Progress in Myocardial Regeneration – Exosomes and iPCs

A New Understanding of Cardiovascular Physiology, Pathology and Future Therapies

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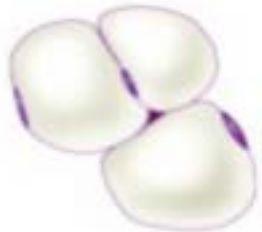
Progenitor Cells for Cardiac Repair-Regeneration

Endothelial Progenitor Cells

HSCs, MSCs, MAPC

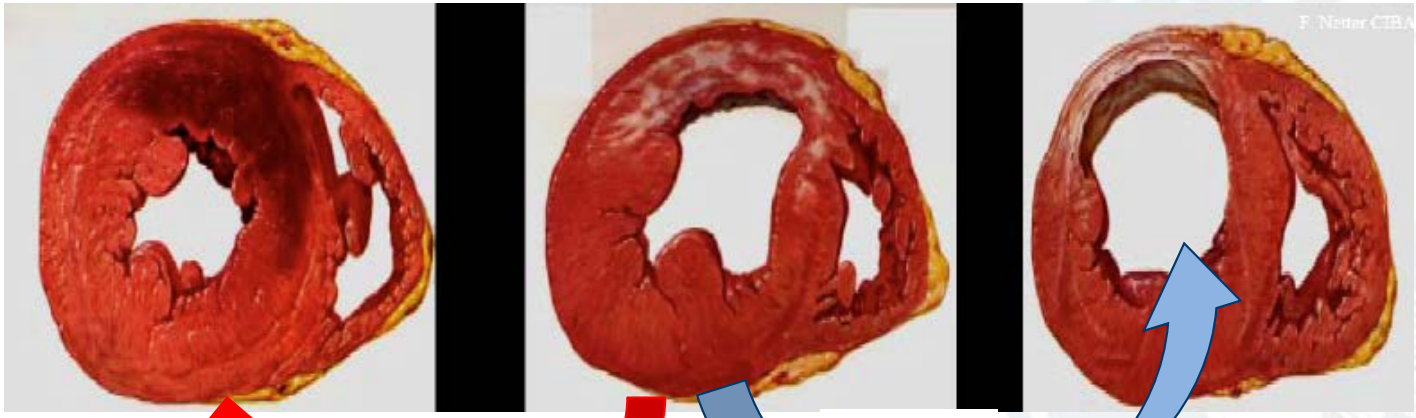
Myoblasts



MSCs ADSCs

ES

STEMI



Chronic Heart Failure

CPC

c-Kit⁺, SP cells, Isl1⁺, SSEA-1⁺, Epicardial cells
Cardiospheres and –derived cells

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Progenitor Cells for Cardiac Repair-Regeneration

BAMI phase 3
FP7-EU

EPCs

HSCs, MSCs,
MAPC

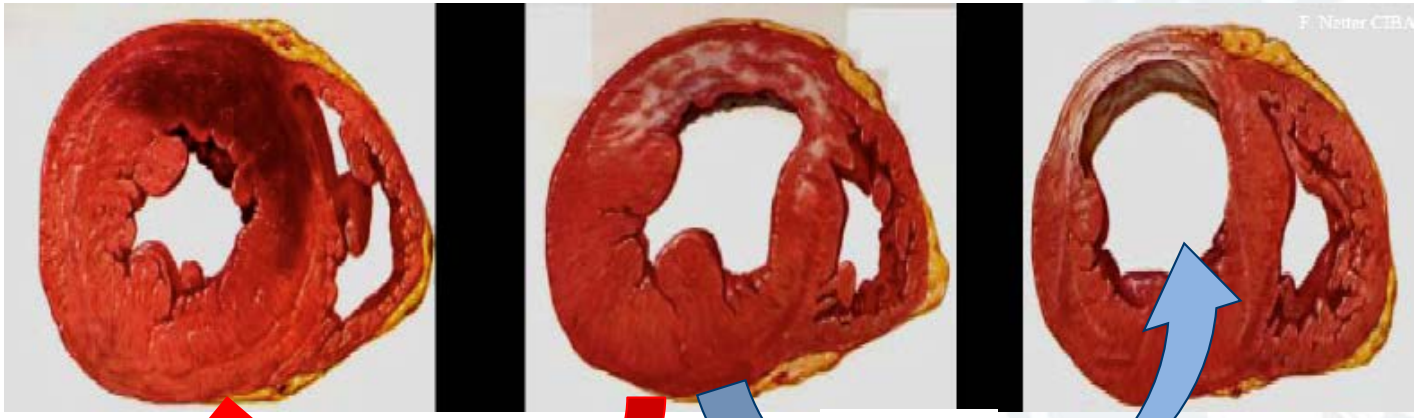
Myoblasts

MSCs
ADSCs

(ES-CM)
iPSC-CM

PARACRINE MODE OF ACTION: EXOSOMES, ...

STEMI



Chronic
Heart Failure

CPC

c-Kit⁺, SP cells, Isl1⁺, SSEA-1⁺, Epicardial cells
Cardiospheres and –derived cells

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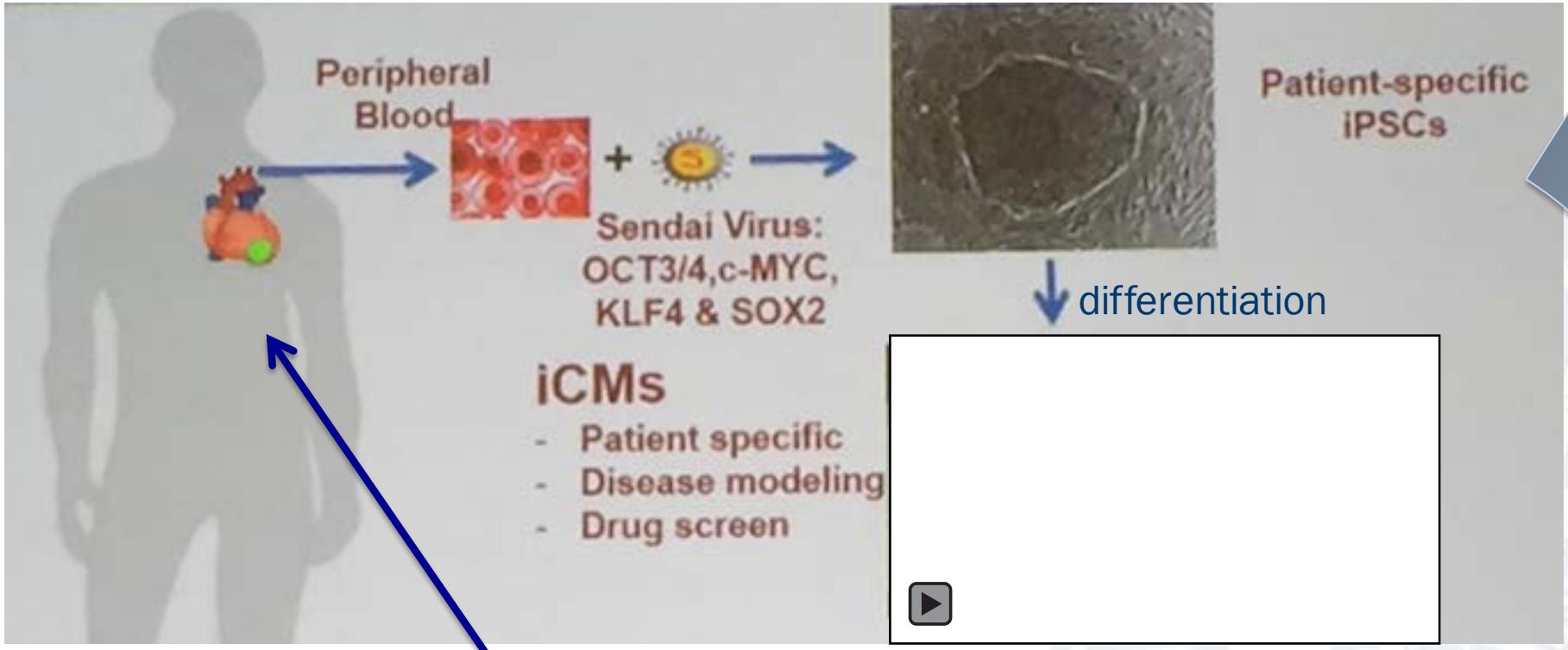
Outline Of Presentation (I): induced Pluripotent Stem Cells, iPSC

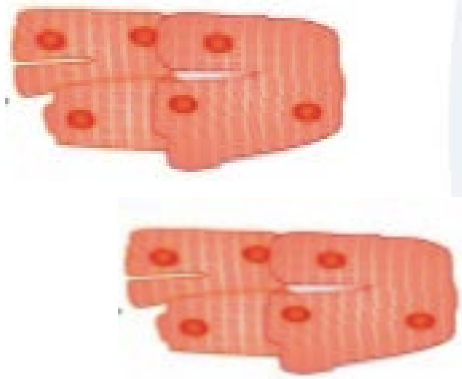
- What are iPSCs?
- Role in Cardiovascular Physiology / Pathology
- Therapeutic potential: how, when, where?

Generation of iPSC-derived Cardiac Myocytes



Personalized therapy
(+/- gene editing)

→ Cardiac regeneration?



expansion ex vivo

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iPSC-derived Human Cardiac Myocytes in Cardiac Regeneration: caveats

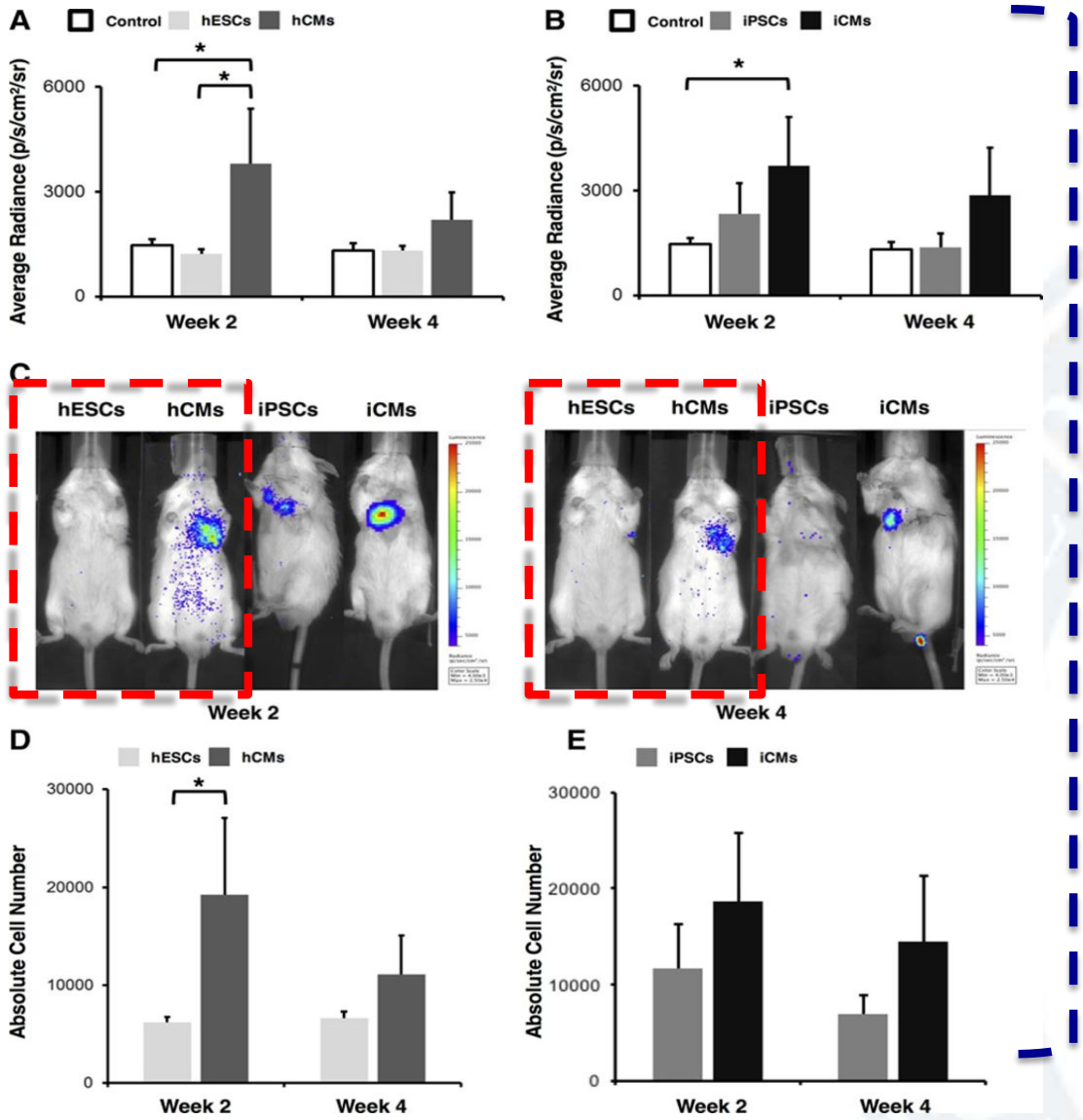
- Cell Survival after Transplantation?
- Maturity of iPSC-derived cardiac myocytes ?
- Arrhythmias ?
- Tumor formation ?

Human ES- and iPS Cell Engraftment in SCID Mice Measured by Bioluminescence Imaging

ES cells

ES-hCM

↑ LVEF & viability



iPS cells

iPS-iCM

↑ LVEF & viability

Tachibana et al.
Circ Res.2017;121:e22-e36.

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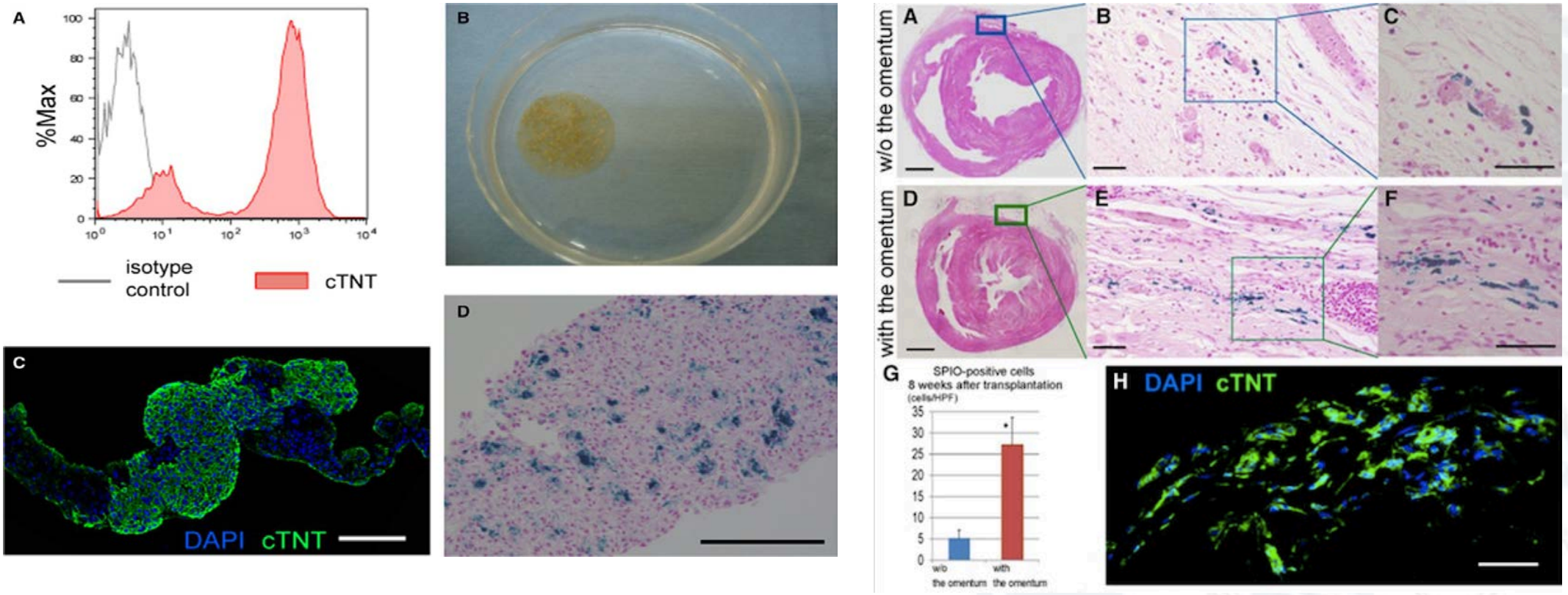
Human induced pluripotent stem cell–derived cardiac myocytes (hiPS-CMs) cell sheet improves survival after transplantation.



Masashi Kawamura et al. Circulation. 2013;128:S87-S94

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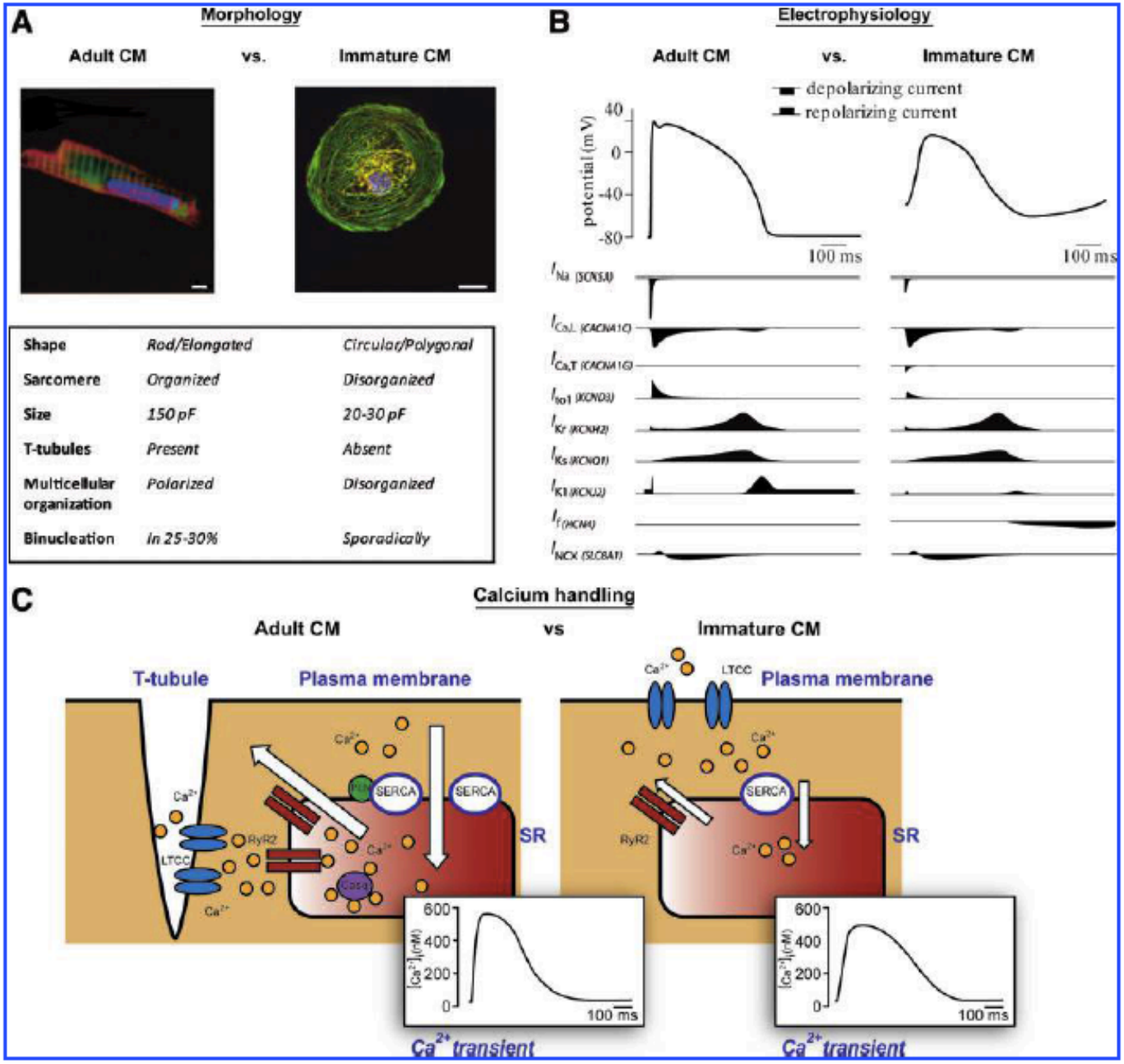
Immaturity of human iPSC-derived Cardiac Myocytes



Shape	*Rod/Elongated*	*Circular/Polygonal*
Sarcomere	*Organized*	*Disorganized*
Size	*150 pF*	*20-30 pF*
T-tubules	*Present*	*Absent*
Multicellular organization	*Polarized*	*Disorganized*
Binucleation	*in 25-30%*	*Sporadically*

Veerman et al.
Stem Cells Dev 2015

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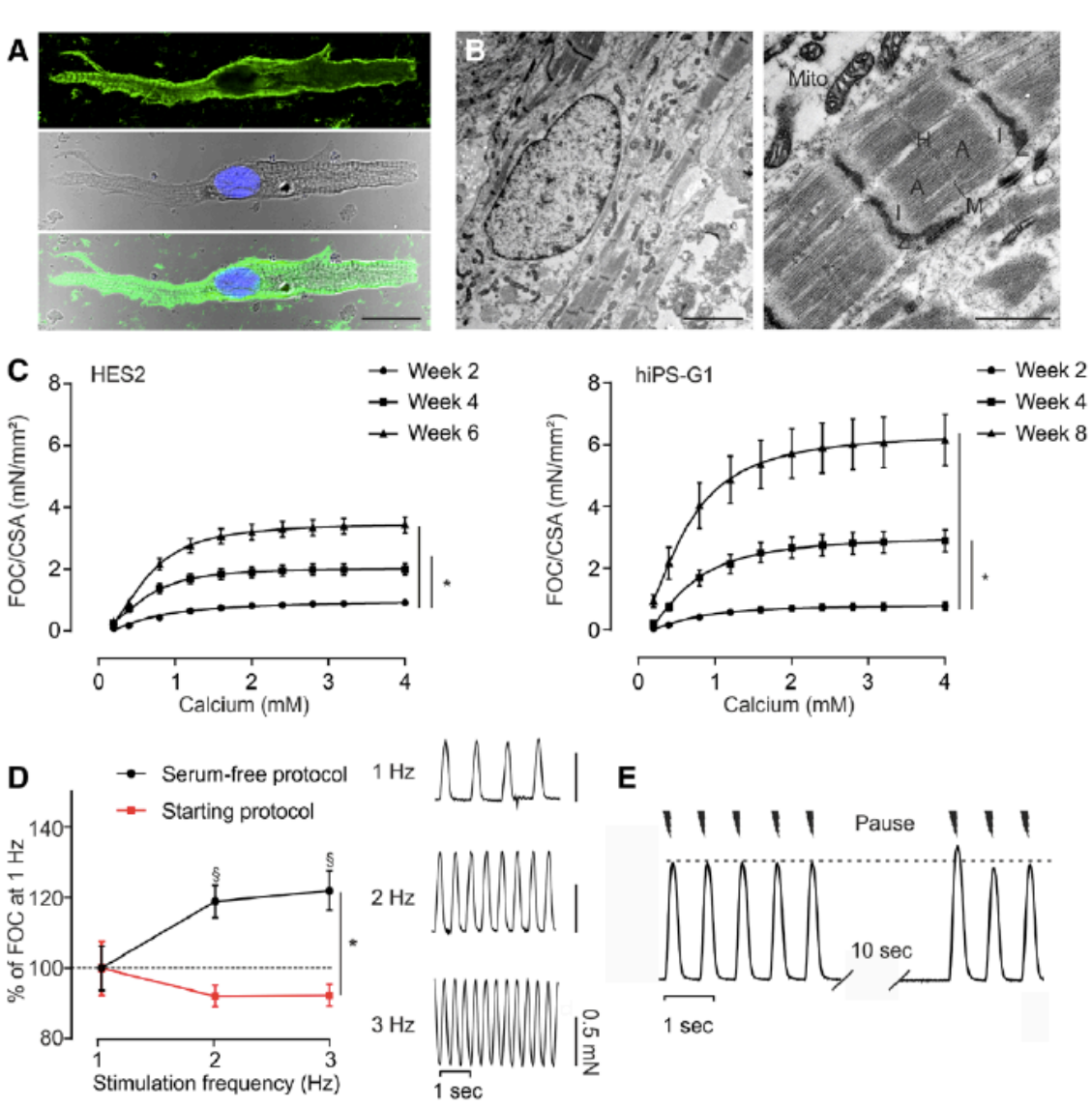
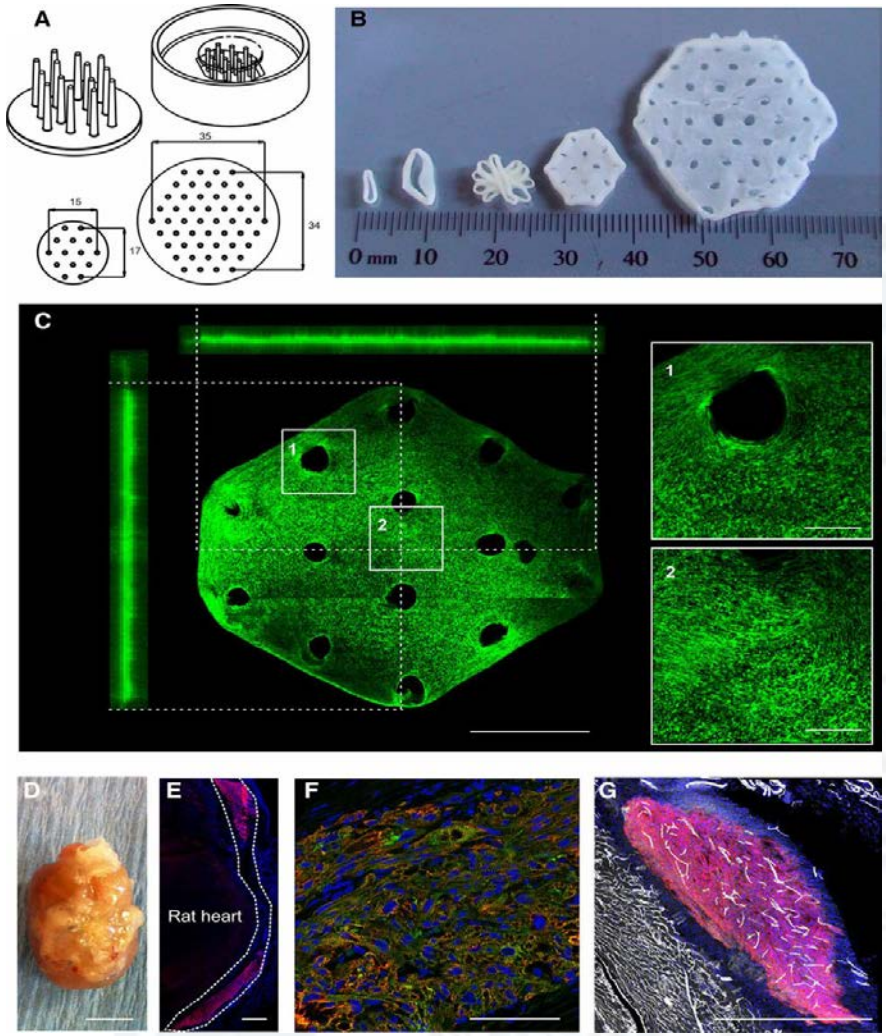
Engineered Human Myocardium for Heart Repair





Malte Tiburcy et al. Circulation. 2017;135:1832-1847

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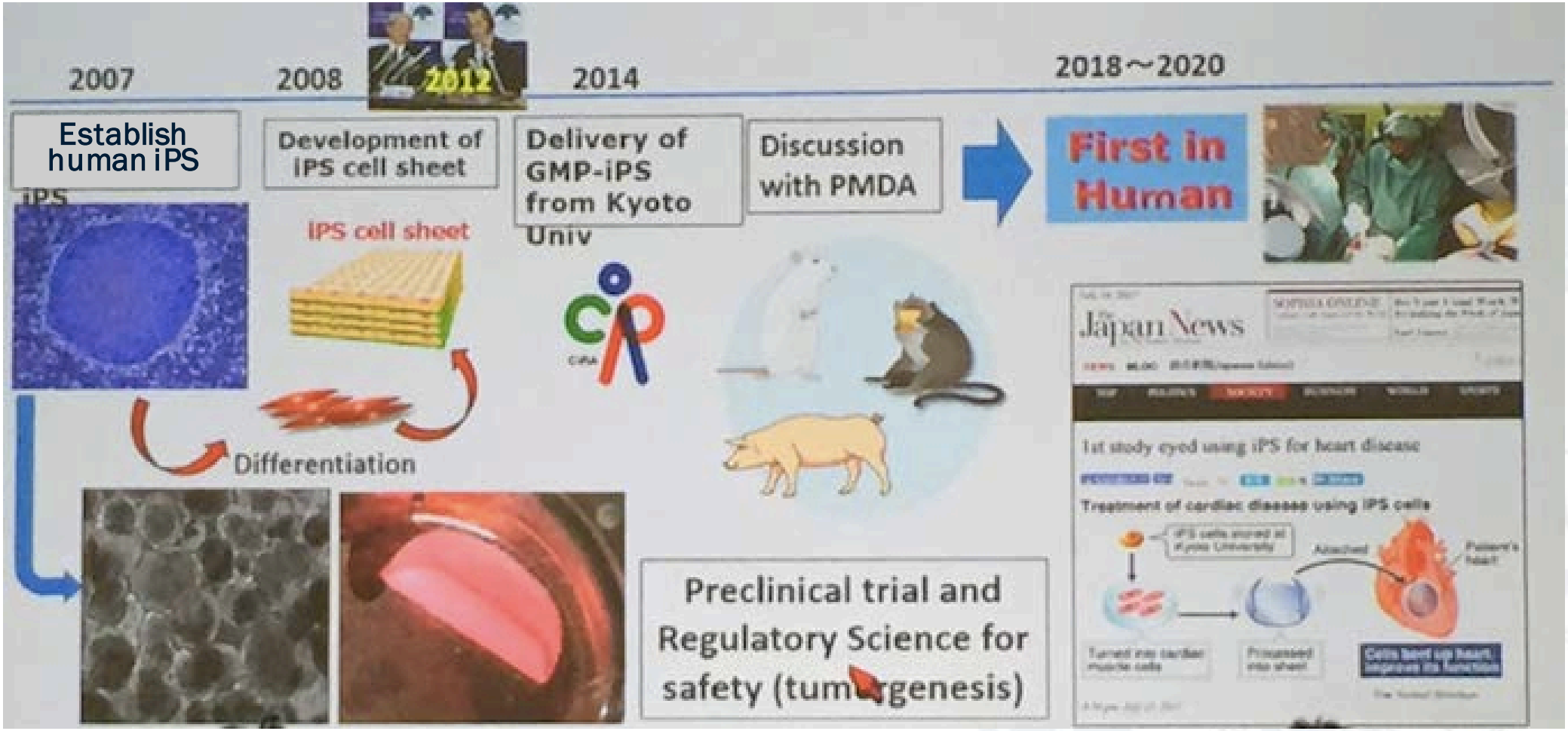
Future Clinical Application of human iPSC-derived cardiac myocytes: EHT



Courtesy of Yoshinori Yoshida, Kyoto, Japan

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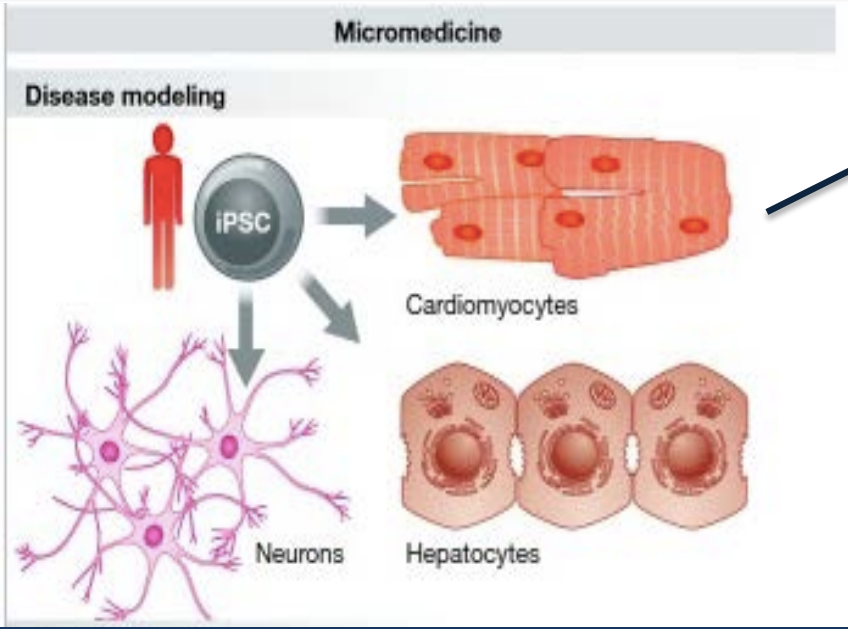
iPSC-derived Human Cardiac Myocytes in Cardiac Regeneration: caveats

- Cell Survival after Transplantation not permanent
- Maturity of iPSC-derived cardiac myocytes incomplete
- Arrhythmias (tbd)
- Tumor formation (tbd)

➔ Major potential in disease modeling

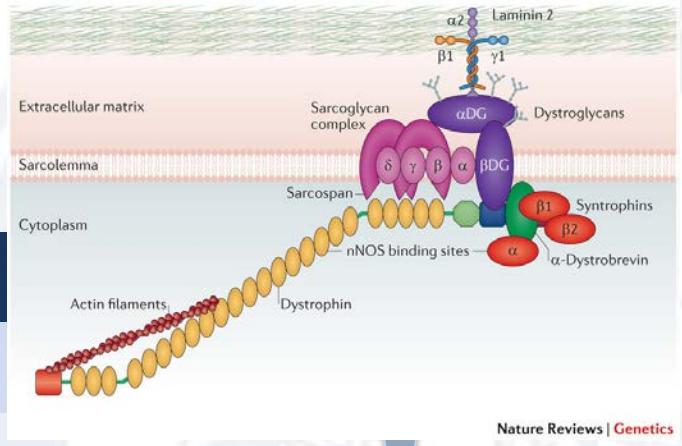
iPSC Application beyond Cardiac Regeneration



Disease modeling:

→ mutations in genes encoding ion channels, sarcomeric proteins,...

- Duchenne Muscular Dystrophy



iPSC Line	Meth	origin	Mutation	Cardiac Function
DMD01	SeV	PBMC	del exon 49-52	normal (13y)
DMD02	SeV	fibrobl	pt mut exon 35	cardiomyopathy (20y)
DMD03	SeV	PBMC	del exon 16-17	normal (8 y)
DMD04	SeV	PBMC	del exon 46-51	cardiomyopathy (15y)
HC01	SeV	fibrobl	NA	normal

(Courtesy of M. Sampaolesi, SCIL 2017)

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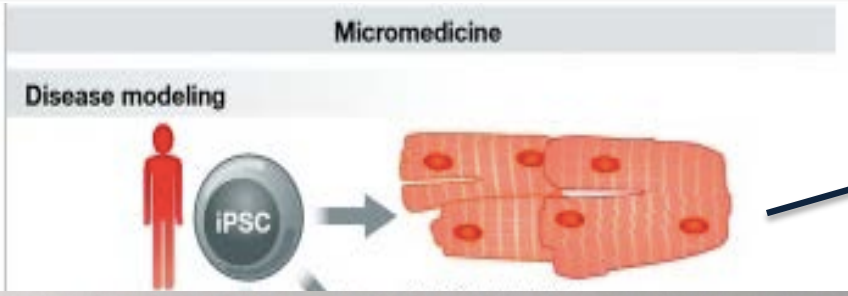
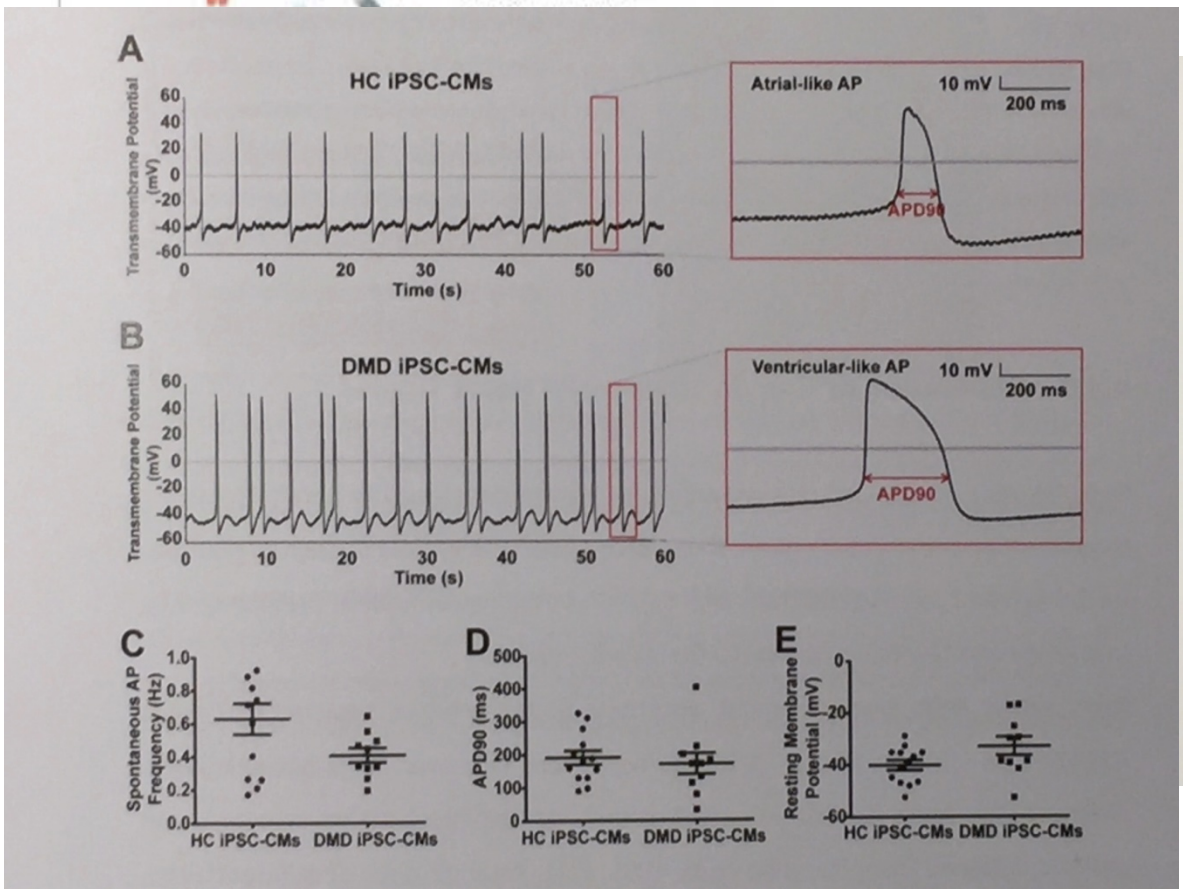
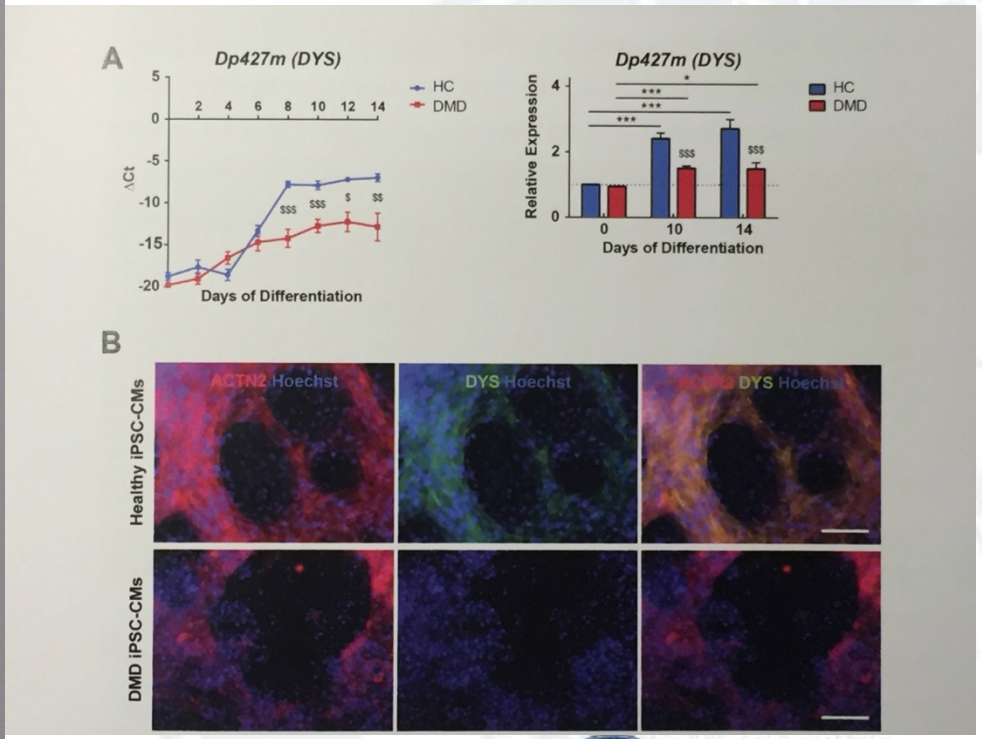
iPSC Application beyond Cardiac Regeneration



Disease modeling:
→ Screening mutations in dystrophin





(Courtesy of M. Sampaolesi, SCIL 2017)

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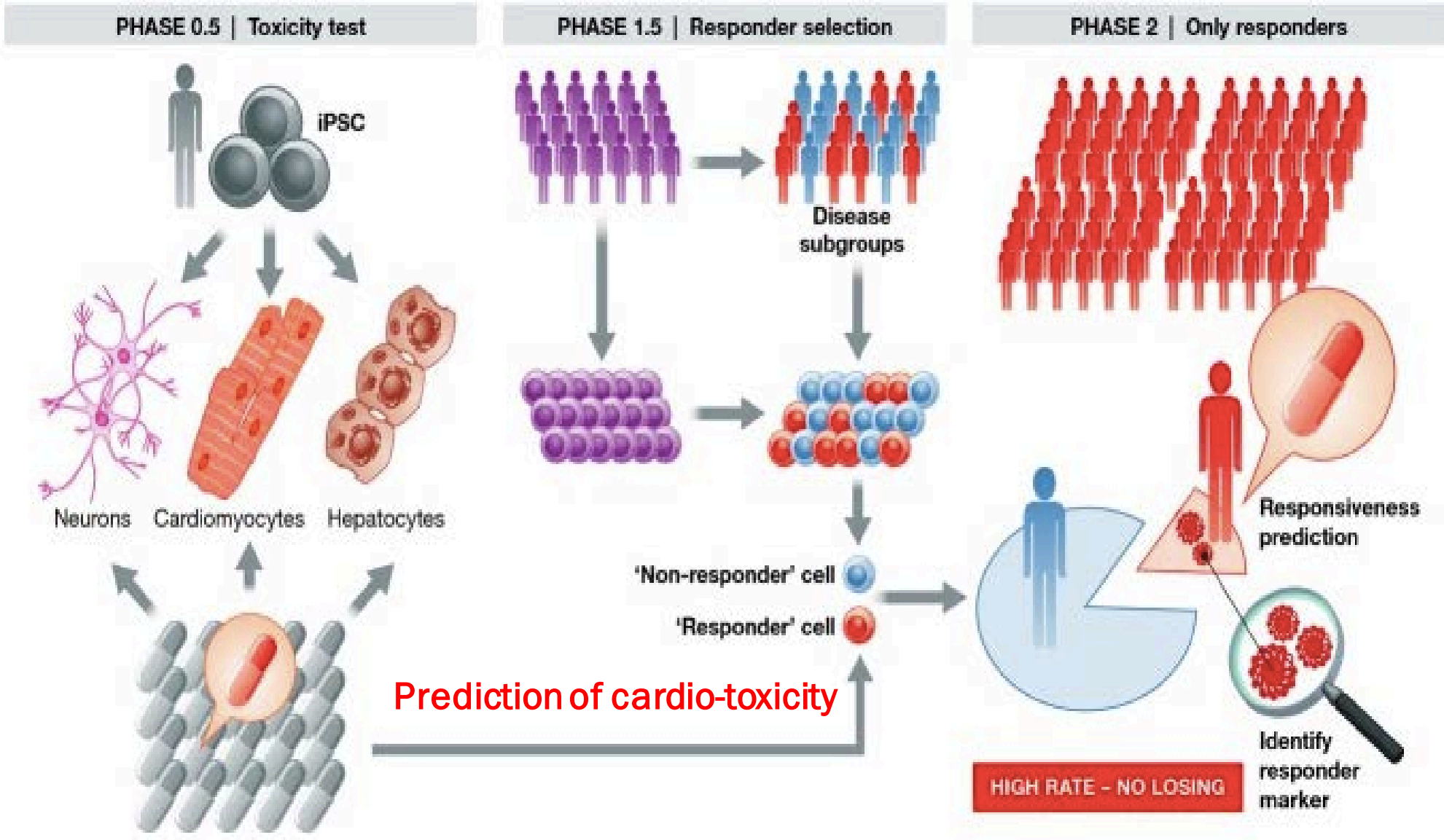
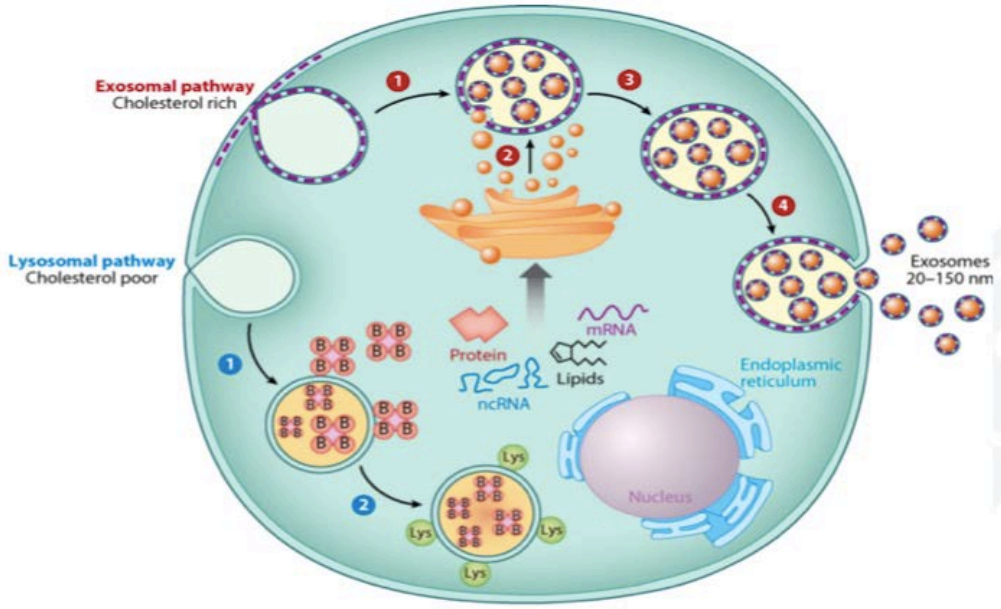
iPSC Application beyond Cardiac Regeneration

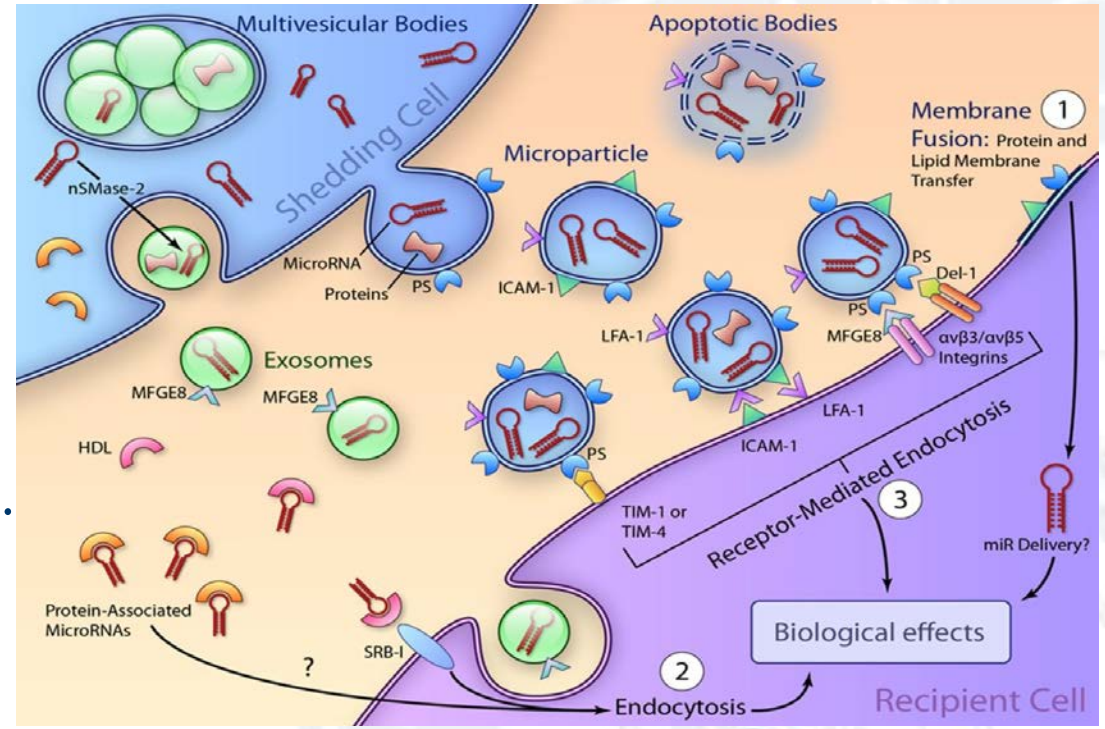


Outline Of Presentation (II): Exosomes

- What are exosomes?
- Role in Cardiovascular Physiology / Pathology
- Therapeutic potential: how, when, where?

What are Exosomes?





Small (100 nm) vesicles:

- cell- and tissue- specificity
- contain proteins, miRNA, mRNA,..
- stable in circulation (capsid)
- rapid uptake by cells
- cell – cell communication

→ mediate paracrine effects of progenitor cells

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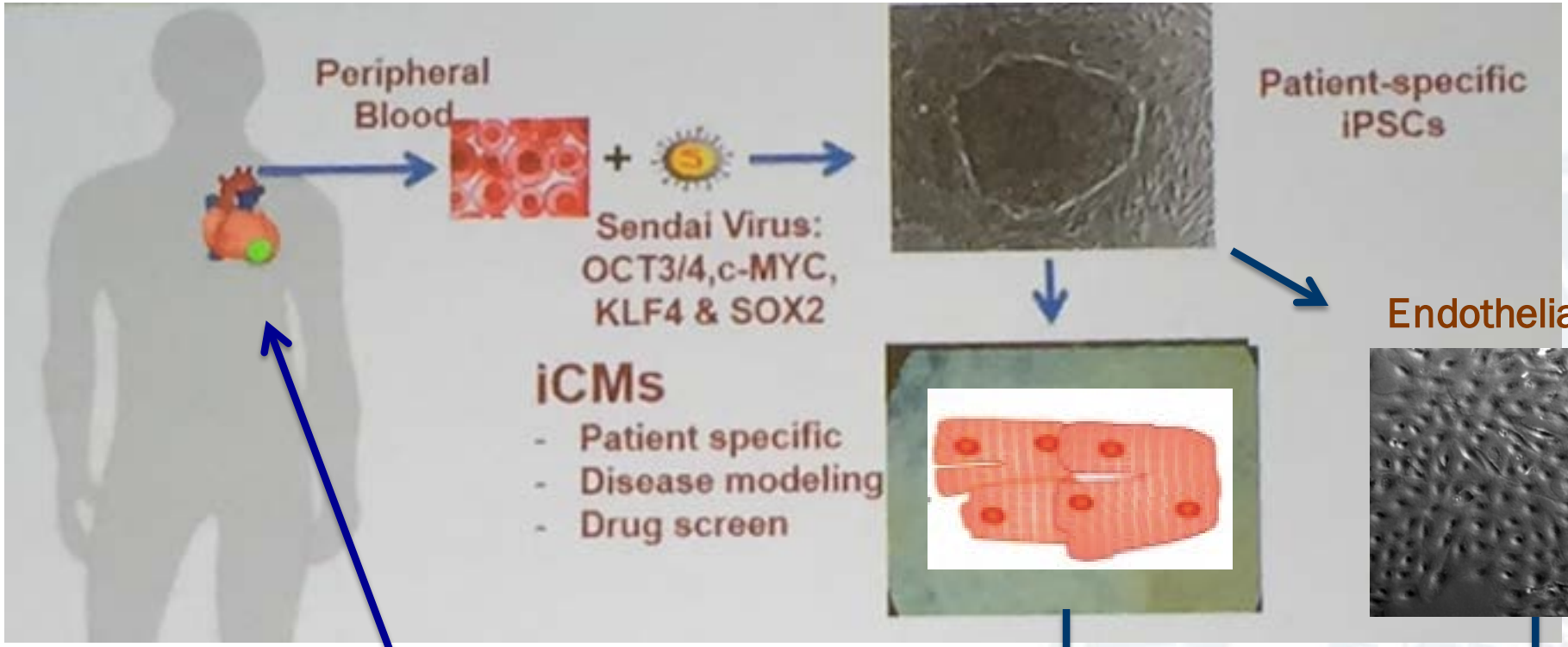
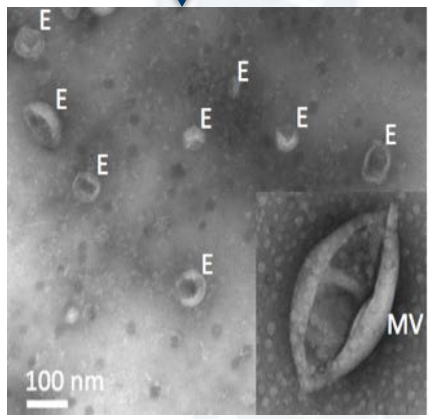
Generation of Exosomes from iPSC-derived Cardiac Myocytes and Endothelial Cells



Personalized therapy?

Exosomes:
- Cell free
- Patient specific
- Disease specific



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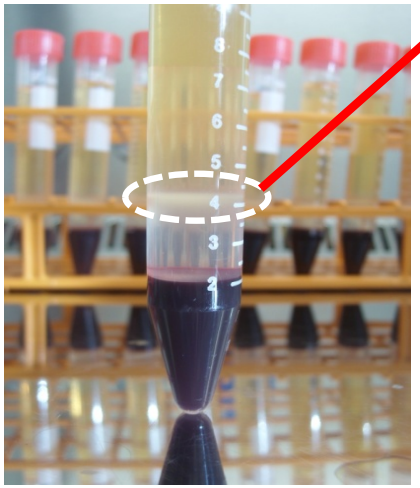
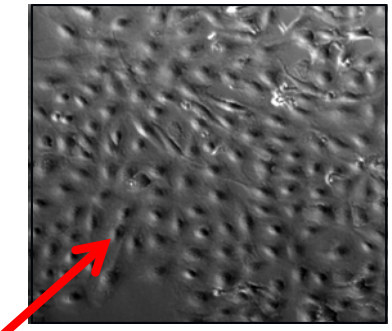
Characterization of human Blood Outgrowth Endothelial Cells (BOEC) and BOEC-derived Exosomes

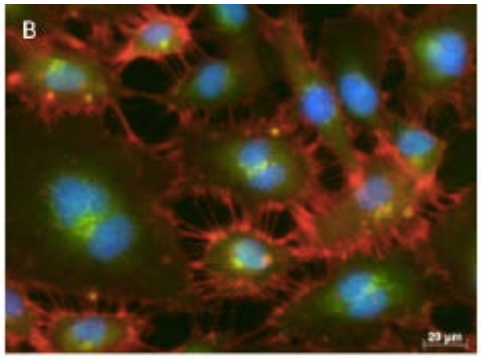
B? **Peripheral Blood derived**

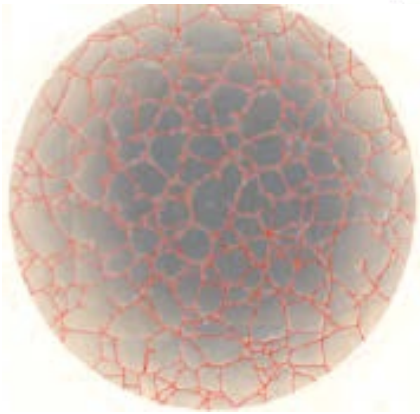
O? **Outgrowth after 2-3 weeks**

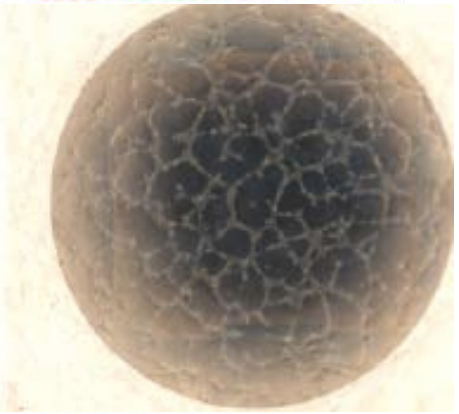
EC? **Endothelial Progenitor Cell characteristics**

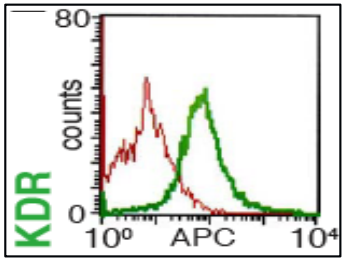


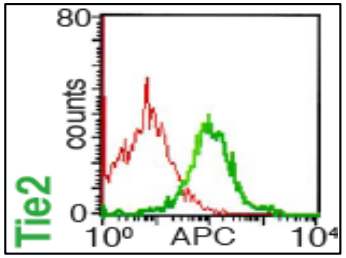


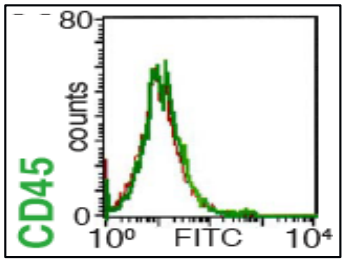






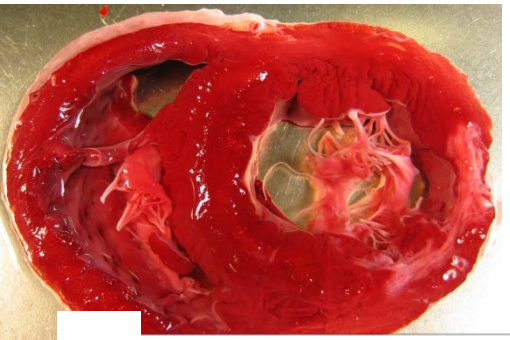


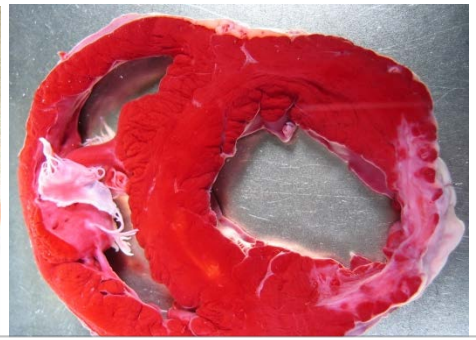




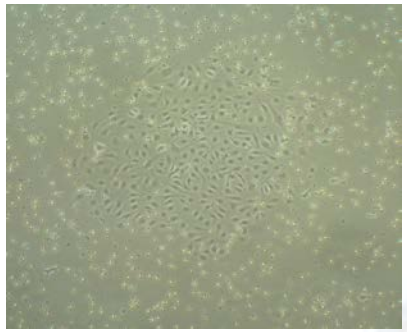
BOECs improve myocardial perfusion and reduce LV remodeling in Ischemic CMP

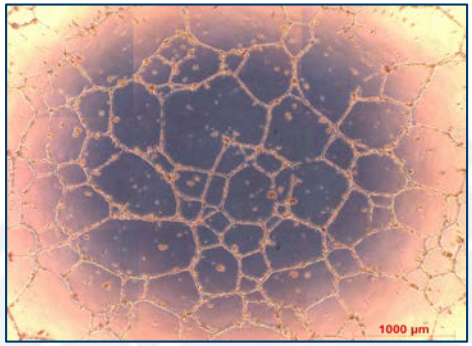
TTC: 6w after IC cell transfer





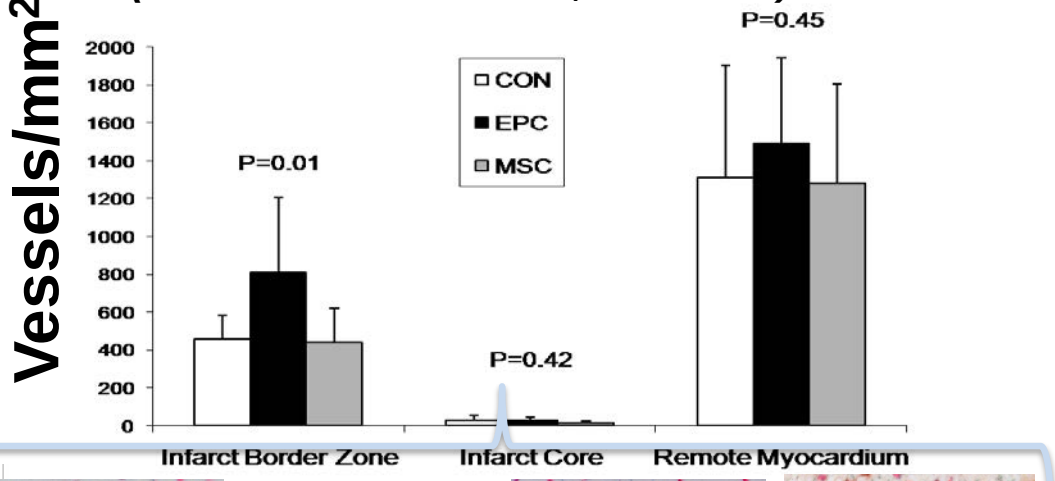
(Dubois et al. JACC 2010;55:2232-43)

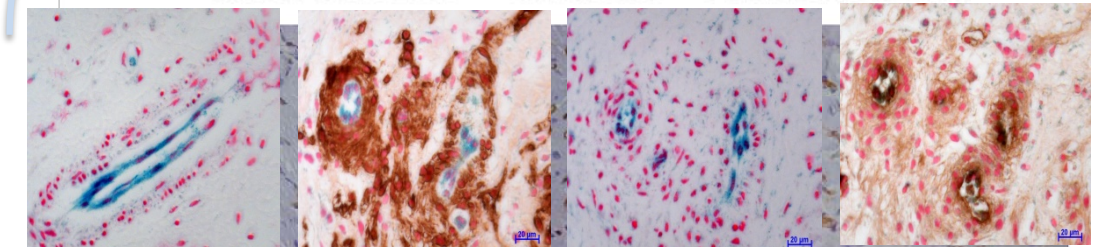




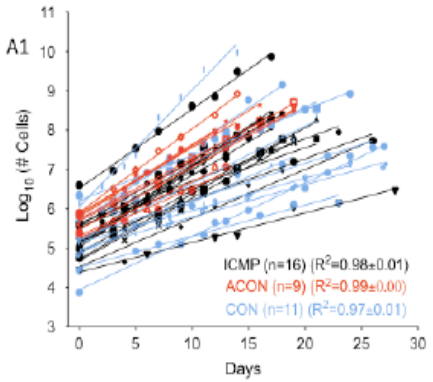
IHD (EF<35%): n=45, Isolation Efficiency 81%

Con: n=32, Isolation Efficiency 71%

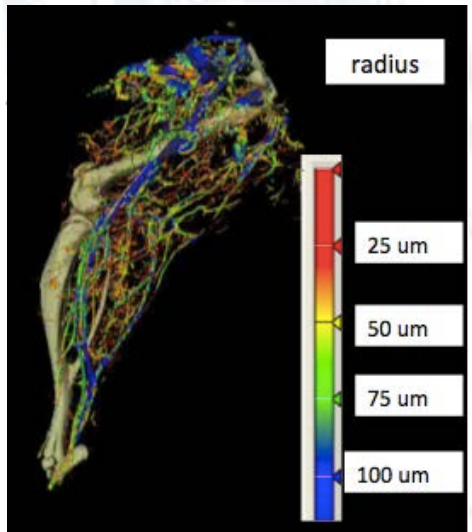




In vitro expansion



(Dauwe et al, JAHA 2016)

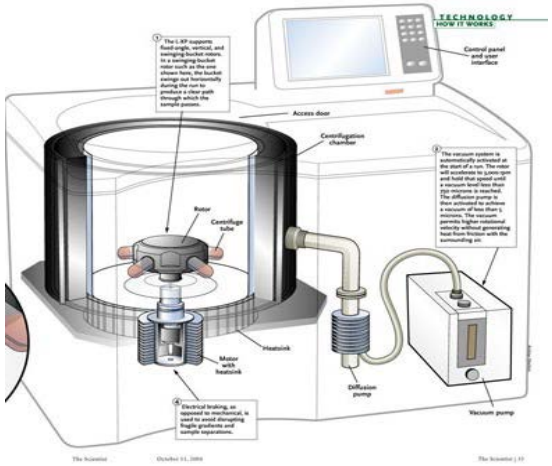


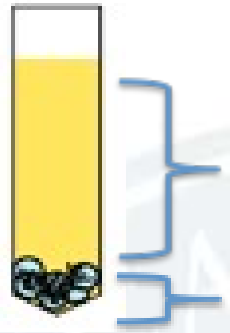
BOEC Exosome Characterization

collect
culture media
(Nxa / Hxa)

10cm Petri Dish
@7ml of culture media
(FBS Depleted Exosomes)

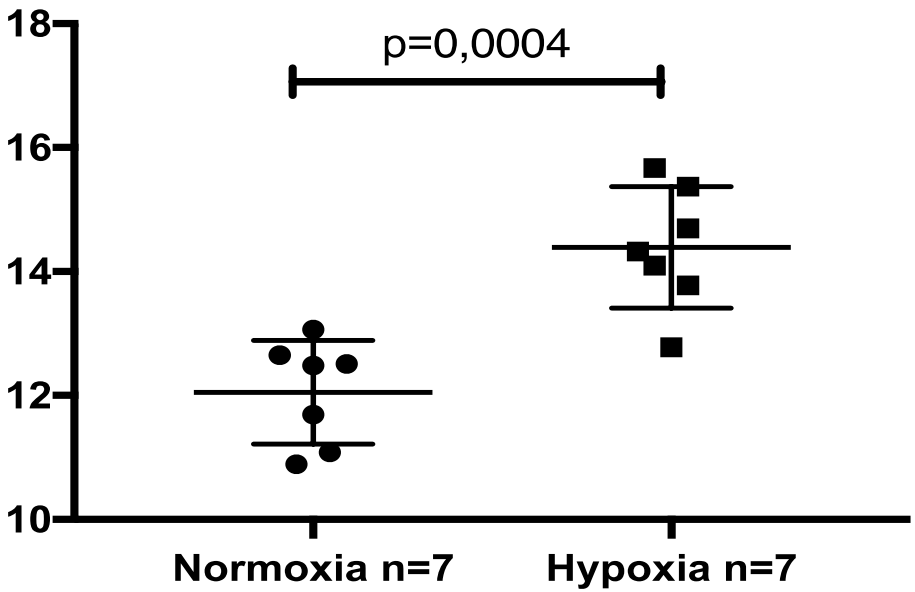
Ultracentrifugation

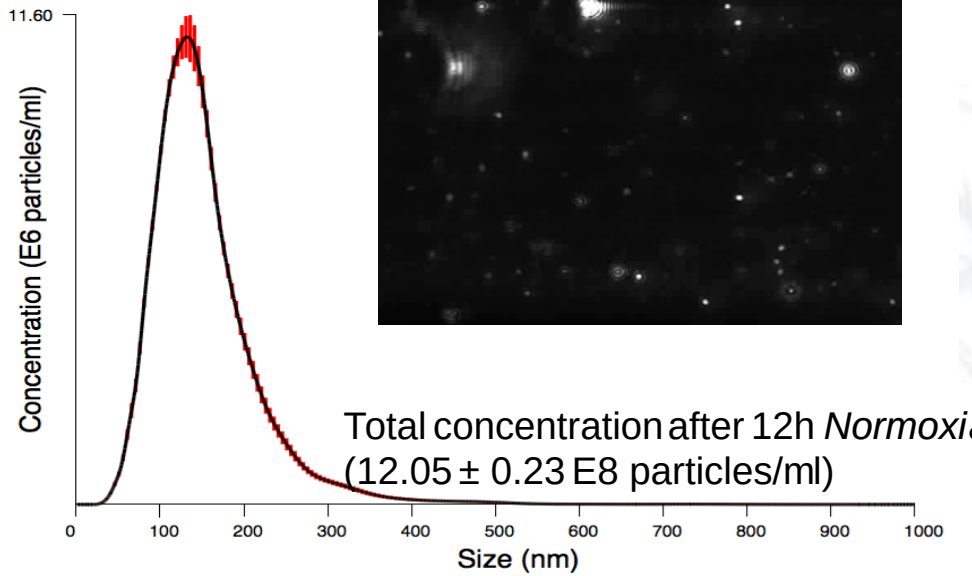




Supernatant

Exosomes
Pellet

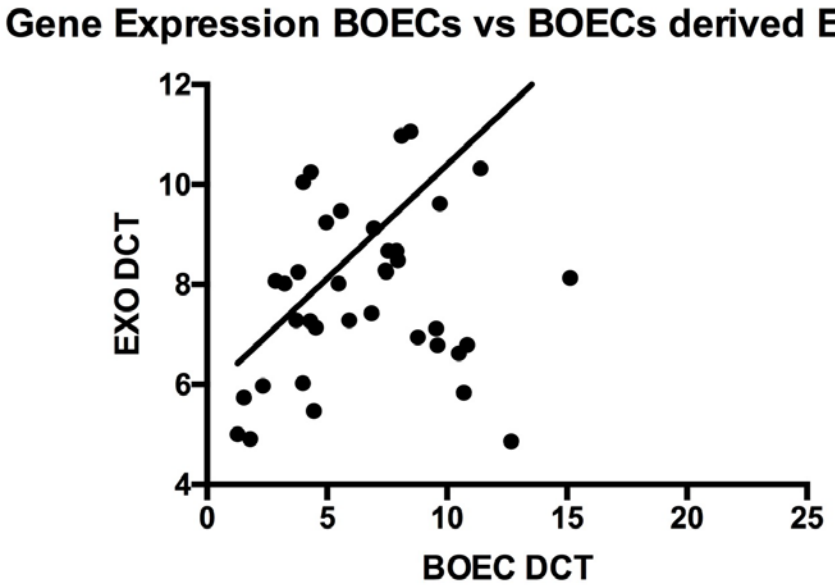




Total concentration after 12h *Normoxia*
(12.05 ± 0.23 E8 particles/ml)

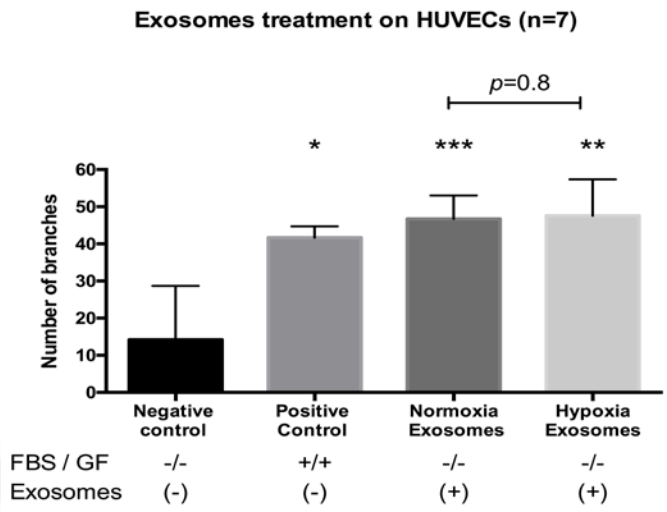
Averaged Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

BOEC-specific Exosomes stimulate 2D Tube Formation of HUVEC in Matrigel

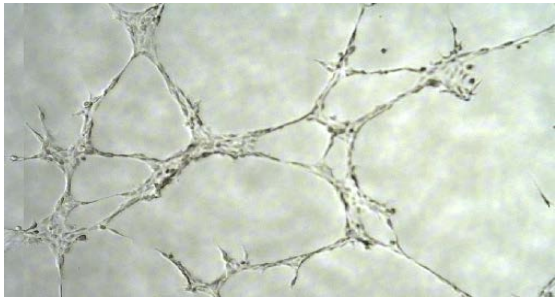


MMP1
MCP1
KGF/FGF2
PLGF
VEGFR2

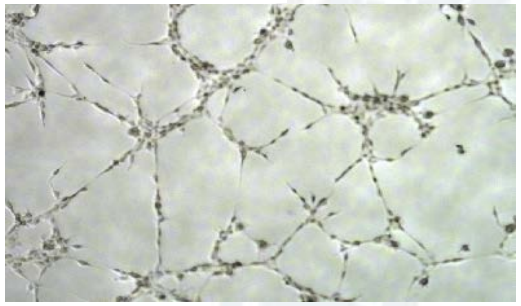
P Value < 0.001
$R^2 = 0.33$



Negative Control
FBS/GF -/-



Positive Control
FBS/GF +/+



Normoxia Exosomes
FBS/GF -/-

Hypoxia Exosomes
FBS/GF -/-

A. Wibowo et al. ESC 2017

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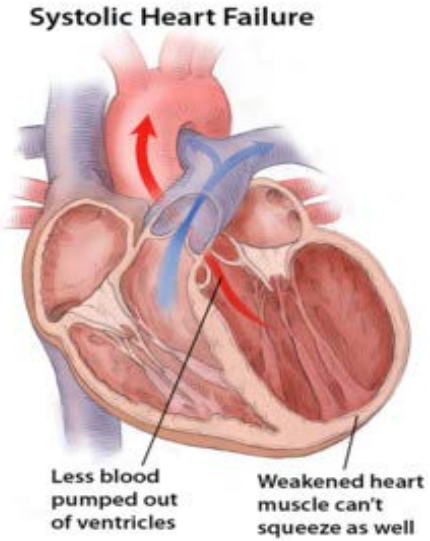
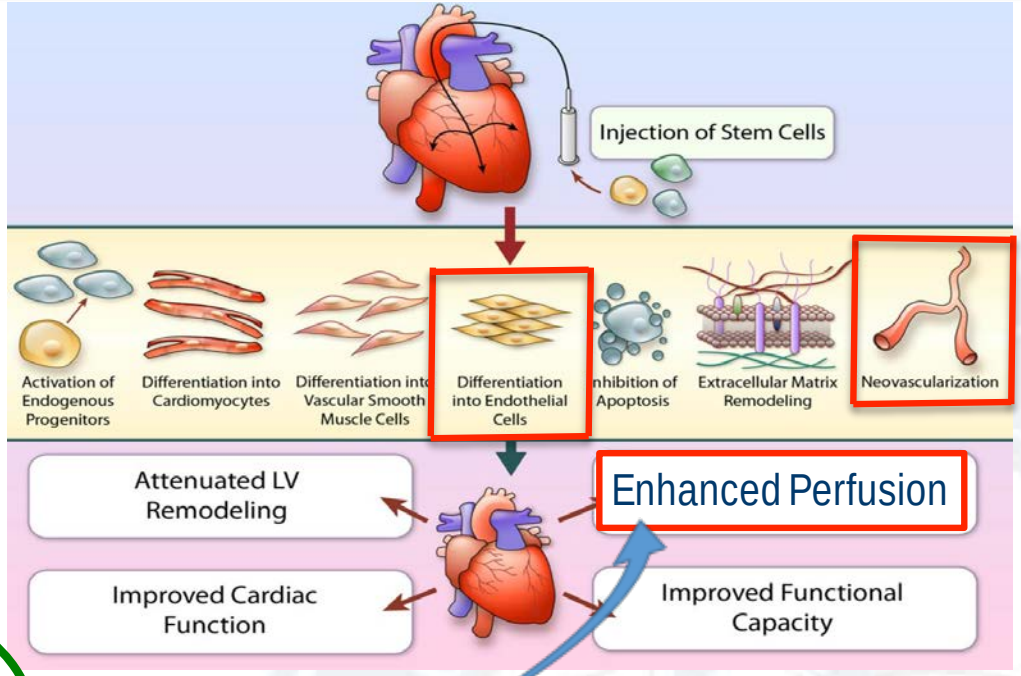
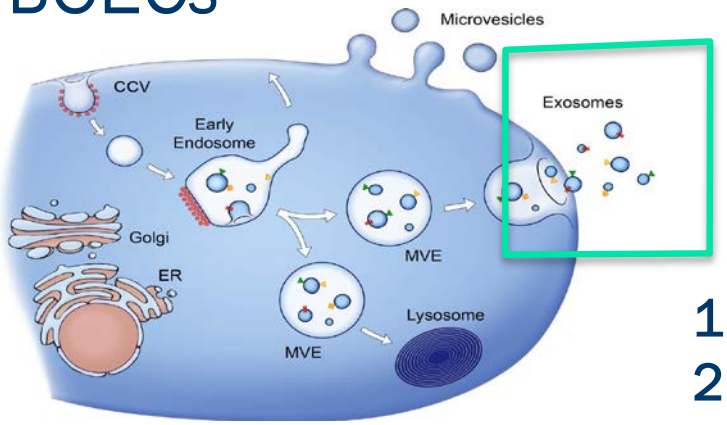
BOEC-derived Exosomes for Cardiac Revascularisation

Myocardial Ischemia





BOECs



Exo_RNA
Exo_Protein

1. Standardization of EXO purification?
2. Optimal concentration and delivery mode?
3. Pathological micro-environment?
4. Exo content and stimulus for release?

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Modified from Sanganalmath, S. Circ Res (2013). Raposo G. J Cell Biol 200(4)

BOEC transfer in Refractory Ischemic Cardiomyopathy

Preclinical Research

Clinical Research

Small animals

Large Animals

Phase Ib

Phase II

BOEC transfer

IMAGING

SAFETY/FEASIBILITY

FIM Phase 1 (safety/feasibility) dose escalation
- ICMP with EF 15-35%
- dose escalation (n=3 pts /dose
FU: 1w – 3, 6 mo – 1 y (PET-MRI)

(Hendrickx, Stem Cells 2010)
(Dauwe, EHJ CV imaging 2015)
(Coppens, Diabetologia 2013)
(Dauwe, JAHA 2016)

Future iPSC and Exosome-based Therapies

- **iPS-derived cardiac myocytes:**

  → Source cells for cardiac regeneration?

  - Paracrine mode of action: immunomodulatory, cytoprotective, pro-angiogenic, anti-fibrotic,…
  - Immature cardiac phenotype: arrhythmogenicity
  - **FIM**: cell sheet with human iPS-CM for ischemic CMP (Japan)

  → Robust potential for disease modeling and precision medicine

  - Cardio-oncology applications

- **Progenitor cell-derived Exosomes:**
  - Mediators of paracrine effects and intercellular signaling molecules
  - Recapitulate benefit of parent progenitor cells in vitro and in vivo
  - **FIM**: - graft versus host disease
    - wound healing in sceroderma (Japan)

Thank you

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