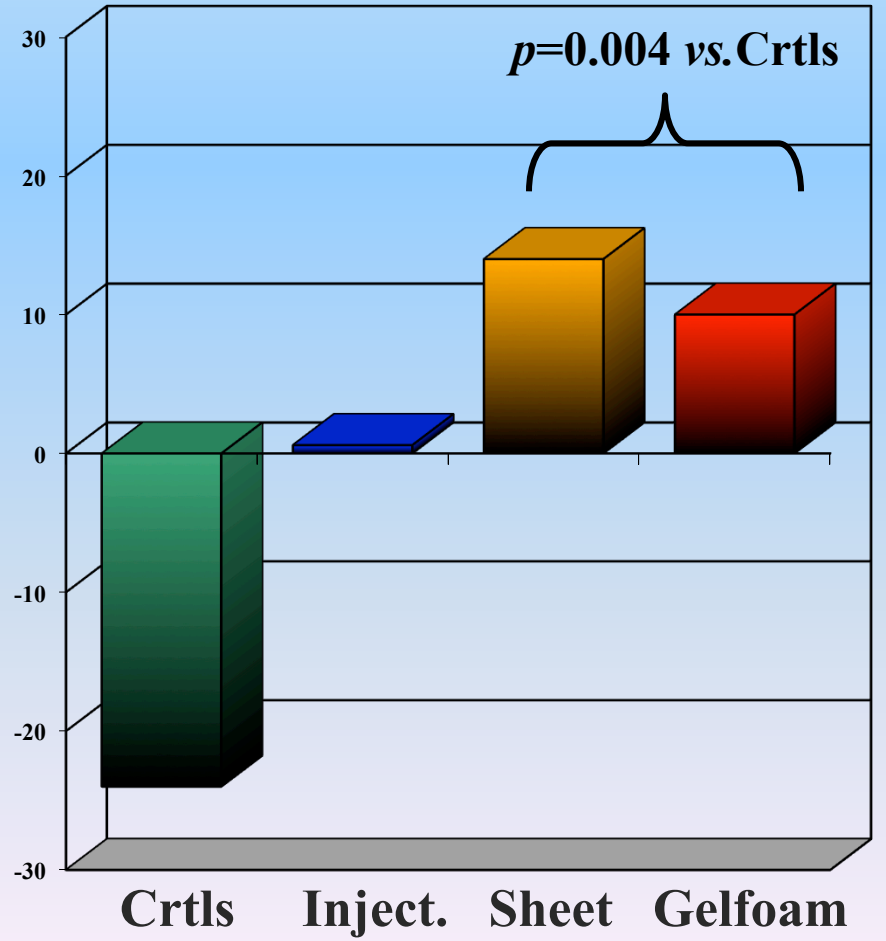
Comparative Assessment of Myoblast Delivery Methods

Rat model of permanent coronary occlusion; Myoblast delivery 2 weeks post MI; Echo assessment of LV function 1 mo. postTx

Baseline EF (%)



End point EF (% change)



Hamdi et al. Ann Thorac Surg 2009;87:1196-203.

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Stem Cells for Heart Failure

Objective	Strategy
▪ Cardiac-committed cells	ESC-derived cardiac progenitors
▪ Epicardial delivery	Fibrin patch
▪ Preservation of early graft survival	Pericardial flap
	+ transient immunosuppression

ESCORT

Roadmap of Preclinical Studies

In vitro studies

- Optimal culture conditions
- Cell characterization (genotype, phenotype, epigenetic status)

In vivo studies

>300 rats

- Cardiac differentiation
- Assessment of scaffolds
- Functional outcome
- Lack of teratoma

54 ID mice

- Lack of teratoma

32 nonhuman primates

- Cardiac differentiation
- Lack of teratoma

4 sheep

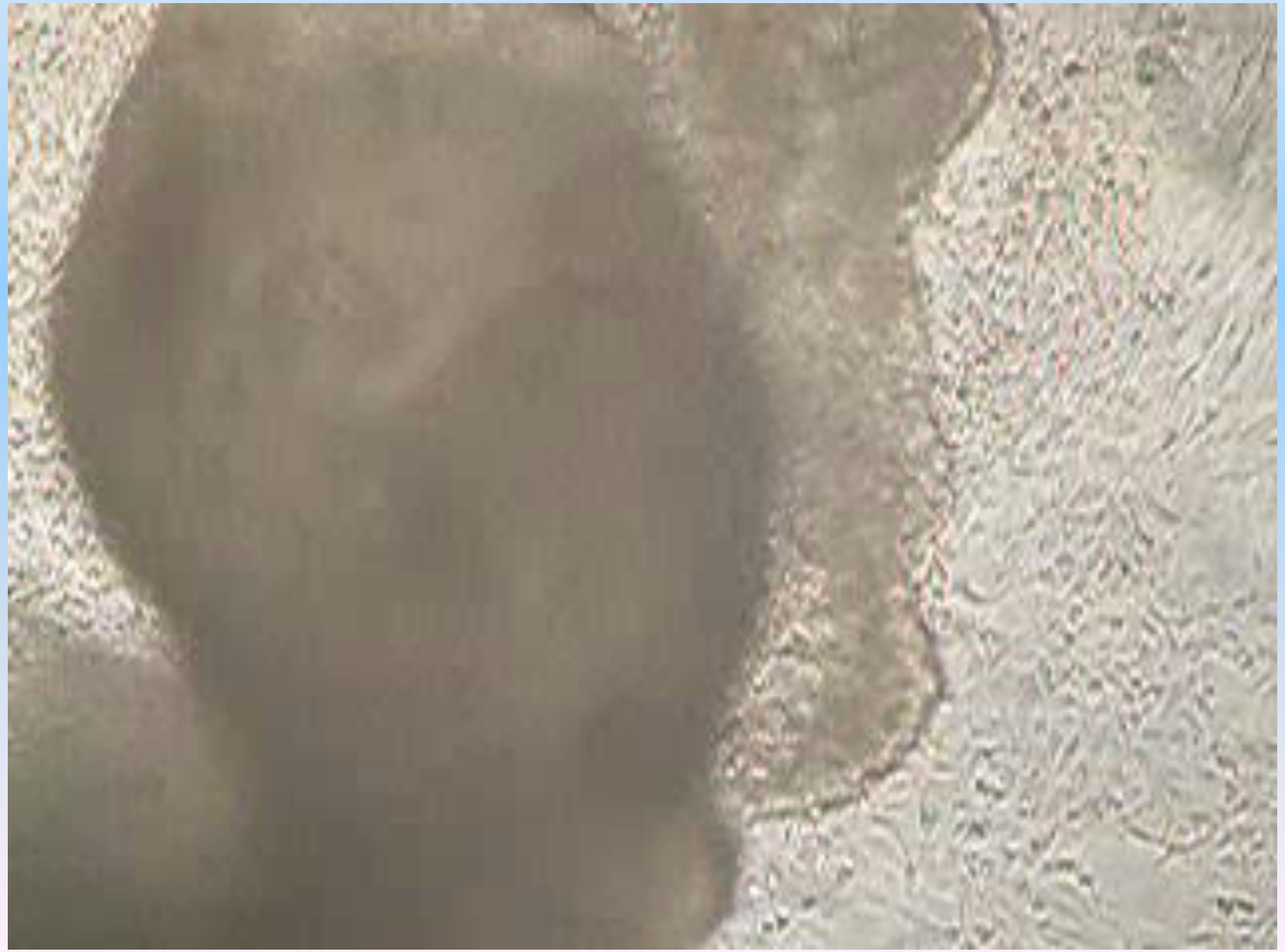
- Surgical training

hESC : Translational Issues

Steps

- Selection of the cell line
- Scale-up of pluripotent ESC and set-up of banks
- Cardiac specification
- Purification
- Safety testing

Beating I6 ES-Derived Embryoid Bodies



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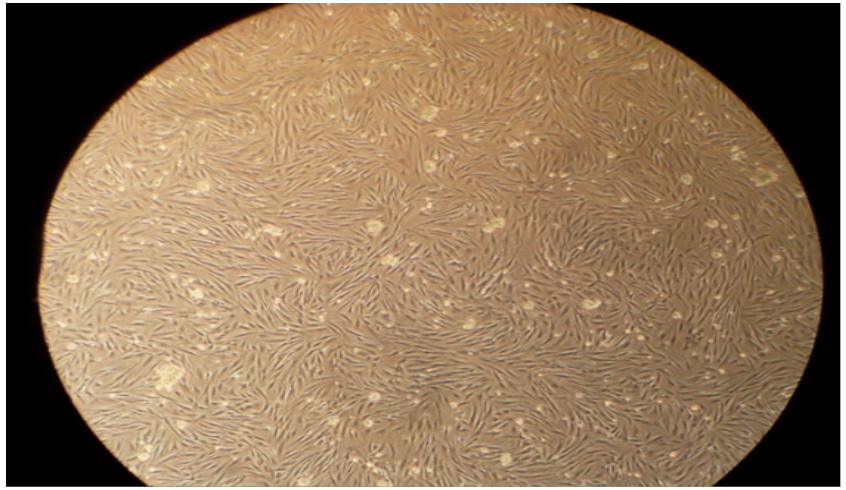
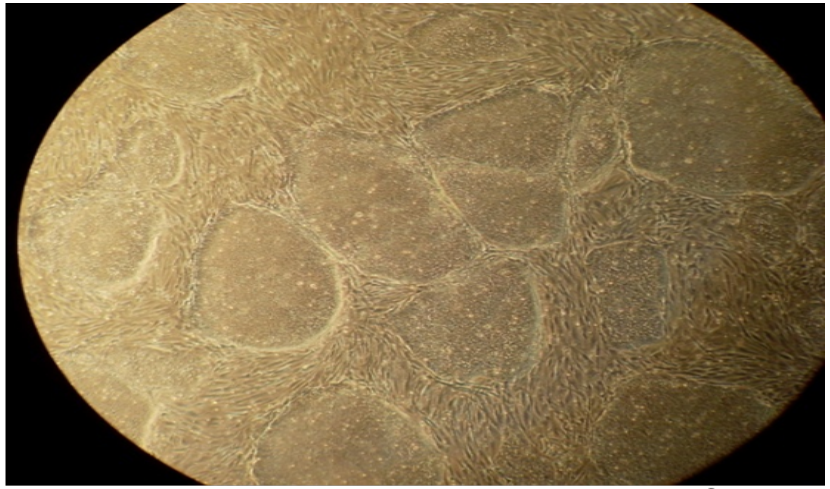
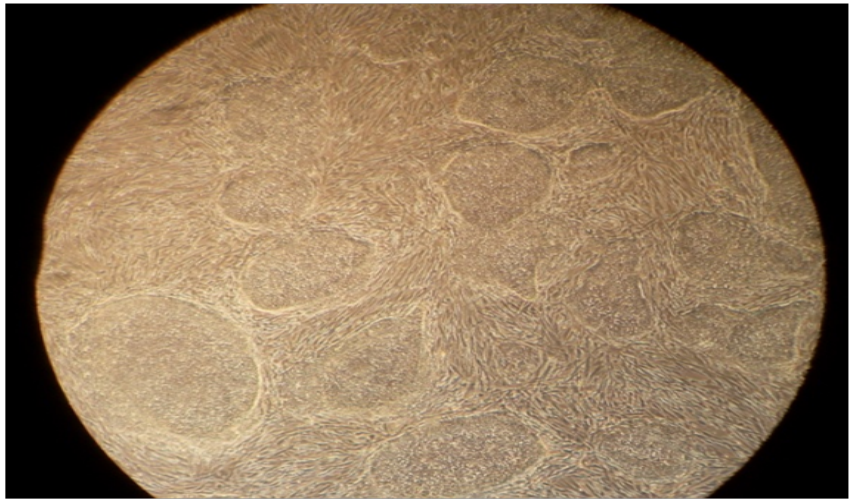


Post P2



Pre-banking (P38)



Pre-late banking (P44)

hESC : Translational Issues

Steps

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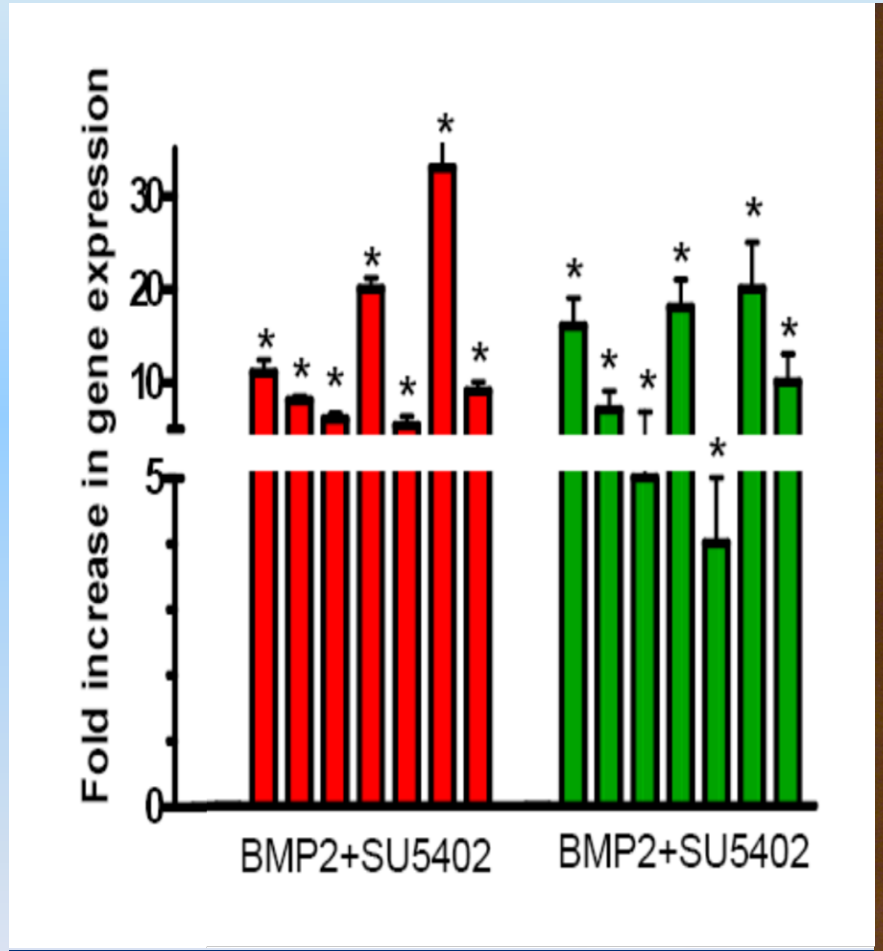
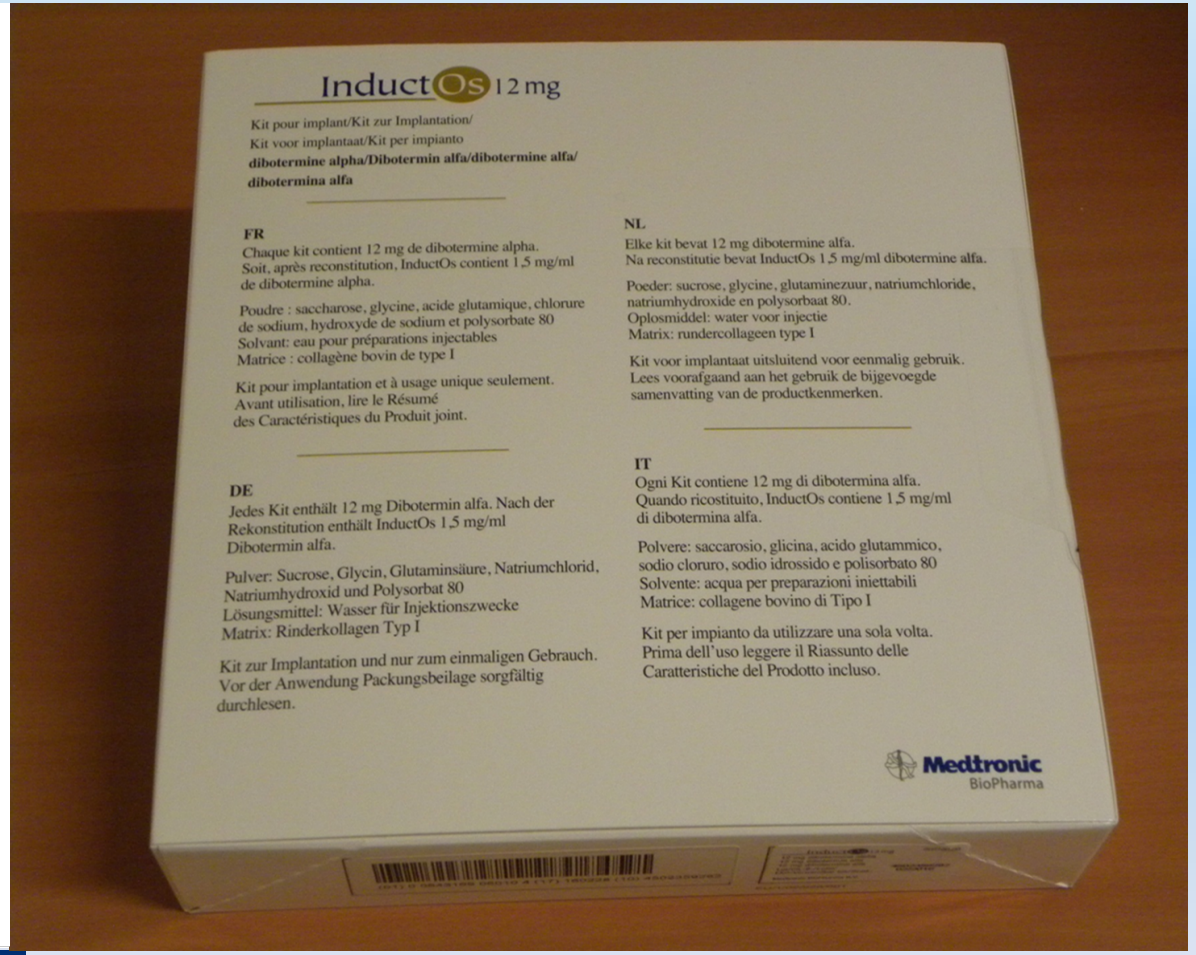
Molecular Regulators of Cardiac Differentiation





4-day exposure to BMP-2 (10 ng/mL) and a FGF inhibitor (SU-5402, 1μM) in insulin-free α-MEM + 2%B27 medium

Alexander & Bruneau, Trends in Molecular Medicine 2010;16:426-34.

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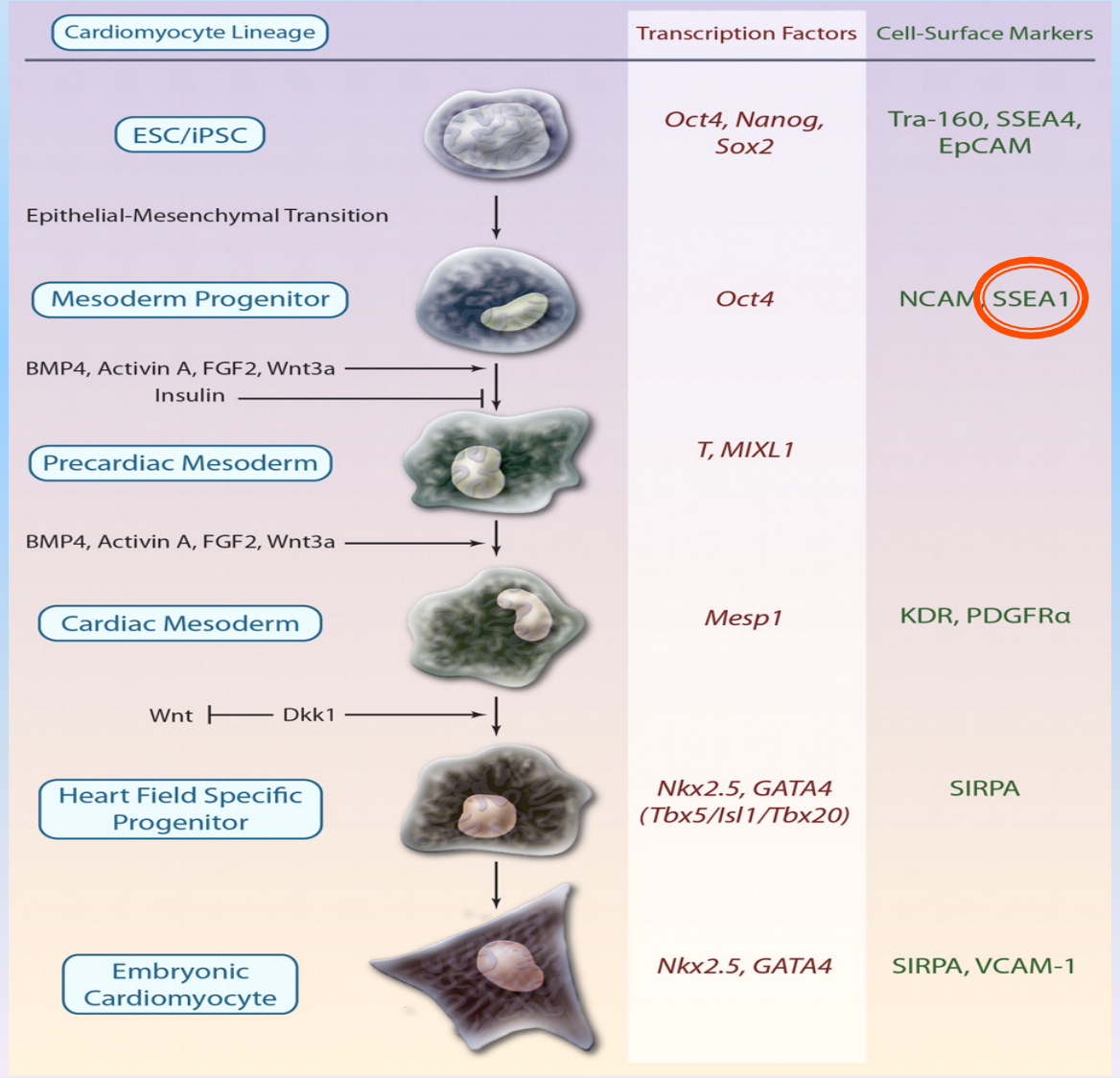
Immunomagnetic Cell Sorting

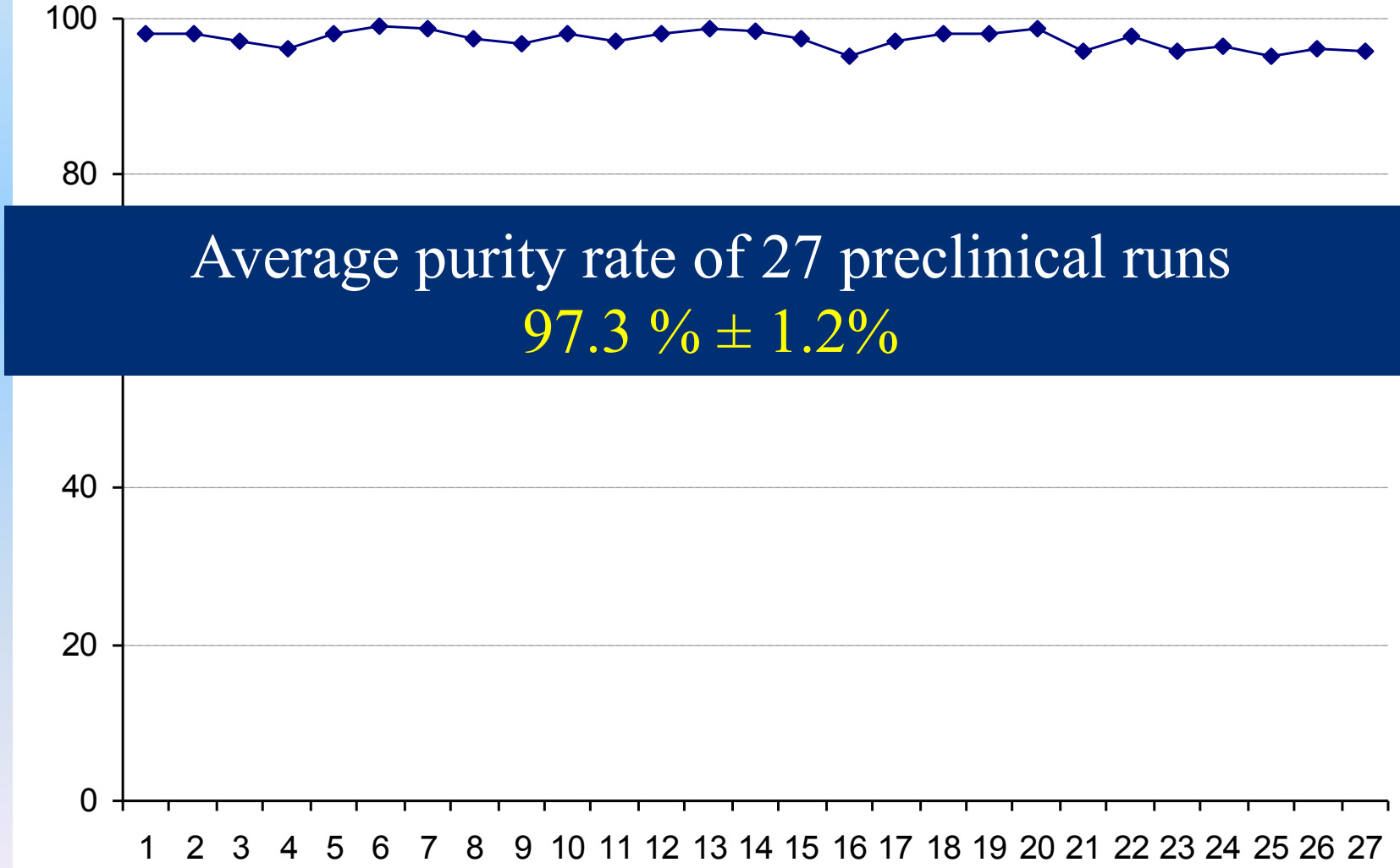
SSEA-1
- Is a surface marker suitable for flow cytometry and accessible to magnetic beads
- Labels cells which have lost pluripotency
- Can be recognized by commercially available antibodies

Model of Differentiation of Human Pluripotent Stem Cells via Sequential Progenitors to Cardiomyocytes



Mummery et al. Circ Research 2012;111:344-58.

Purity Rate of the Sorted SSEA-1^{+} Progenitor Cell Population

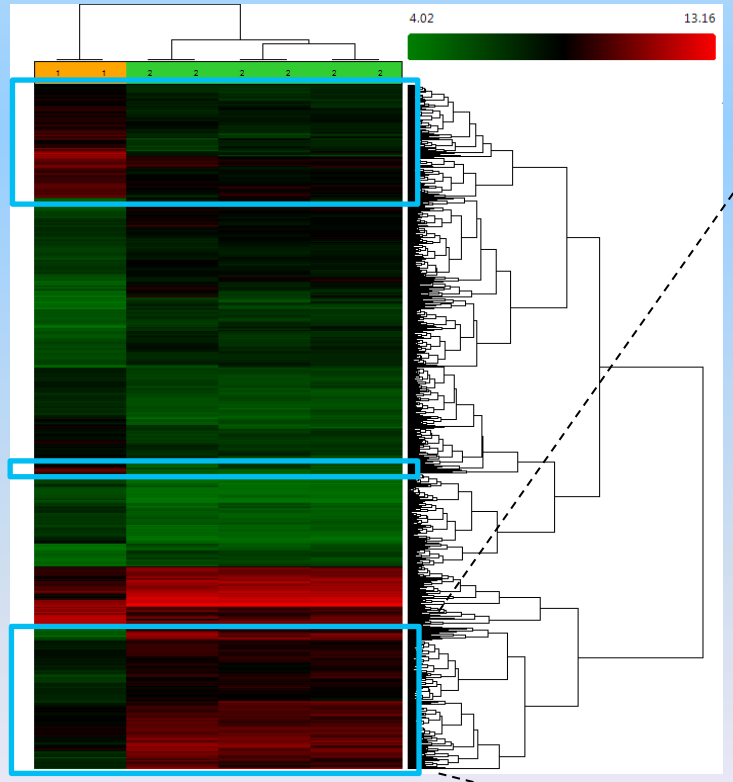


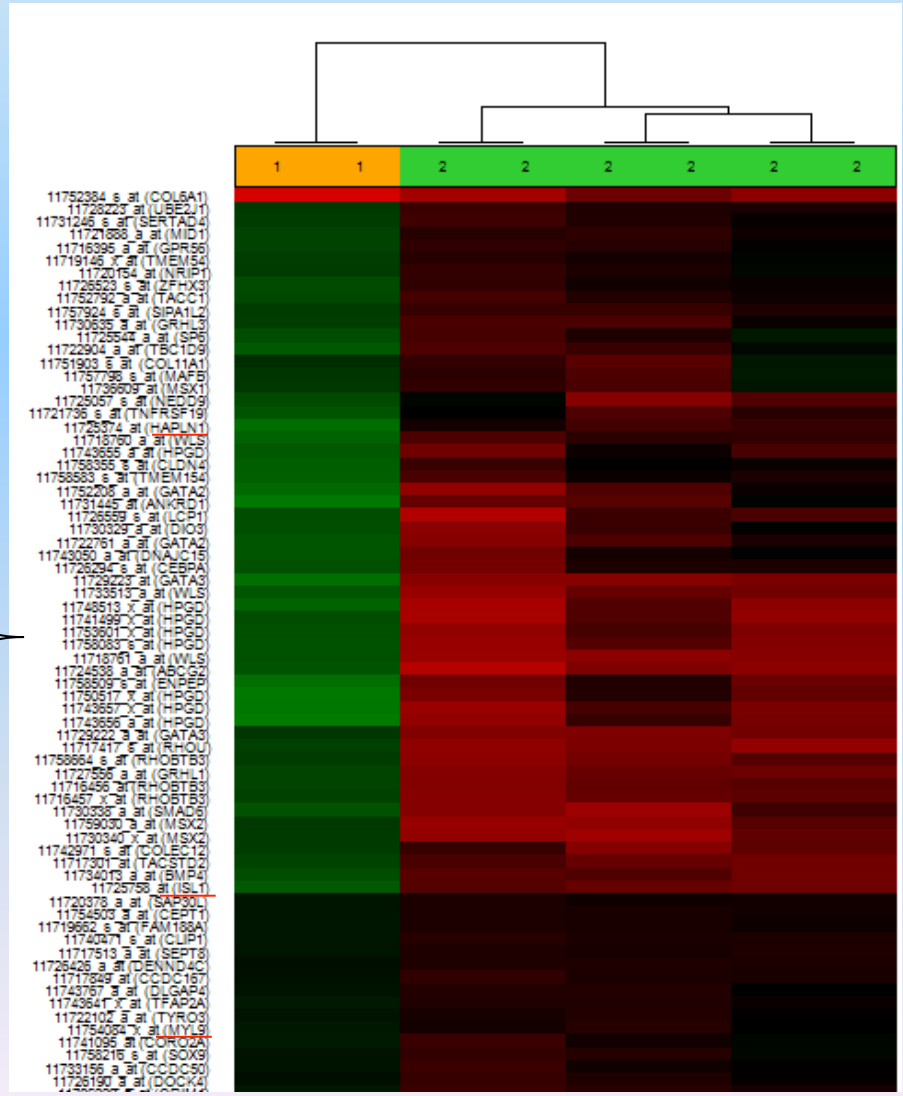
Average purity rate of 27 preclinical runs

97.3 % ± 1.2%

Heat Map of the Expression of Genes in Pluripotent I6 ESC *vs* SSEA-1^{+} Committed Progenitors : Mesodermal/Cardiac Genes

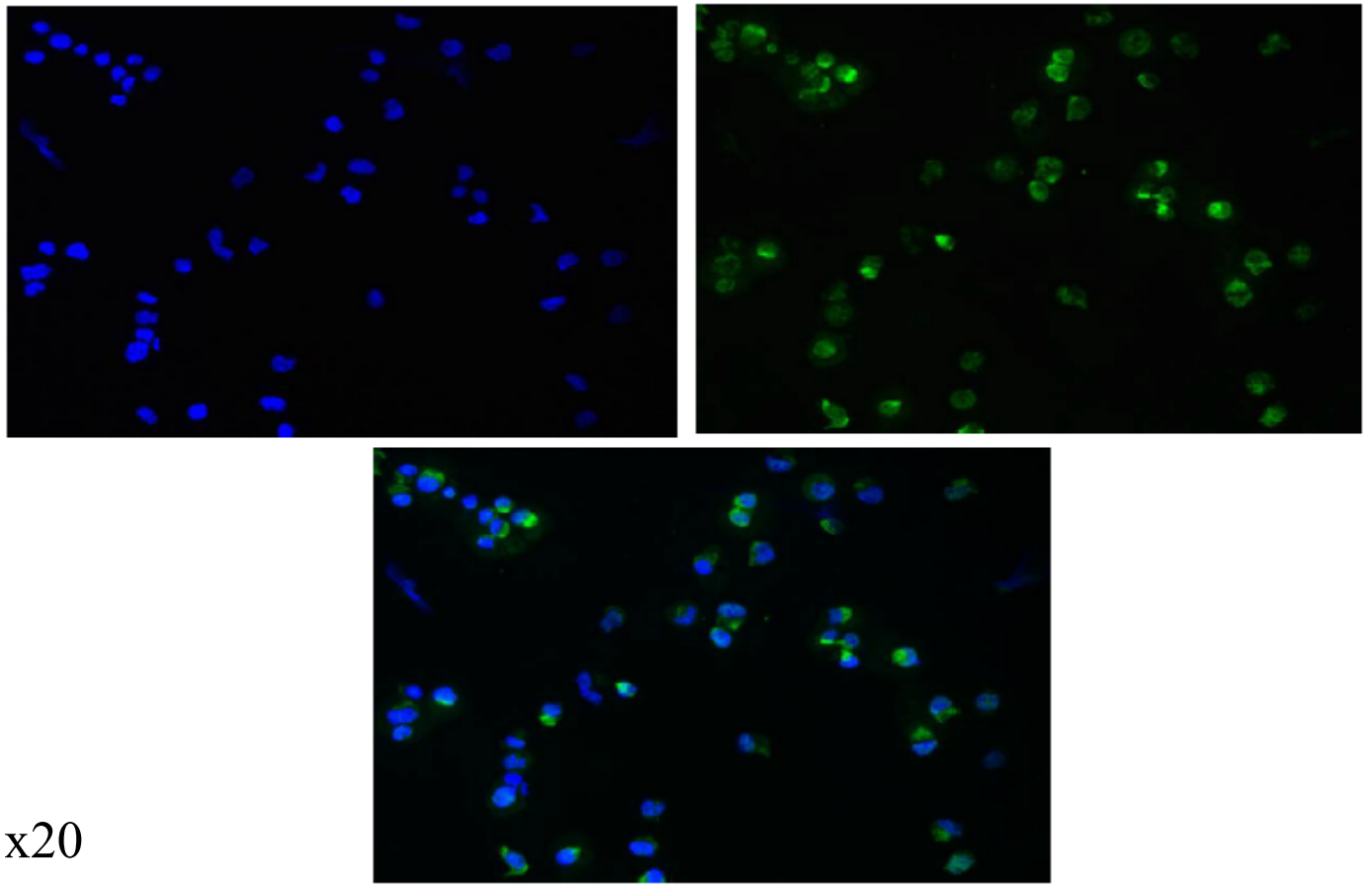
Two data sets for each of 3 sorting procedures





Immunostaining-Based Assessment of Cell Identity

Expression of *Isl-1* immediately after sorting (cytospin)



x20

hESC : Translational Issues

Steps

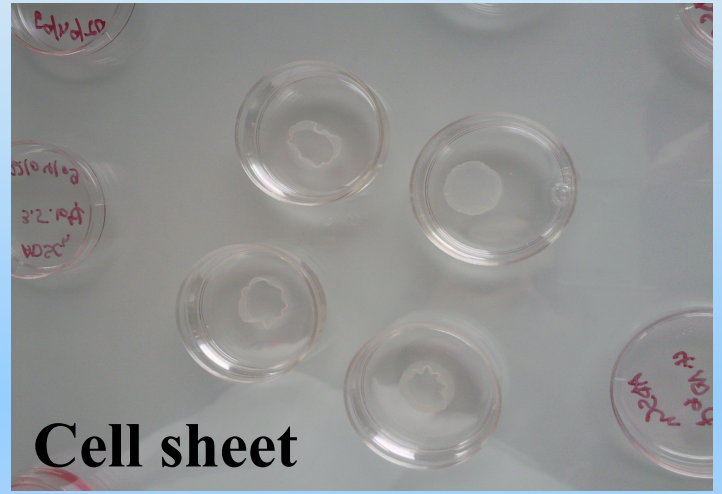
- Selection of the cell line
- Scale-up of pluripotent ESC and set-up of banks
- Cardiac specification
- Purification
- Safety testing

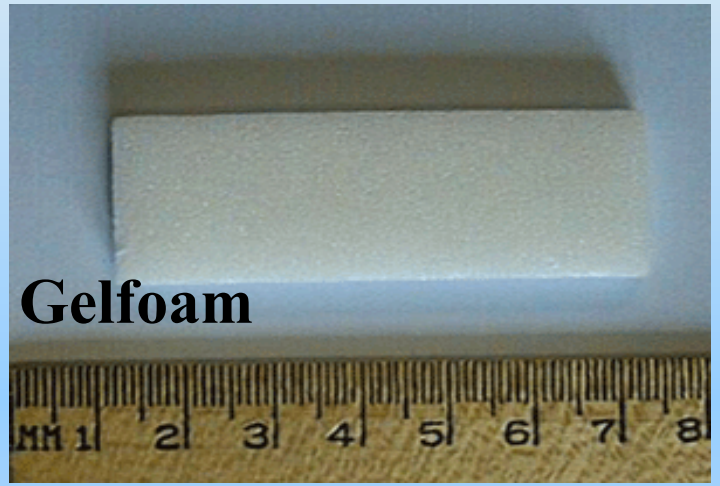
hESC Translational Issues : Safety Testing

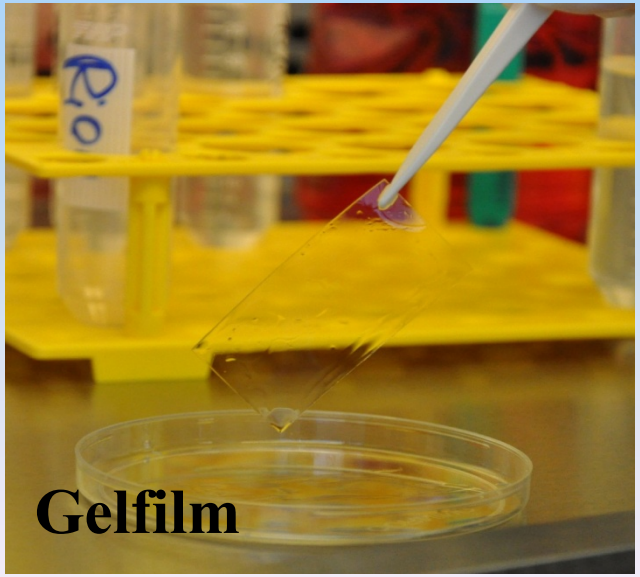
Safety area	Targets
Virology	Cell banks (MCB/WCB, LPCB), antibody, SSEA-1$^+$ progenitors
Cyto	tors ys)
Onc	G2 -/- γc-/- C5-/-) injected with pluripotent ESC, SSEA-1$^+$ progenitors (with spiking experiments), **SSEA-1$^-$ cells**

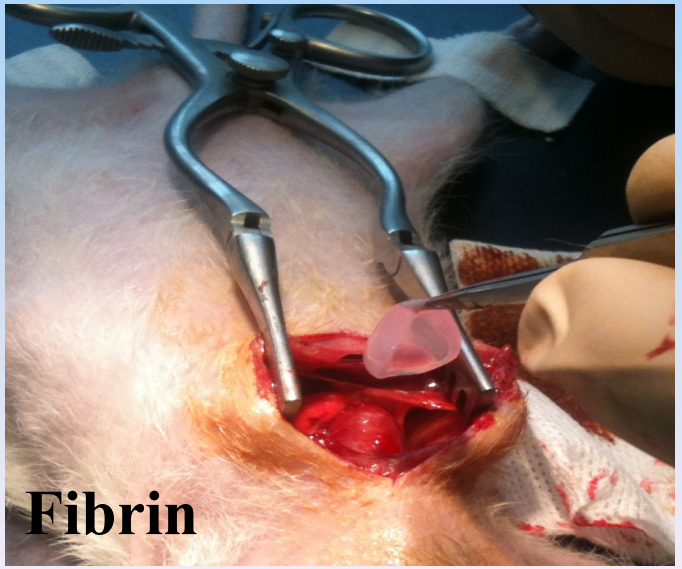
- No contamination
- No genetic abnormalities
- No teratoma after appropriate purification

Evolution of Tested Scaffolds









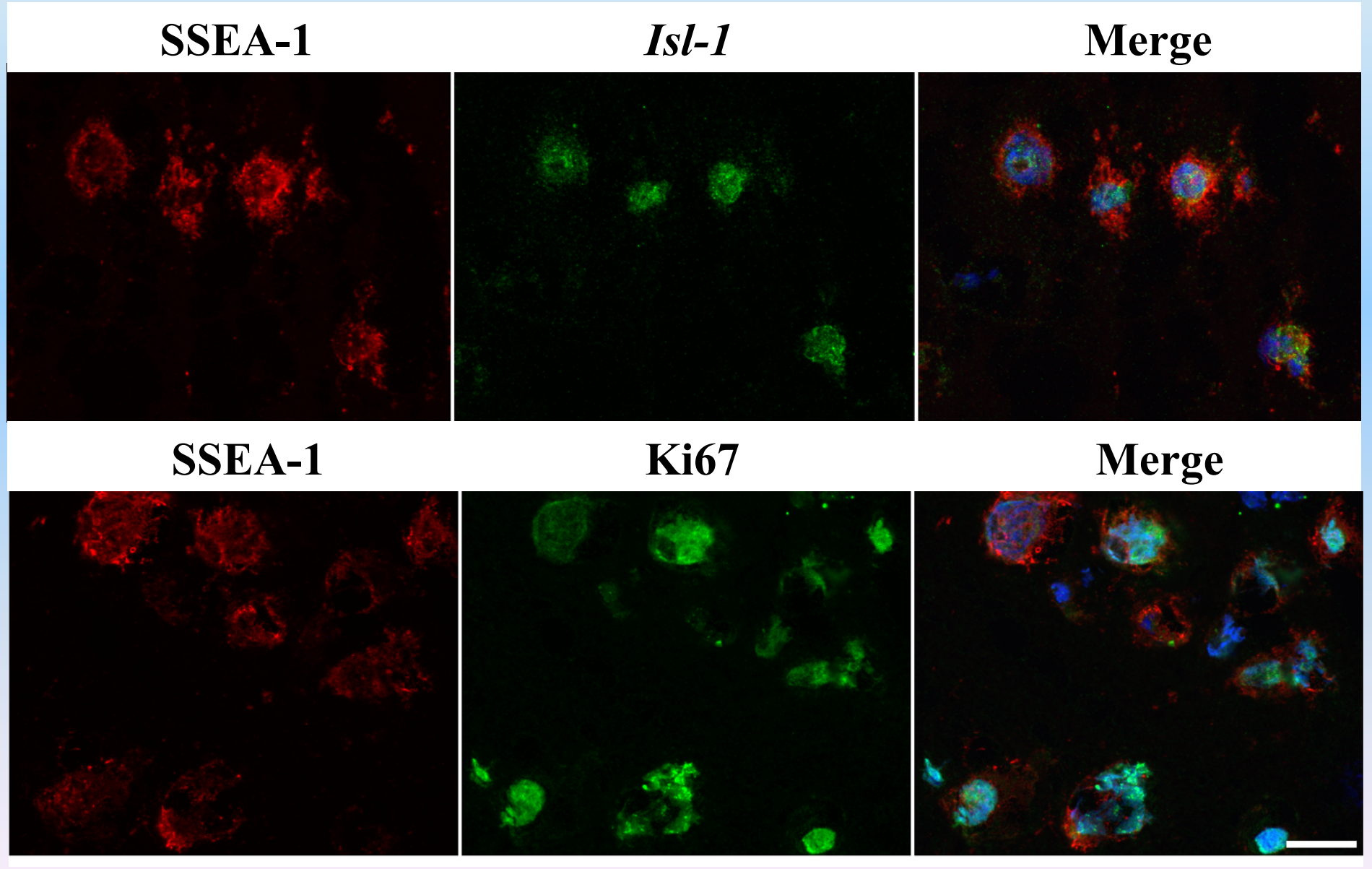
Cardiac Patches

Advantages of Fibrin

- Biocompatibility
- Tunability of mechanical properties
- Angiogeneic potential
- Marketed approval for human use

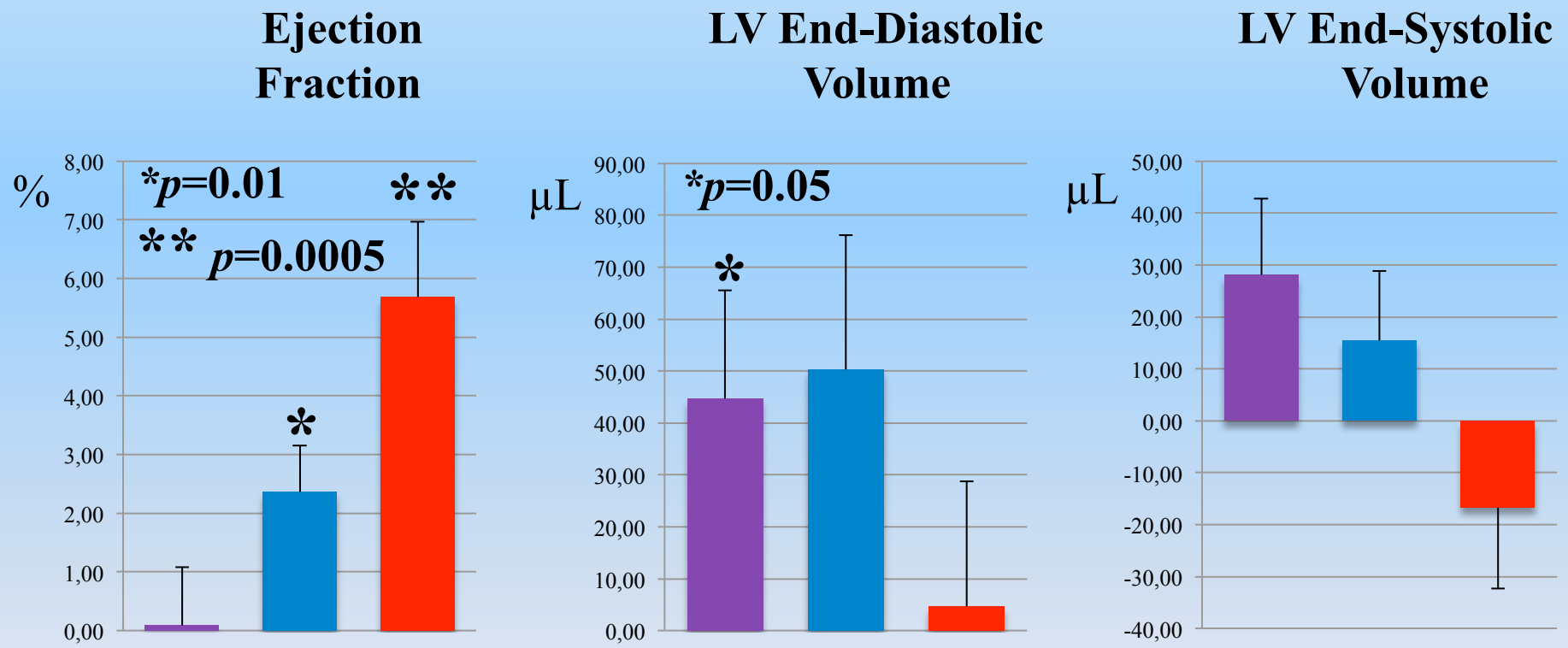
Manufacture of the Fibrin Patch

Imaging of SSEA-1^{+} Progenitor Cells Embedded Into a Fibrin Scaffold



Tx of ESC-Derived SSEA-1^{+} Progenitor Cells Embedded Into a Fibrin Scaffold

Rat model of chronic MI
Absolute Changes Between 4-month and Baseline Data



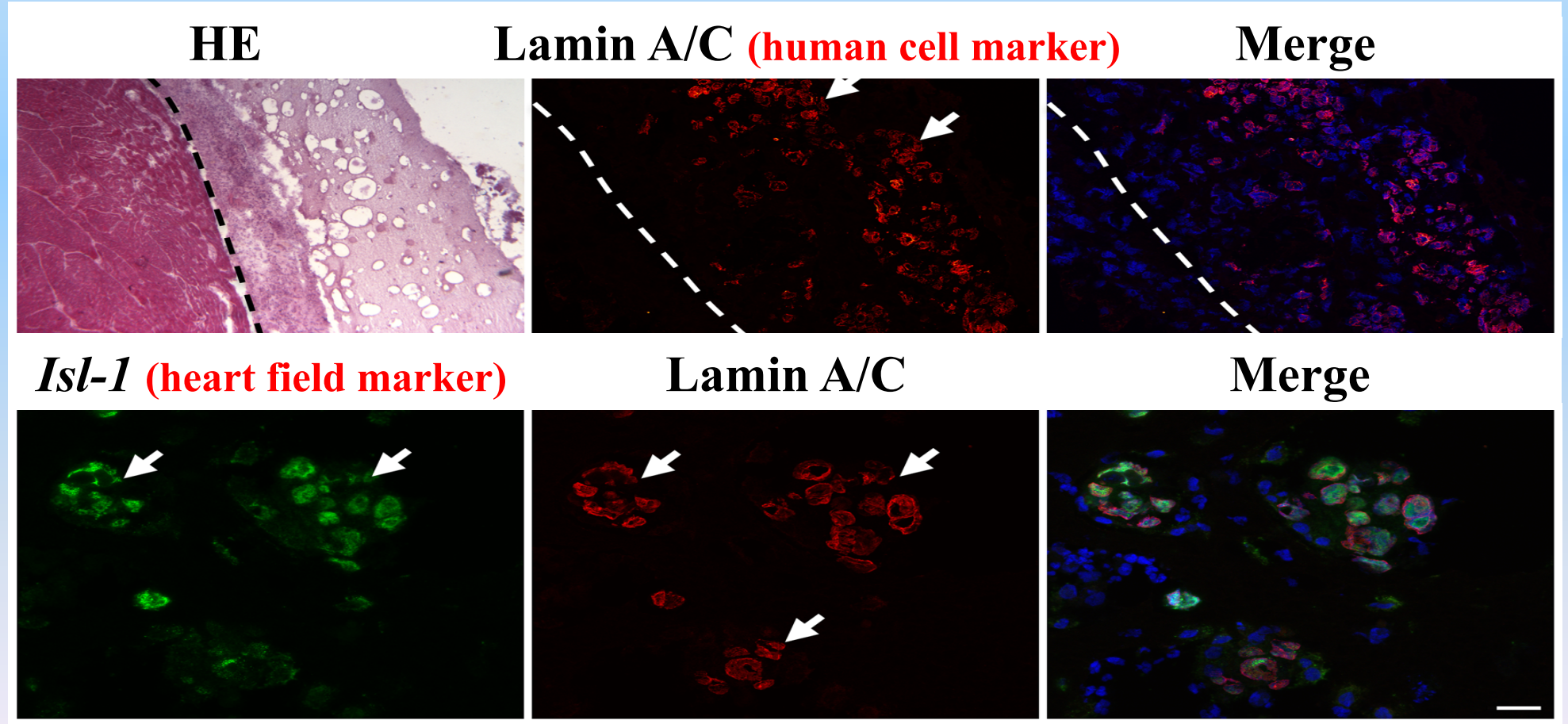
Sham (n=13) **Controls (cell-free fibrin scaffold, n=12)**

Treated (SSEA-1^{+} progenitors+fibrin scaffold, n=16)

Bellamy et al. J Heart Lung Transplant (on-line, November 7, 2014)

Imaging of SSEA-1^{+} Progenitor Cells Embedded Into a Fibrin Scaffold Following Epicardial Scaffold Delivery Onto an Infarcted Rat Heart

48-Hour Data

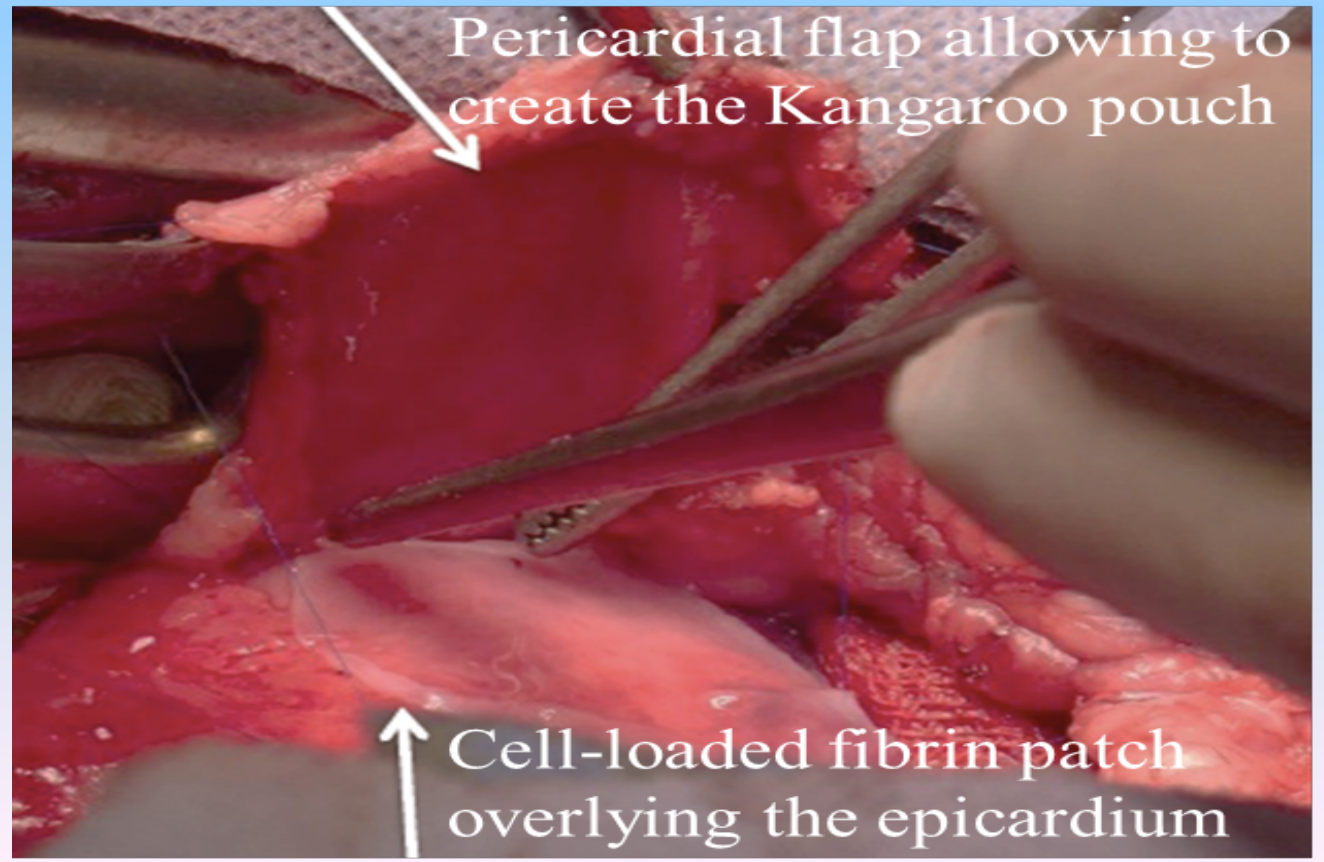


—— Upper panel : 150μm (HE); 40μm (Lamin A/C, Merge)
Lower panel: 20μm

Stem Cells for Heart Failure

Enhancement of Graft Survival

Provision of a Trophic Support:
The pericardium as a natural bioreactor



Delayed Rejection:
Short-term immunosuppression

Immunosuppression Regimen

- Ciclosporine (1mg/kg/day with target trough levels of 100-150 ng/mL)
- Mycophenolate mofetyl (2g/day for 1 month, then1g/day)
- 2-month treatment, currently shortened to 1 month

ESCORT

Embryonic **S**tem **C**ell f**O**r **R**egenerative **T**herapy (NCT02057900)

Inclusion Criteria

- Age between 18 and 81
- Severe LV dysfunction (LVEF ≤ 35%)
- History of MI (> 6 mo.)
- Disabling functional limitation (angina and/or NYHA Class III/IV heart failure) despite optimal medical therapy
- Previous implantation of an ICD ± CRT
- Indication for a conventional coronary and/or valve procedure
- Contra-indication to heart transplantation

ESCORT

End Points

- Primary : Safety

✓Intraoperative : arrhythmias, bleeding

✓Postoperative (early : 1 year) : MACE, tumour

✓Postoperative (late : 5 years) : tumour

- Secondary :

❖Feasibility

❖Efficacy :

✓Local and regional LV function

✓Viability of the grafted area

✓Functional status

✓MACE

ESCORT Trial : Flow Chart

End point	Pre (-1M)	T0	1W	2W	3W	M1	M3	M6	M12
Clinical/Biol/Radio	X					X	X	X	X
VO_2max	X								X
Walking test (6min)	X								X
Echo	X					X	X	X	X
ICD	X			X		X	X	X	X
Holter EKG 24 h	X								X
Nuclear angiogram	X								X
PET scan (oncology)	X							X	
Bodyscan	X								X
Allo-immunization	X						X	X	±X
ELISPOT InFγ*	X			X		X	X	X	X

* Cell responses to EBV and CMV viruses

ESCORT Trial

Case Report

- 68-year old insulin-dependent diabetic woman
- History of infero-lateral myocardial infarction
- Clinical symptoms of heart failure (NYHA Class III)
- LVEF: 26%
- Coronary angiogram : critical left main stenosis and Cx complete occlusion

ESCORT Trial

Release Criteria

- Cell number : 4 x 10^6
- Specification rate : 64%
- Viability rate : 96.1%
- Purity rate (%SSEA-1^+ cells) : 99%

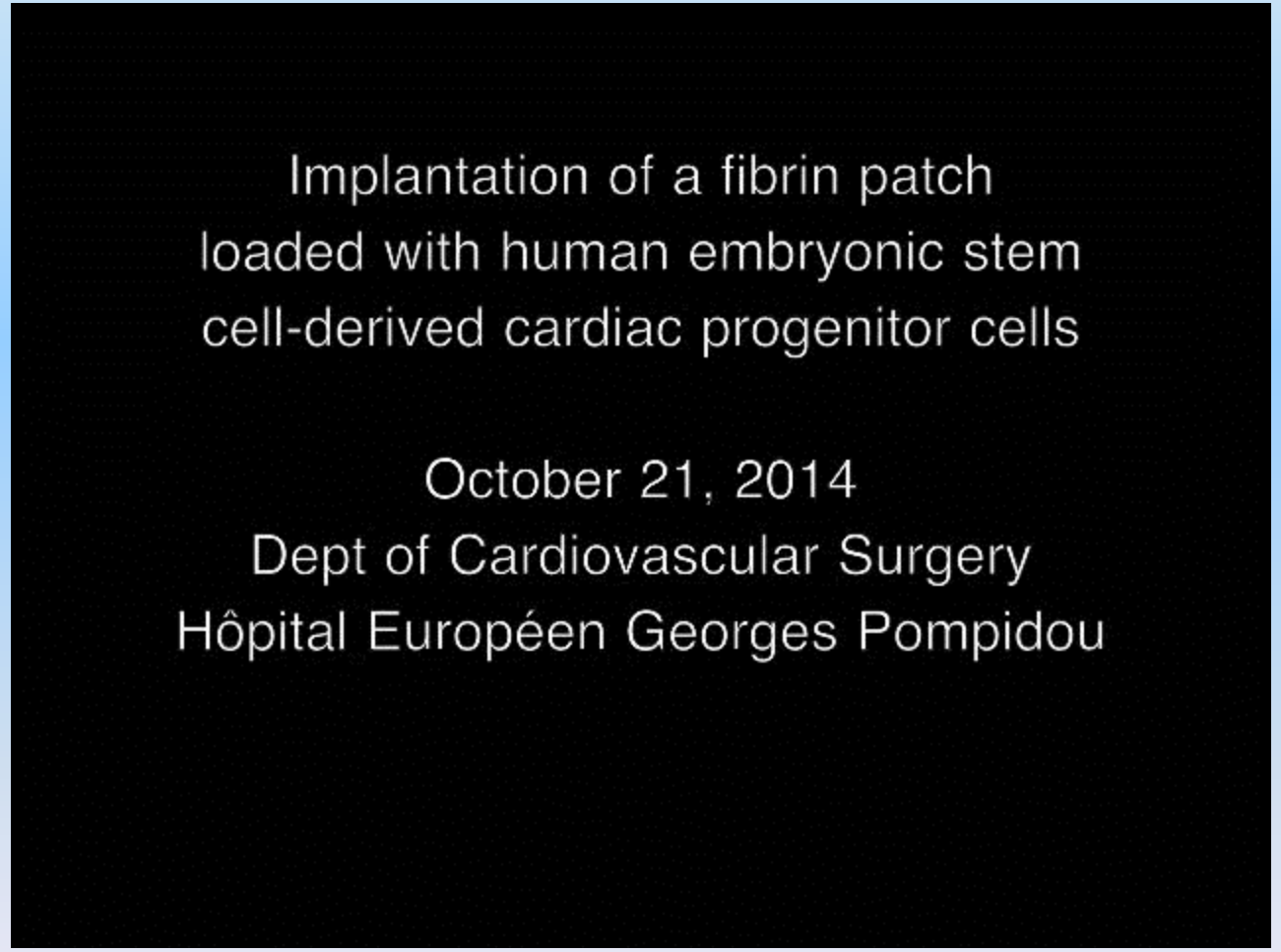
✓ *Nanog* (SSEA-1^+/I6): 0.009
✓ *Sox 2* (SSEA-1^+/I6): 0.036

Markers of pluripotency

✓ *Isl-1* (SSEA-1^+/I6): 159

Marker of heart fields

✓


Implantation of a fibrin patch
loaded with human embryonic stem
cell-derived cardiac progenitor cells
October 21, 2014
Dept of Cardiovascular Surgery
Hôpital Européen Georges Pompidou

ESCORT Trial

Early Postoperative Course

- Uneventful weaning from CPB
- Early postoperative extubation (2 hours and 30 minutes)
- Stable hemodynamics under a short-duration moderate/minimal inotropic support
- No bleeding (370 mL/24 first PO hours)
- Peak TnI level : 3.8 ng/mL

ESCORT Trial

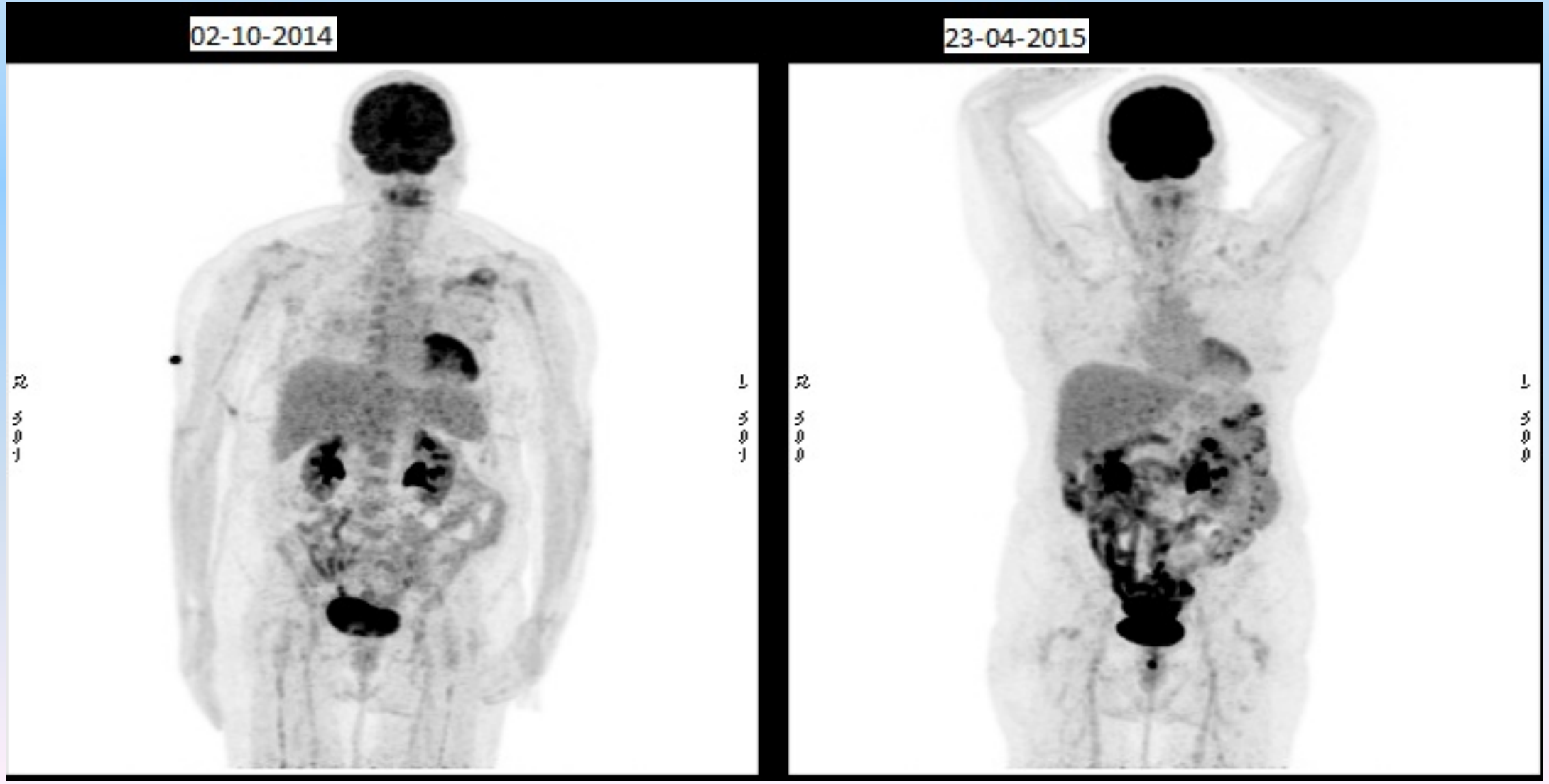
6-month Follow-Up : Safety Data

Absence of adverse events

- No arrhythmias (interrogation of ICD)
- No immunosuppressive drug-related complications
- No new morphological cardiac abnormality
- Partial (1 antigen) alloimmunization to the grafted cells

^{18}FDG Pet Scan

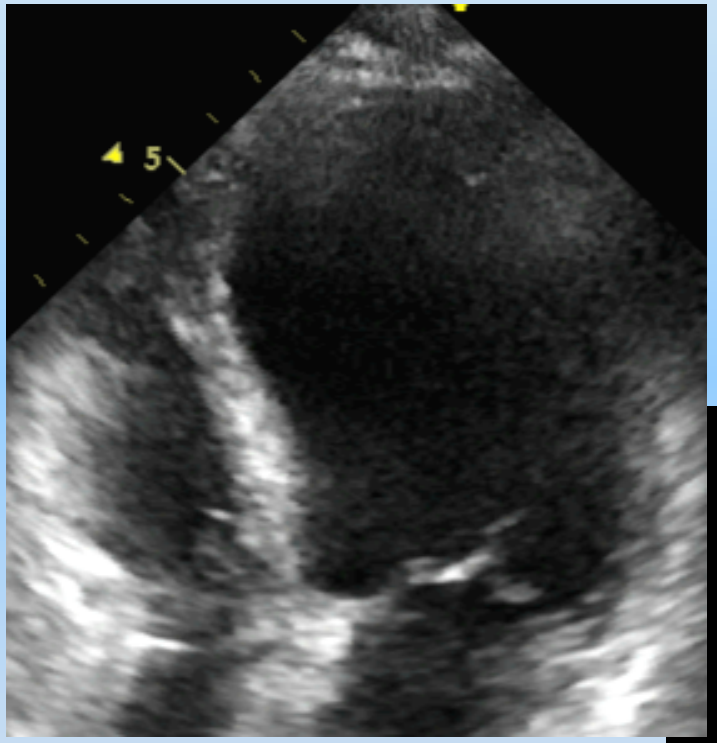
Pre-Op **6 months**



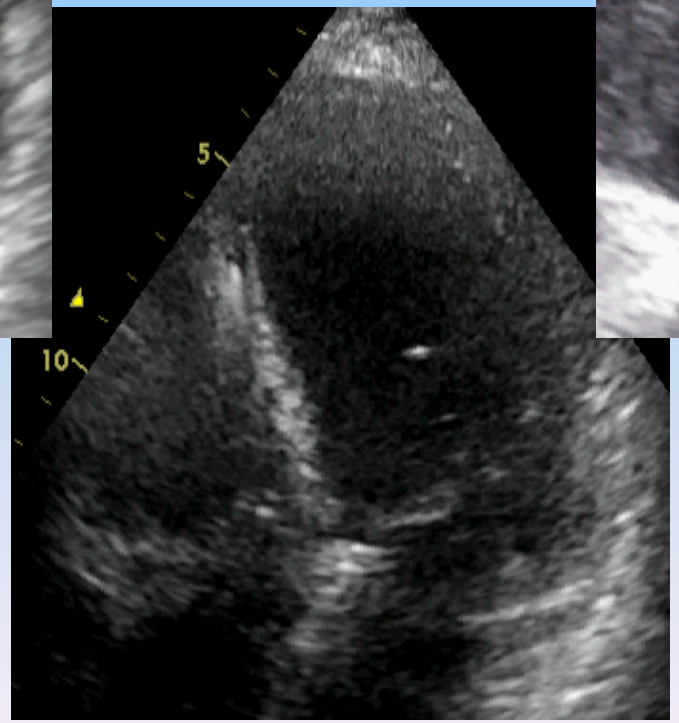
Functional Status

Echo 2D	Pre-op	M2	M3	M6
LVEDV (mL)	161	160	134	135
LVESV (mL)	117	117	85	84
LVEF (%)	26	27	36	38
NYHA Class	III	II	II	I
6mn WT(m)	350		467	

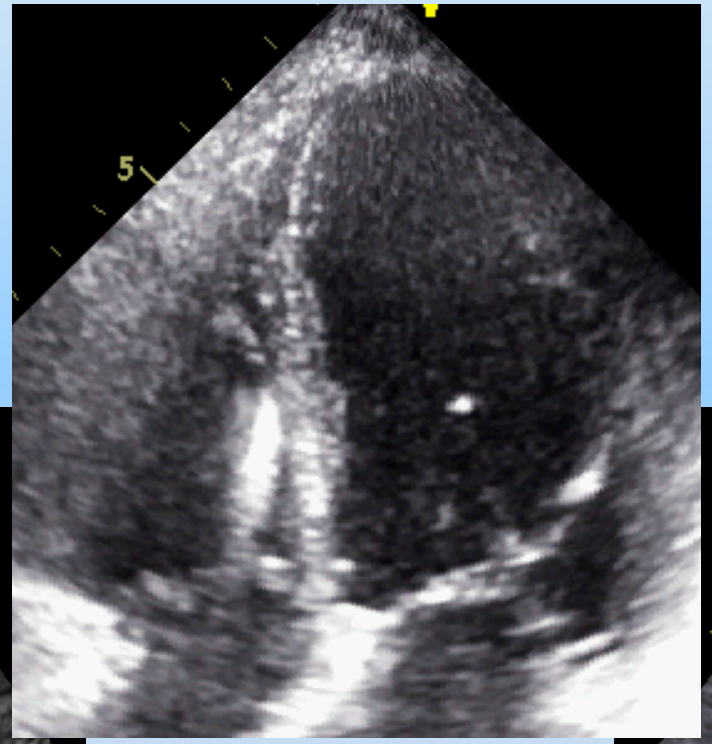
LV Volume/Function
(Apical 4-chamber view)



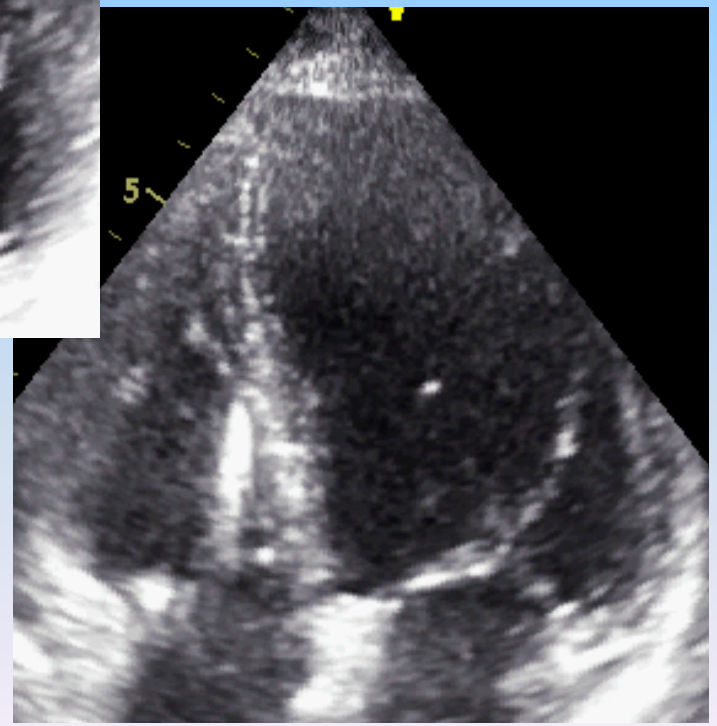
Pre-op



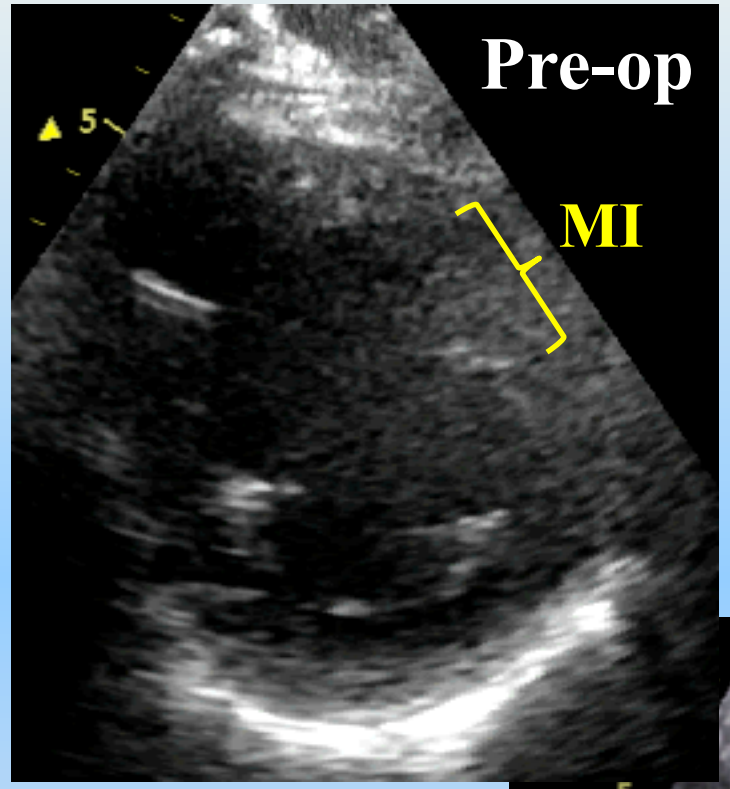
1 month

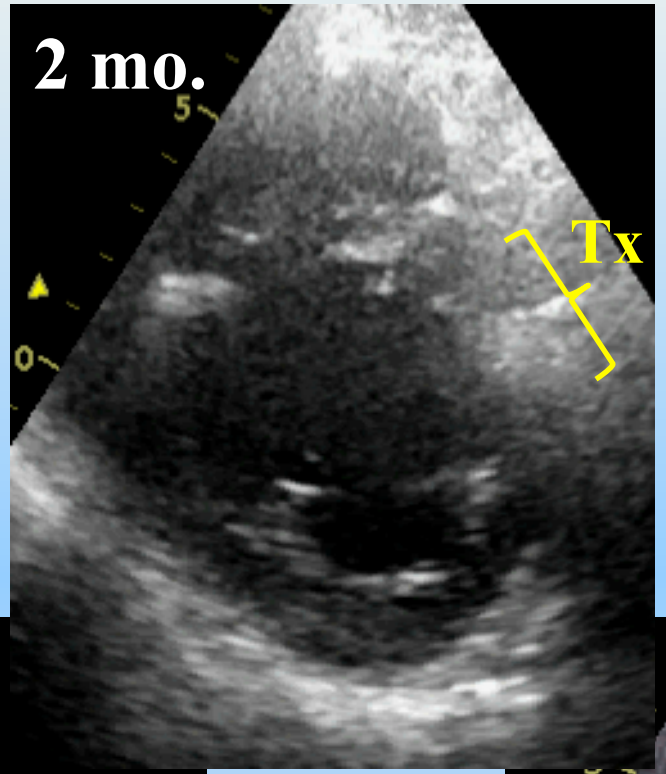


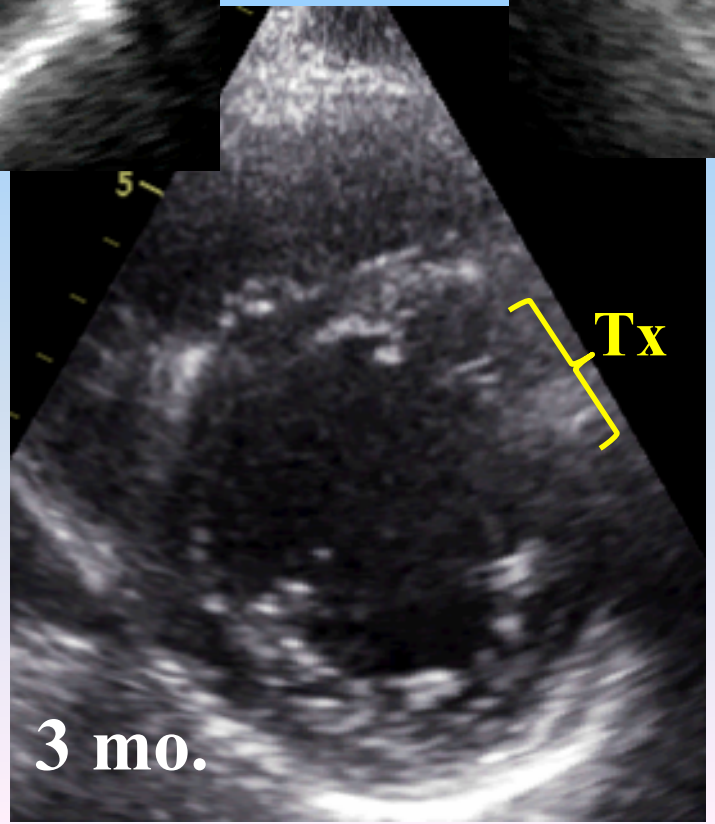
2 months

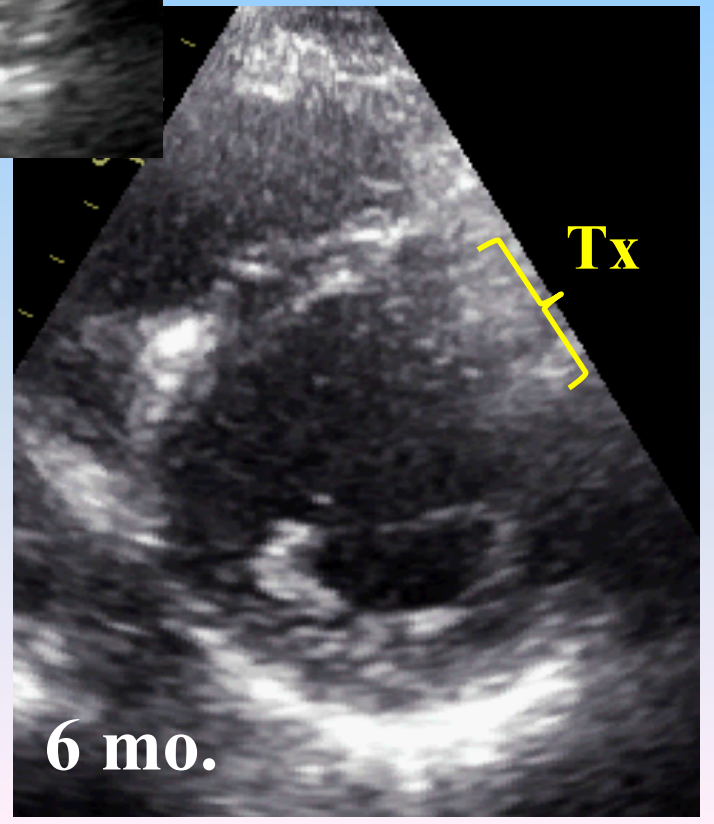


6 months









2D-E Displaying the Infarcted Non-viable Transplanted Area *(Parasternal LA view; Anterolateral LV segments)*

ESCORT Trial

Areas of Further Research/Improvement

- Feeder-free cultures
- Differentiation towards a more mature cardiac phenotype ?
- Negative type of immunomagnetic selection or microfluidics-based method
- More « biomimetic » patch material
- Expeditious assessment of lot release criteria
- Exclusive use of cell-derived factors/microvesicles?

Those Who Did It : INSERM U 970 (Paris Research Cardiovascular Center)


André Tomescot


David Kalfa


Damélys Calderon


Marie-Cécile Périer


Chantal Mandet


Hadhami Hamdi


Agnès Maurel


Etienne Puymirat


Hany Nematalla


Valérie Bellamy

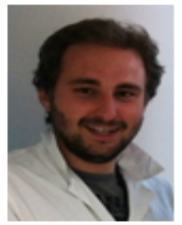

Patrick Bruneval


Margaux Pontailler


Camille Marijon


Susanne Proksch


Mathieu Pieronne


Joevin Sourdon


Nisa Renault


Bertrand Léobon


Kasra Azamoush


Yohan Farouz


Laetitia Pidial


Aurélie Chaudeurge


Anaïs Kervadec

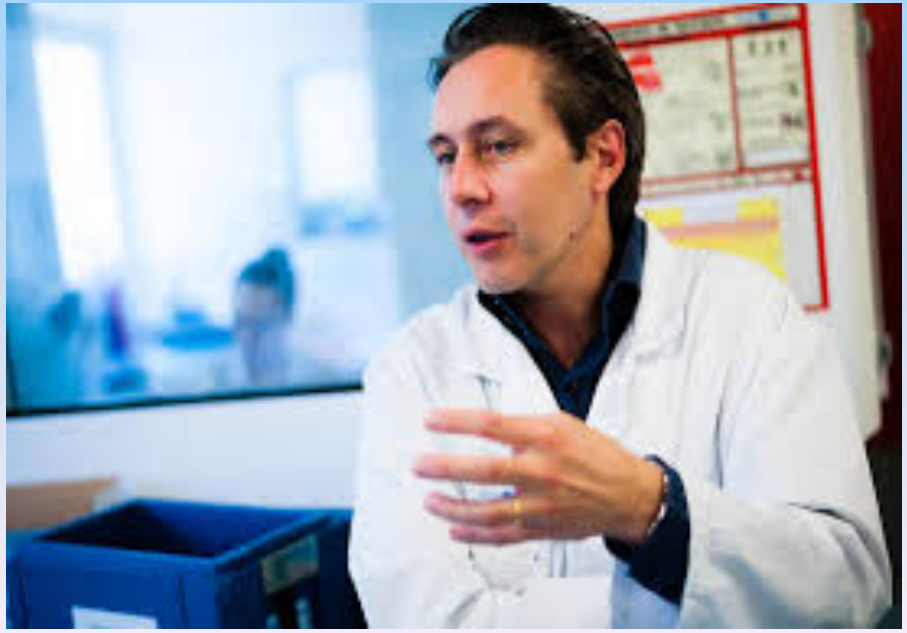

Benjamin Brinon


Akira Furuta

Hôpital Saint-Louis

Centre Hospitalo-Universitaire et de Recherche

Department of Cell and Tissue Biotherapies



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