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stem cells




Proarrhythmic modeling in pluripotent stem cell-derived cardiomyocytes

No conflict Of Interest

Peter Sartipy, Ph.D.

The Frontiers in CardioVascular Biology 2012 meeting
London, 30 March - 1 April

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Cellectis group

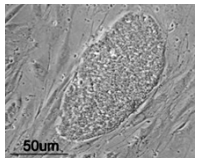
Research tools

Production tools

Agrobiotech

Therapeutics

Stem cells



cellectis bioresearch

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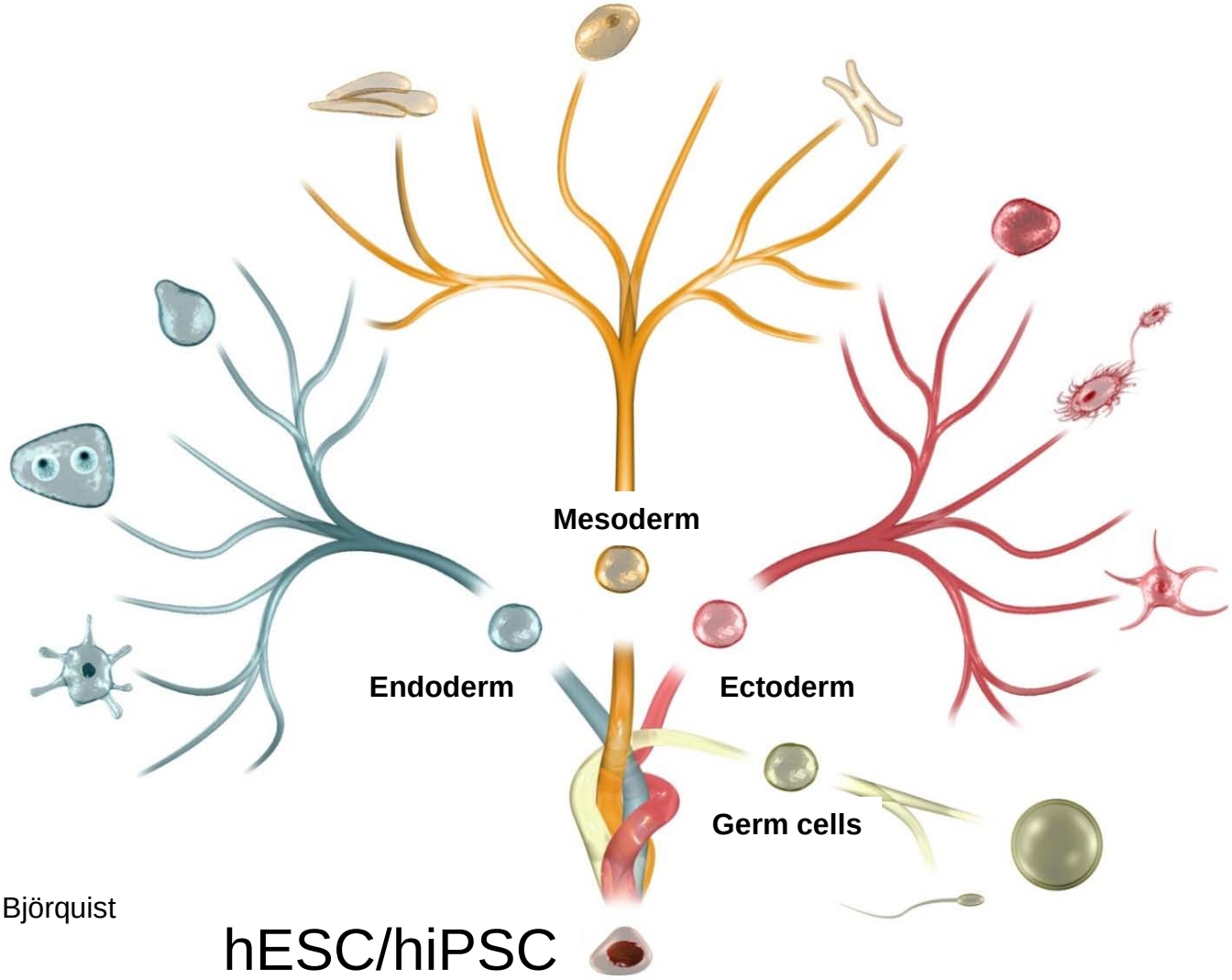
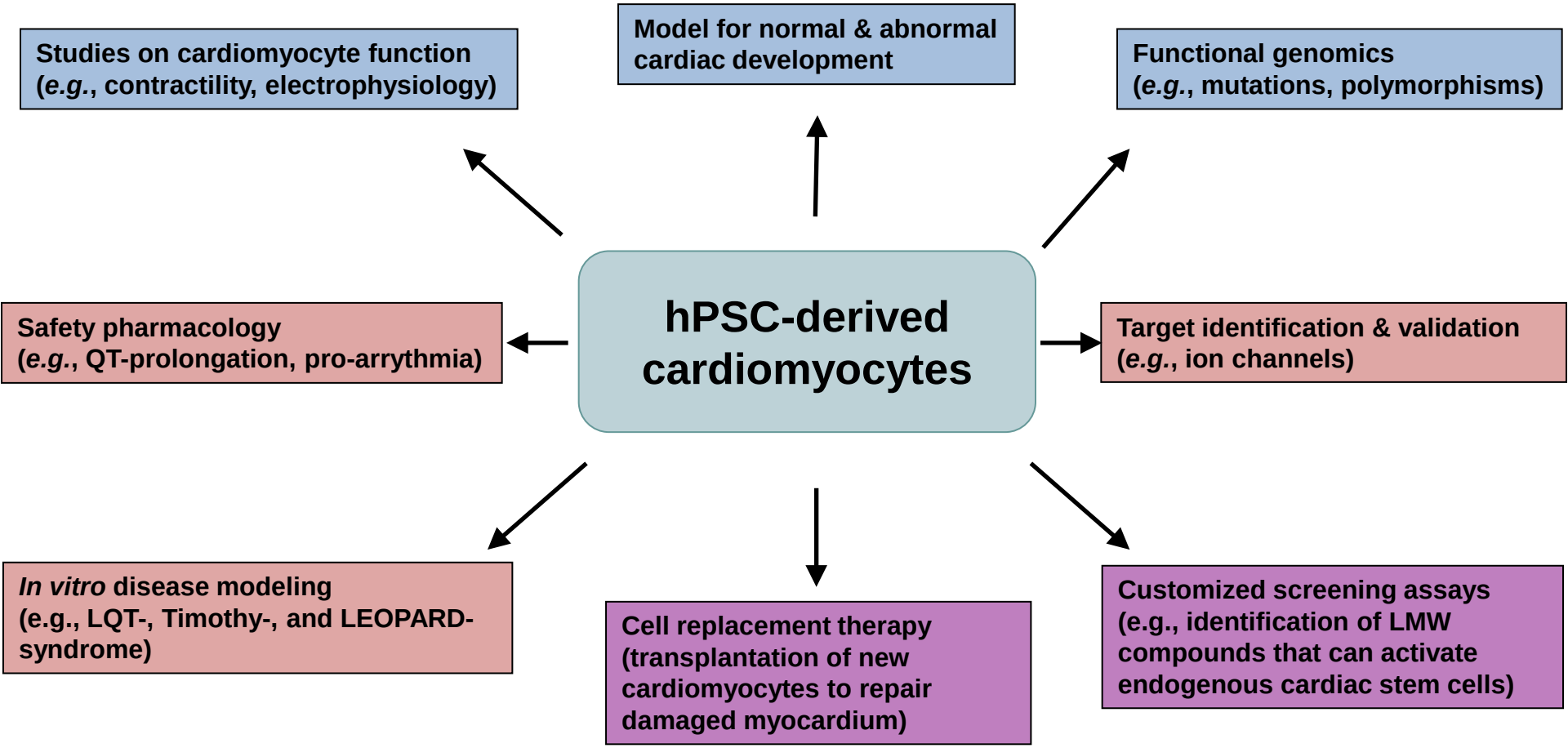
The potential of human pluripotent stem cells



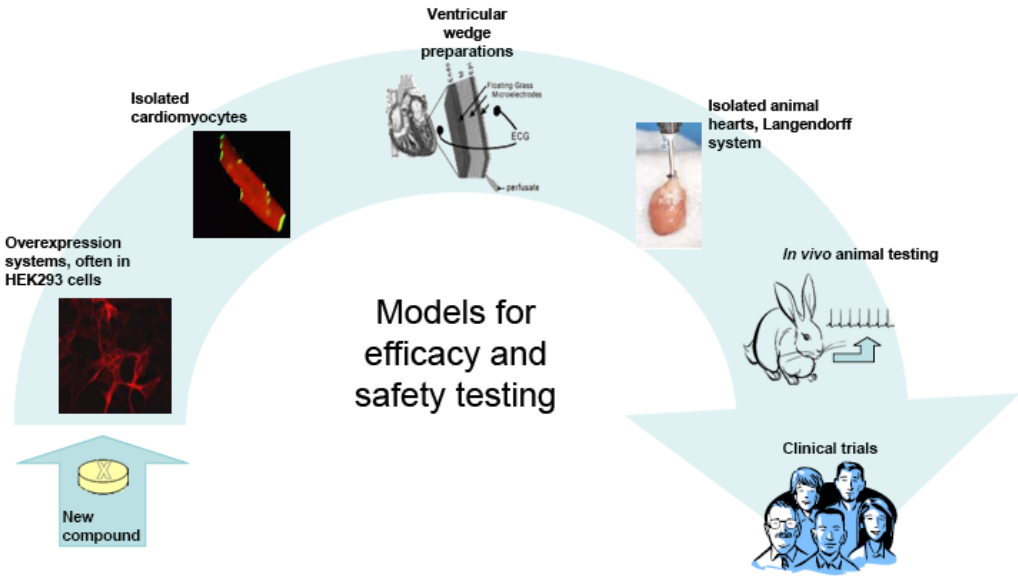
Jensen, Hyllner & Björquist
Review JCP 2009

Applications for human cardiomyocytes



Models for efficacy and safety testing during preclinical drug discovery

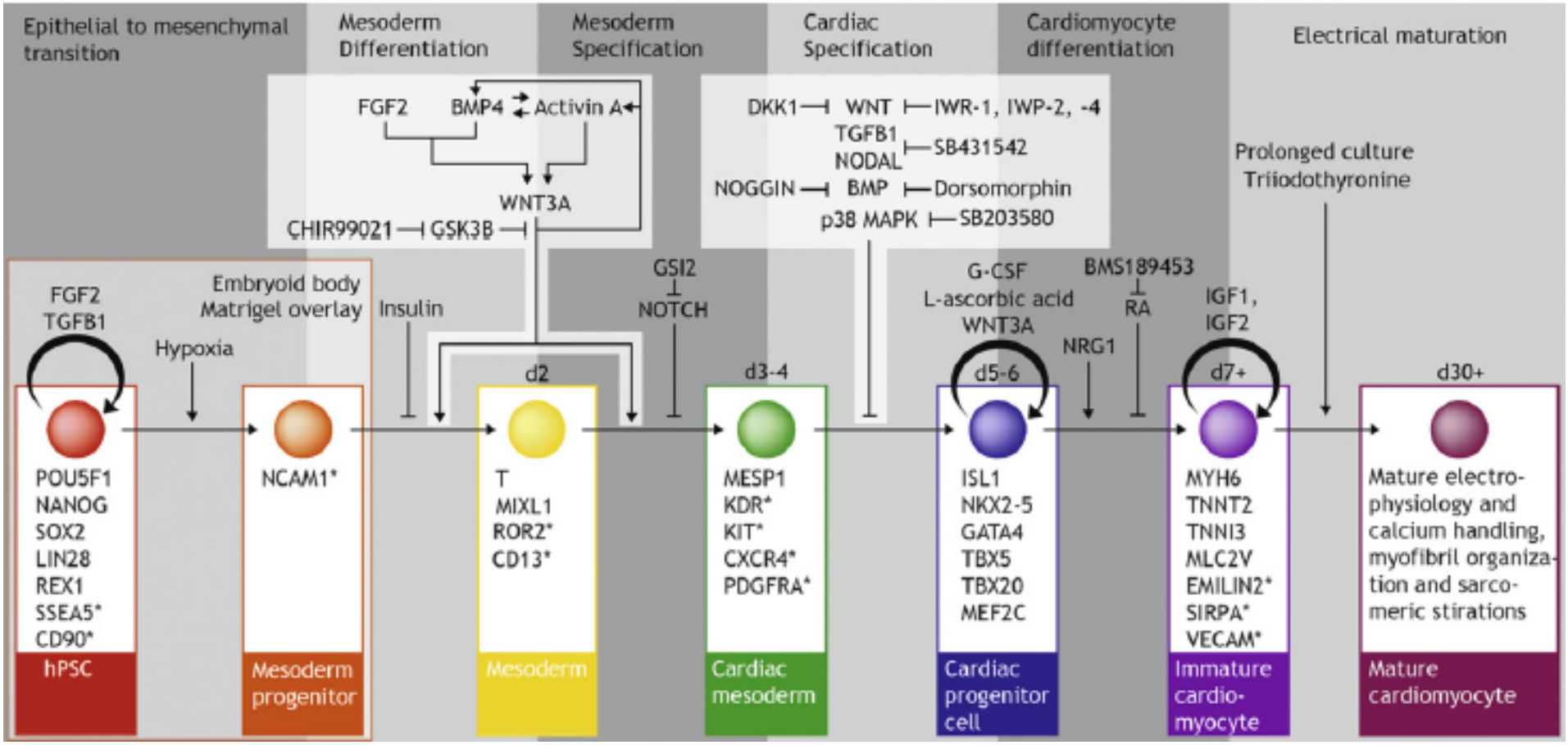
- **Cell lines** (*e.g.,* HL-1, transformed HEK and CHO cells)
- **Primary cardiomyocytes** (*e.g.,* rat, guinea pig, rabbit, dog)
- **Engineered cardiac tissue** (*e.g.,* dissociated embryonic chicken or neonatal rat hearts)
- **Explanted hearts and cardiac tissue** (*e.g.,* dog and guinea pig)
- **Small animal models** (*e.g.*, rabbit, guinea pig, rat, mice)
- **Large animal models** (*e.g.*, dog, monkey, pig)



Jonsson et al Exp Opin Drug Discov 2009

Urgent need for human cardiomyocytes!

Factors involved in cardiac hPSC differentiation



Burridge et al Cell Stem Cell 2012

Interline variability in cardiac differentiation

- Genetic background
- Derivation method
- Culture conditions and passage number
- Efficacy of reprogramming and cell type of origin
- Levels of expression of endogenous growth factors/receptors
- Epigenetic status
- Kinetics of differentiation

➢ Optimization of exogenous and endogenous signaling will ultimately determine the efficiency of cardiac differentiation

Molecular and functional properties of hPSC-derived cardiomyocytes

- Express cardiac markers and ion-channels (*e.g.*, hERG, L-type calcium channel, voltage-gated potassium channels, voltage-gated sodium channels, funny channels).

- Display ventricular-, atrial-, and nodal-like action potentials.

- Functional blocking of ion-channels:
  - hERG-channel blockers (e.g., E-4031, Astemizole, Cisapride, Terfenadine)
  - Funny-channels (e.g., Zatebradine)
  - Sodium channels (e.g., lidocain)
  - Calcium channels (e.g., verapamil)

- Responsive to pharmacological stimuli
  - β-Adrenergic stimulation (e.g., isoprotenerol, adrenalin)
  - α-Adrenergic stimulation (e.g., phenylephrine)
  - Muscarinic stimulation (e.g., carbachol, acetylcholine, atropin)

Developmental status of hPSC-derived CMs

hPSC-derived CMs are in some aspects immature and display a fetal phenotype:

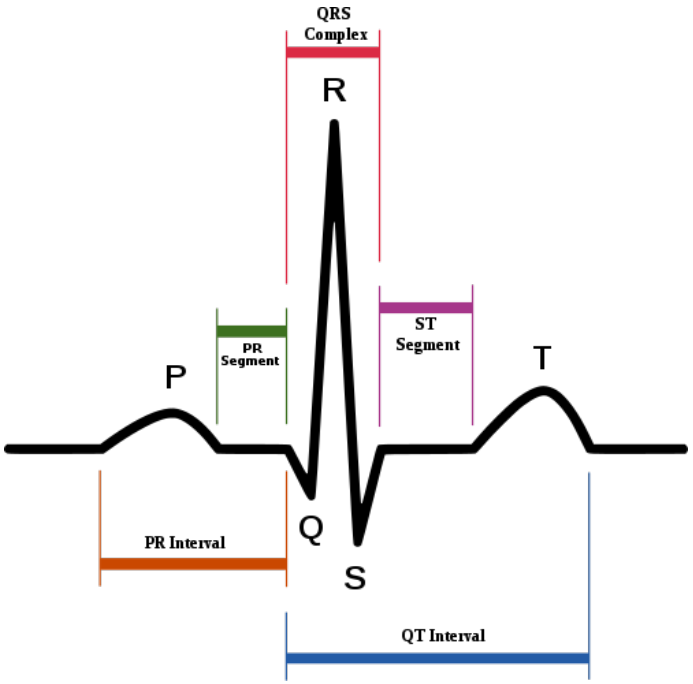
- Spontaneous contraction (large Na^+ current and I_f, low I_{K1})
- Fetal-like electrophysiological properties (lower upstroke velocities, relatively positive resting membrane potential)
- Fetal-like structural properties (low amounts of myofibrils, unaligned and randomly distributed)
- Fetal-like Ca^{2+} handling and SR status

The fetal phenotype may:

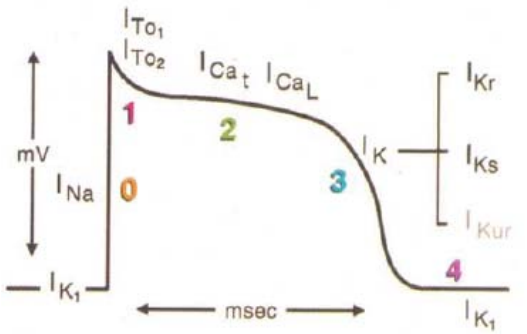
- Reduce the general applicability of current hPSC-derived CMs for in vitro drug testing
- Be beneficial for cell transplantation in regenerative medicine

Cardiotoxicity endpoints

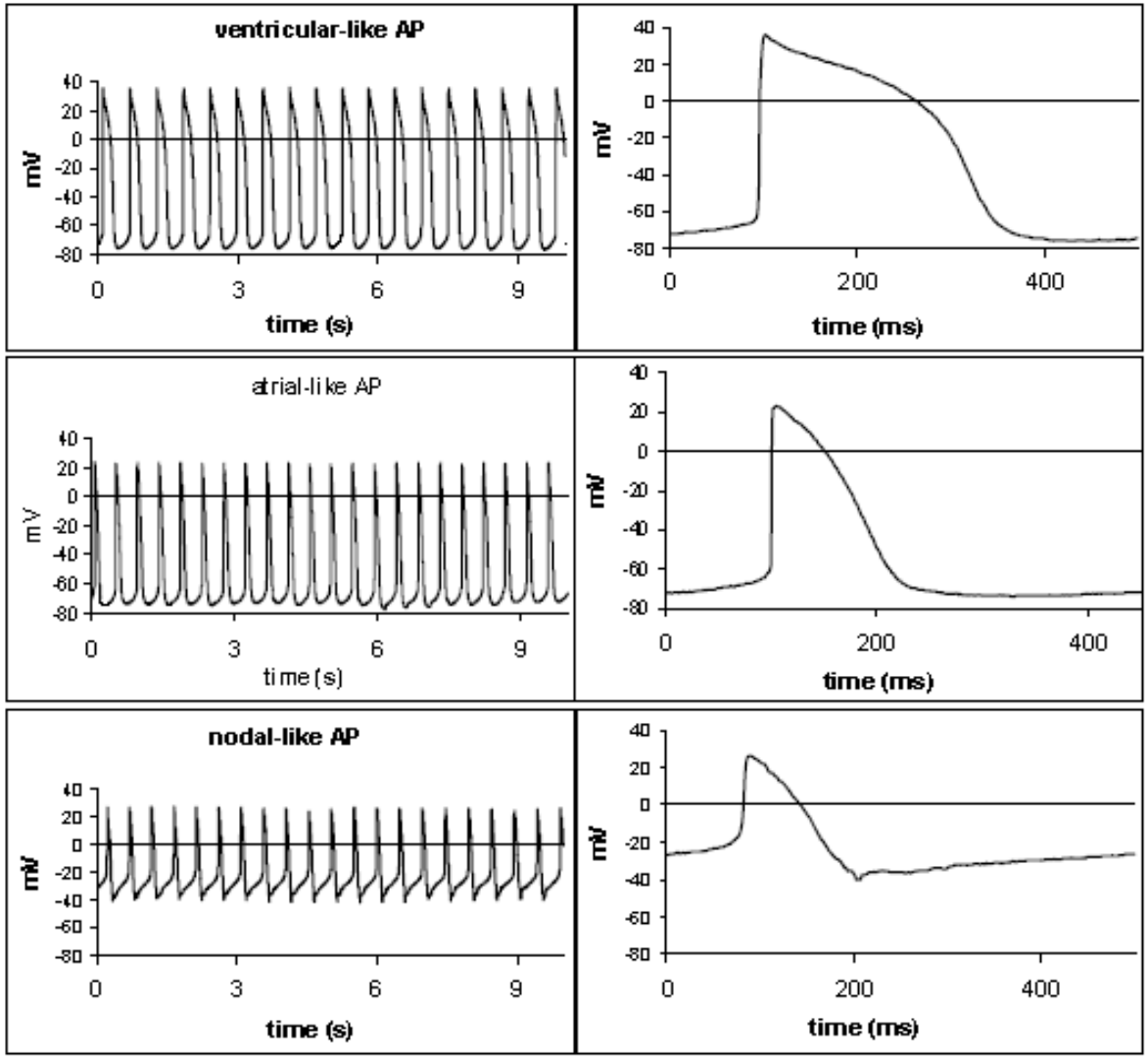
- Biomarker of life threatening ventricular arrhythmia
  - QT prolongation
  - hERG
  - Other risks
- Predictive biomarkers of drug-induced myocardial injury
  - -Troponin
- Contractility
  - morphological changes, hypertrophy
  - Ca $^{2+}$ dysregulation



Schematic representation of a normal ECG trace

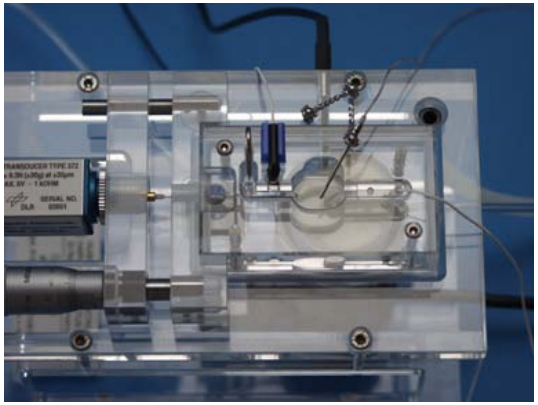


Action potential recordings in hES-CMC™
Demonstrating the presence of cardiac phenotypes



Jonsson et al, Stem Cell Res 2010

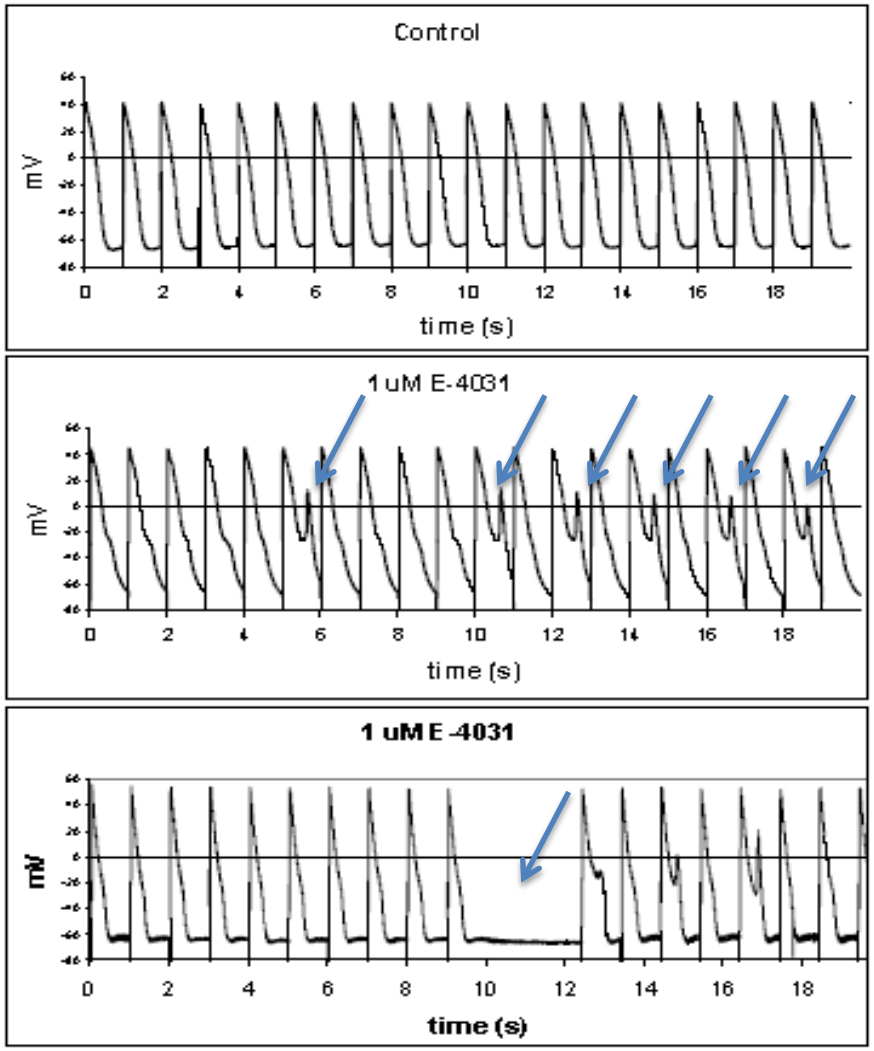
TAP recordings



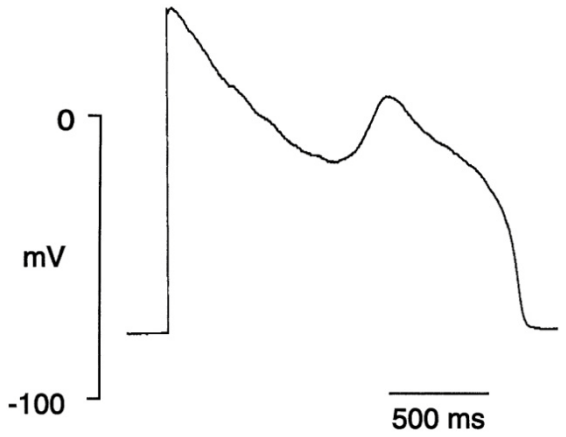
AstraZeneca

University Medical Center Utrecht, Utrecht, The Netherlands

hERG channel block in hES-CMC™ causes EADs



EADs (early afterdepolarizations) can result in torsades de pointes, tachycardia, and other arrhythmias

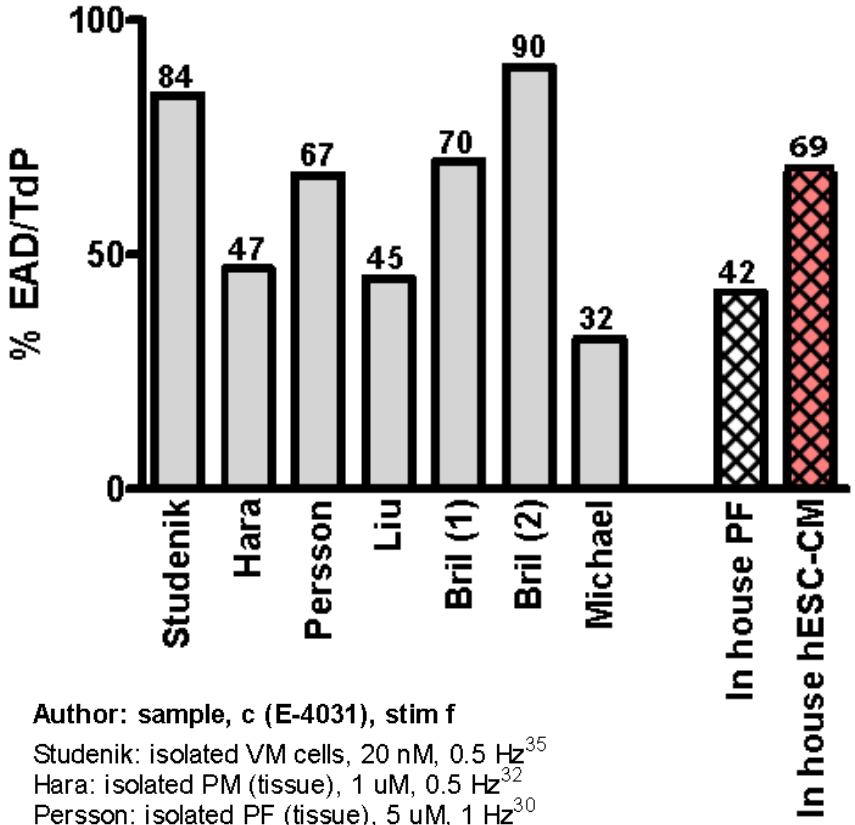


Occurance of EADs in human ventricular cardiomyocytes
From: Pelzmann et al 1997 Cardiovasc Res

Jonsson et al, Stem Cell Res 2010

University Medical Center Utrecht, Utrecht, The Netherlands

Incidence of EADs or TdP after E-4031 administration
Direct comparison of hES-CMC™ with established models



Author: sample, c (E-4031), stim f

Studenik: isolated VM cells, 20 nM, 0.5 Hz[35]
Hara: isolated PM (tissue), 1 uM, 0.5 Hz[32]
Persson: isolated PF (tissue), 5 uM, 1 Hz[30]
Liu: Langendorff heart, 0.5 uM, >1Hz[33]
Bril (1): in vivo (methoxamine), 10 ug/kg/min[31]
Bril (2): in vivo (methoxamine), 20 ug/kg/min[31]
Michael: in vivo (phenylephrine), 10 nmol/kg/min[34]

In house PF: isolated tissue, 1 uM, 1 Hz
In house hESC-CM: cell clusters, 1 uM, 1 Hz

Method:

Multicellular preparations: a) hES-CMC™
b) Rabbit PF

TAP (transmembrane action potential) rec.

Electrical pacing at 1 and 2 Hz

1 µM E-4031 (I_{Kr} blocker)

Jonsson et al Stem Cell Res 2010

University Medical Center Utrecht, Utrecht, The Netherlands

Comparison of I_{Kr} blocking drugs in 5 screening models of pro-arrhythmia

- Moxifloxacin was used as ”Negative control” (fourth generation synthetic fluoroquinolone antibacterial agent developed by Bayer AG)
- Tested in 5 different models
  - Chronic AV block (CAVB) dog
  - Methoxamine sensitized rabbit
  - hES-CMC™
  - Isolated dog CM
  - Isolated rabbit CM
- TdP and EADs as arrhythmogenic outcome
- Low (therapeutic) concentration: 5-10 µM
- High (supratherapeutic) concentration: 100 µM

L Nalos et al 2012 Br J Pharmacol

University Medical Center Utrecht, Utrecht, The Netherlands
AstraZeneca R&D, Mölndal, Sweden
Cellartis AB, Göteborg, Sweden
NOTOX B.V., DL 's-Hertogenbosch, The Netherlands.

IKr blocking (moxifloxacin): Results & Conclusion

Arrhythmia incidence (TdP/EAD)	Moxifloxacin		Dofetilide/E-4031
	Low dose	High dose	
CAVB dog	0%[a]		76%[h]/100%[i]
Methoxamine rabbit	0%[b]	17%[d]	83%[f]
hES-CMC	0%[c]	18%[e]	50%[g]
Isolated rabbit CM	23%[c]	33%[e]	63%[g]
Isolated CAVB CM	35%[c]		60%[g]

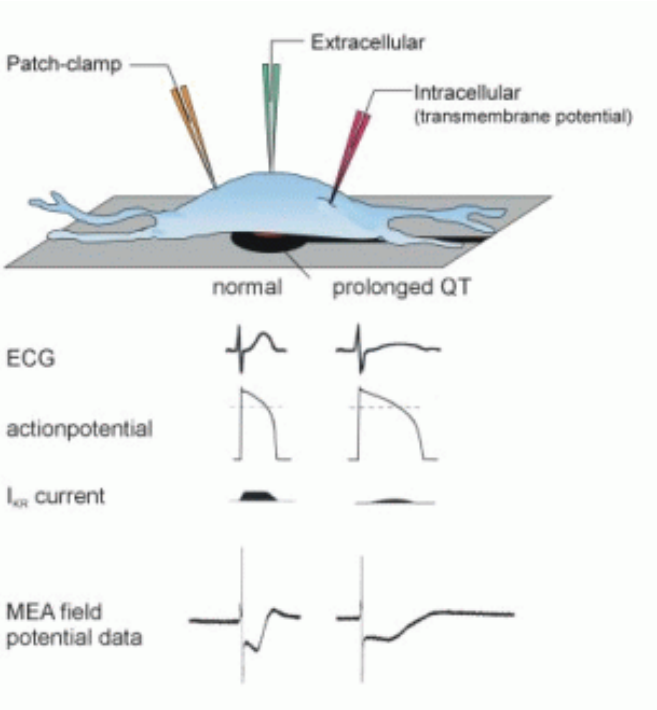
[a]2 mg/kg (10μM), [b]0.1 mg/kg/min (9±3μM), [c]10μM, [d]3 mg/kg/min (107±15μM), [e]100μM, [f]10 μg/kg/min, [g]1μM; [h]Oros *et al*., 2008, [i]Thomsen *et al*., 2006.

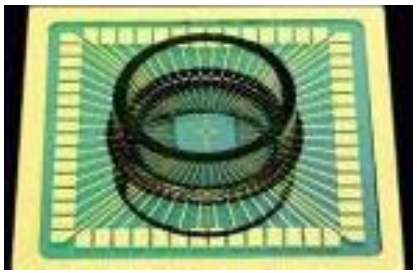
The very sensitive isolated cardiac myocyte assay lacks specificity to discriminate between safe and unsafe drugs. However, the selective response obtained from the hES-CMC model was comparable to the *in vivo* animal models.

Adapted from Nalos et al, 2012

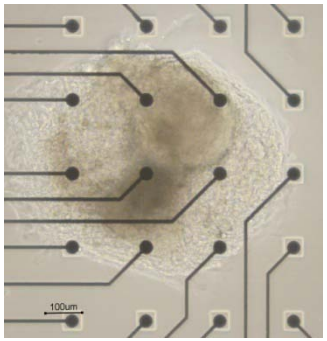
- University Medical Center Utrecht, Utrecht, The Netherlands
- NOTOX B.V., DL 's-Hertogenbosch, The Netherlands.

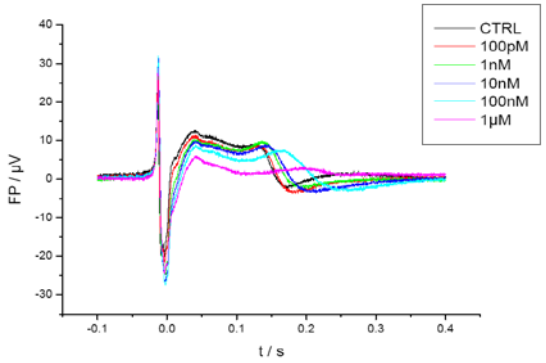
hPSC-derived cardiomyocyte clusters: MEA technology



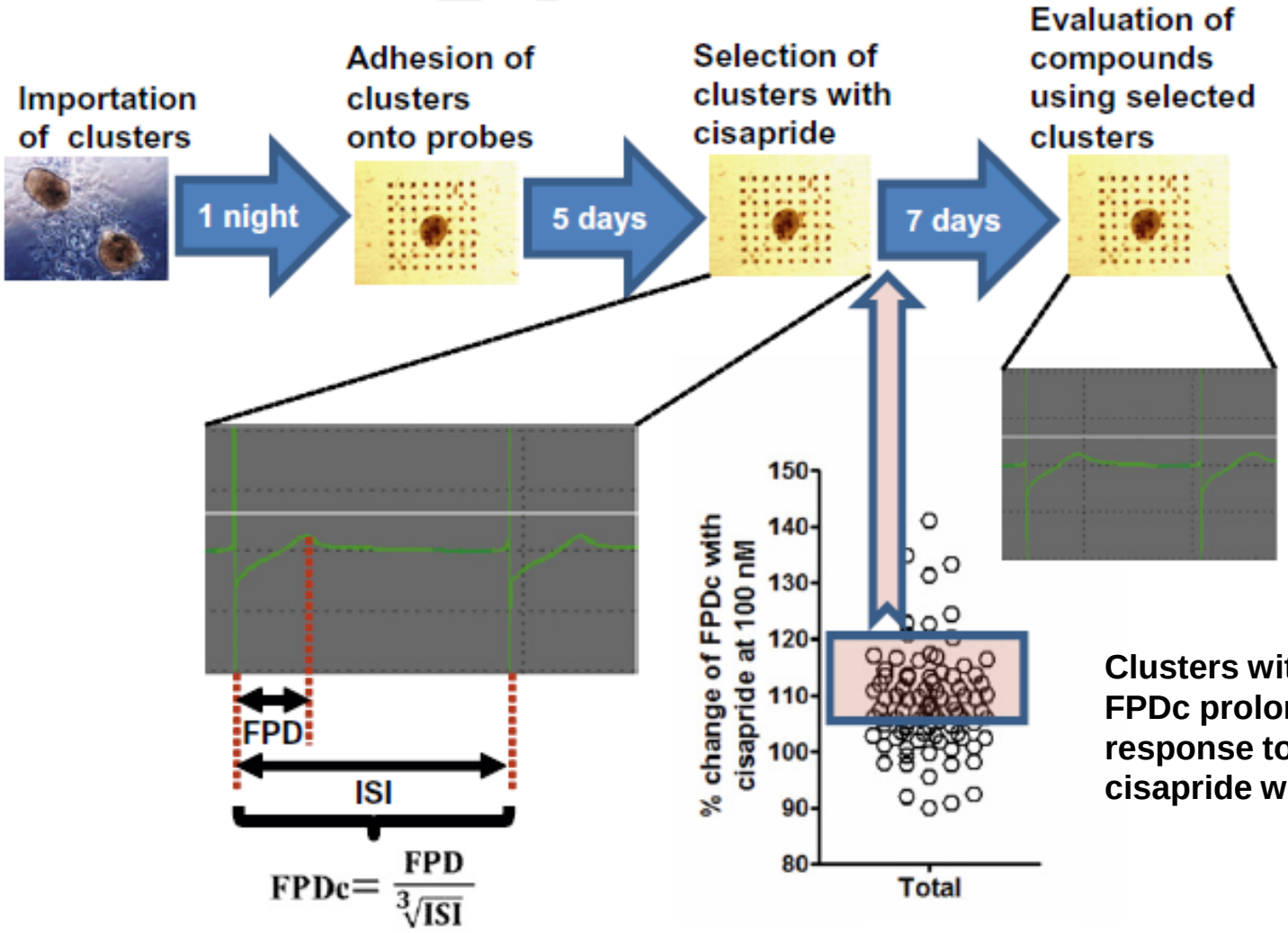


- Signal retrieval is non invasive
- Field potential measured (QT-prolongation, arrhythmia)
- Interpretation is similar to ECG





Pharmacological selection of hES-CMC™ for assessment of drug induced QT interval changes



Clusters with 5–20% FPDc prolongation in response to 100nM cisapride were selected.

Yamazaki et al 2011 *Toxicol In Vitro*

Changes in FPDc in hES-CMC™ treated with reference compounds

A. hERG channel blockers

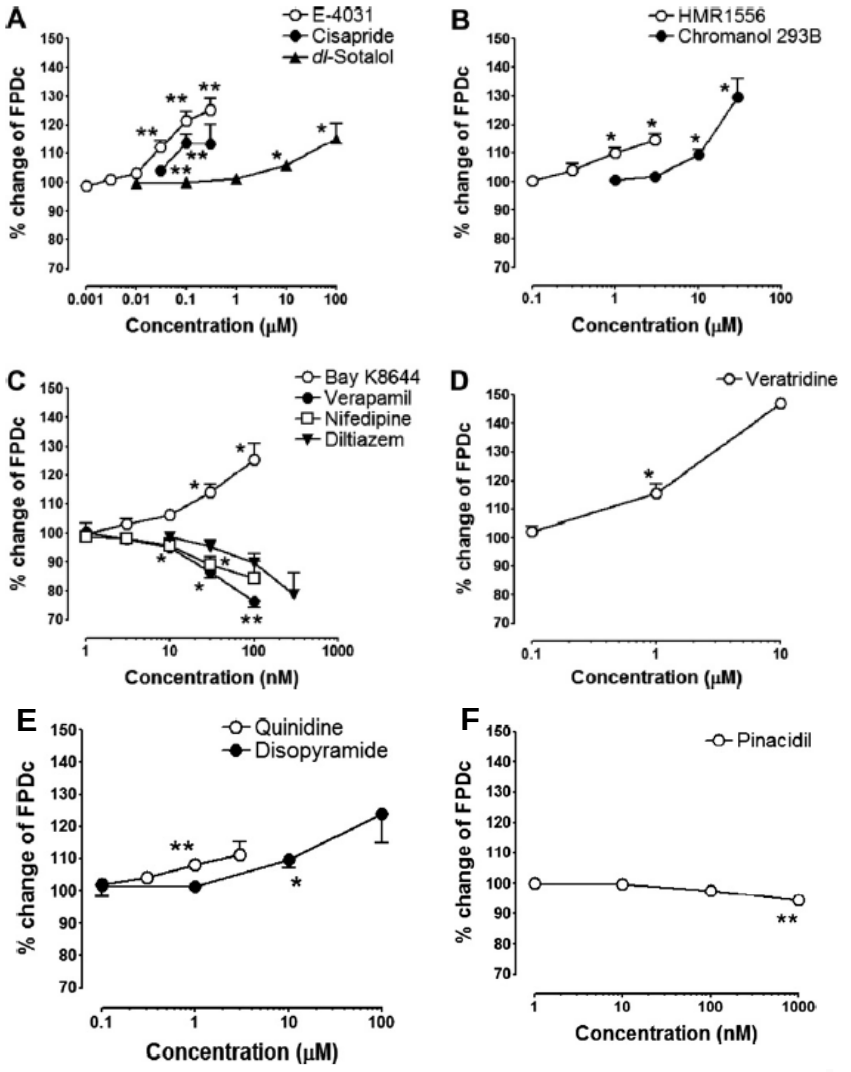
B. I_{Ks} blockers

C. Ca^{2+}-channel activator and blockers

D. Na^{+} channel opener

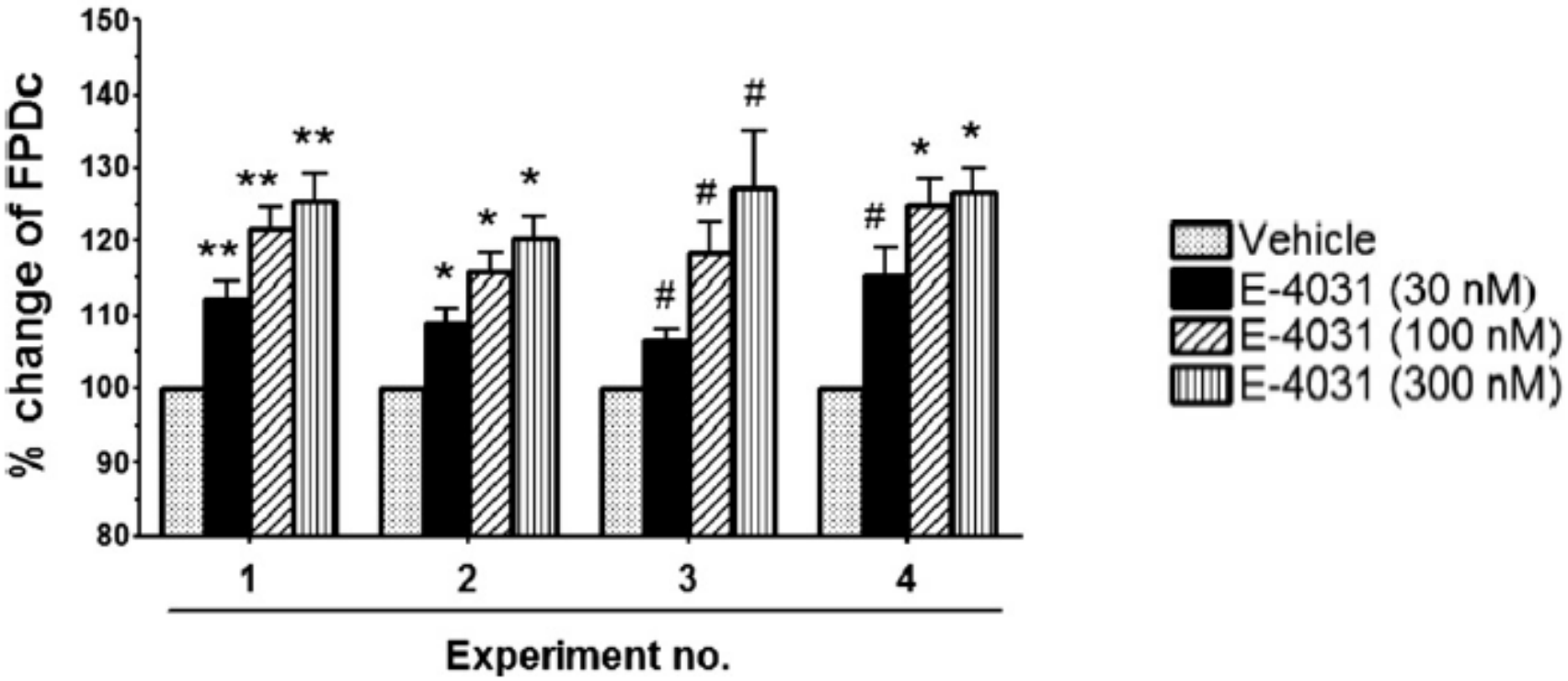
E. Class Ia antiarrhythmic drugs

F. K_{ATP} channel opener



Adapted from: Yamazaki et al 2011 *Toxicol In Vitro*

Reproducible results between different experiments and individuals



Experiments 1 and 4, and Experiments 2 and 3 were performed by different persons

Yamazaki et al 2011 *Toxicol In Vitro*

Conclusions

- hPSC can be differentiated to CMs with relatively high yield.

- Differences between hESC/hiPSC lines in terms of CM-differentiation efficiency.

- hPSC-CMs display many critical functional properties of human CMs, but in some aspects, hPSC-CMs display a fetal phenotype.

- hPSC-CMs can be used for drug testing. Especially, effects of I_{Kr} blockers can be predicted. Standardization of assays/cells is required.

- More research is needed to achieve the “adult” CM phenotype and to generate preparations of pure ventricular-, atrial-, and nodal-like CMs

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thank you …

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