

Combining electrophysiology and contractility recordings for more complete assessment of hiPSC-CMs



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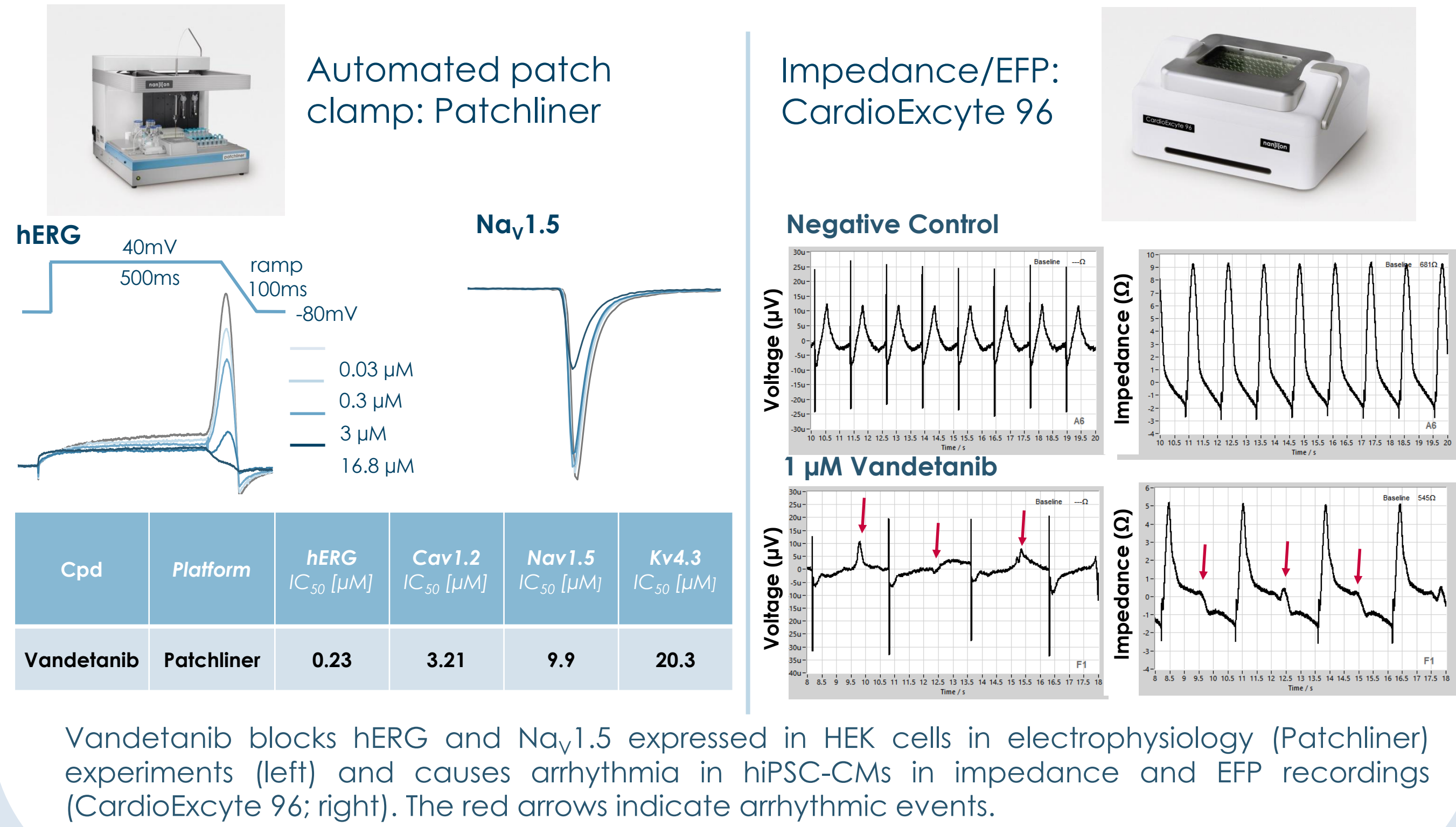
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Introduction

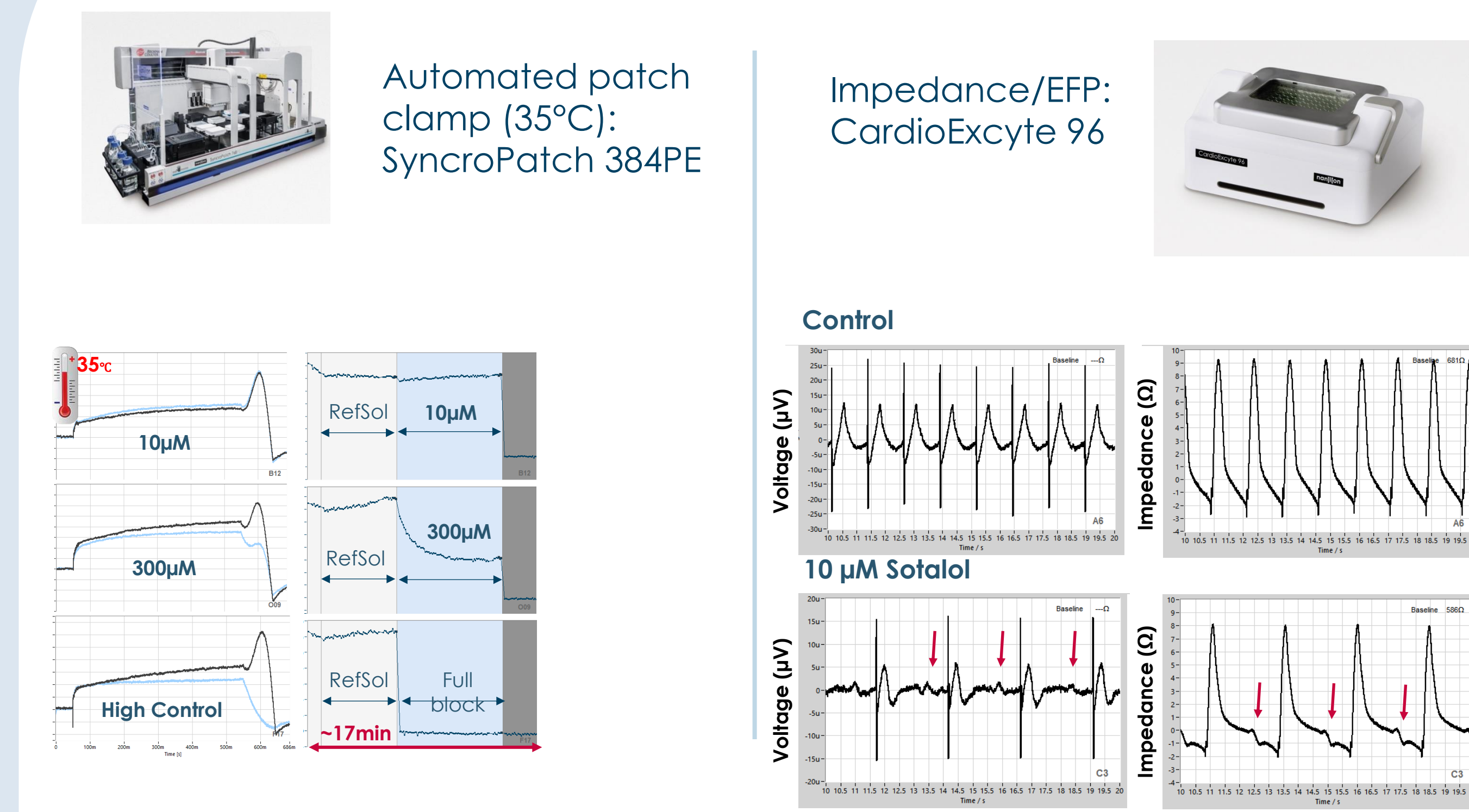
Human induced pluripotent stem cells (hiPSCs) are becoming increasingly important for cardiac safety testing due to their recapitulation of native behaviour, relative abundance and ease-of-use. We combine automated patch clamp (APC), impedance and extracellular field potential (EFP) measurements to study hiPSC-derived cardiomyocytes (hiPSC-CMs) from different sources. In line with the comprehensive in vitro proarrhythmia assay (CiPA) initiative, cardiac ion channels expressed in heterologous expression systems have been recorded on APC devices at room temperature and at physiological temperature and the effect of different pro-arrhythmic compounds on ion channels was investigated. In addition hiPSC-CMs were used on APC platforms recording from 8 or 384 wells simultaneously. Voltage-gated Na⁺ (Na_V), Ca²⁺ (Ca_V) and K⁺ (K_V) currents were recorded in voltage-clamp and action potentials recorded in current clamp mode. Using the dynamic clamp technique coupled with an APC device, electrically modelled I_{K1} was injected into the cell under current clamp conditions and this resulted in a more hyperpolarized and stable resting membrane potential.

Within the myocyte phase II study of the CiPA initiative, a device combining impedance-based contractility and extracellular field potential (EFP) recordings was used to investigate the effects of different compounds deemed low, intermediate and high risk by the FDA. Different hiPSC-CMs were used in a large cross-site evaluation and the results are summarised here.

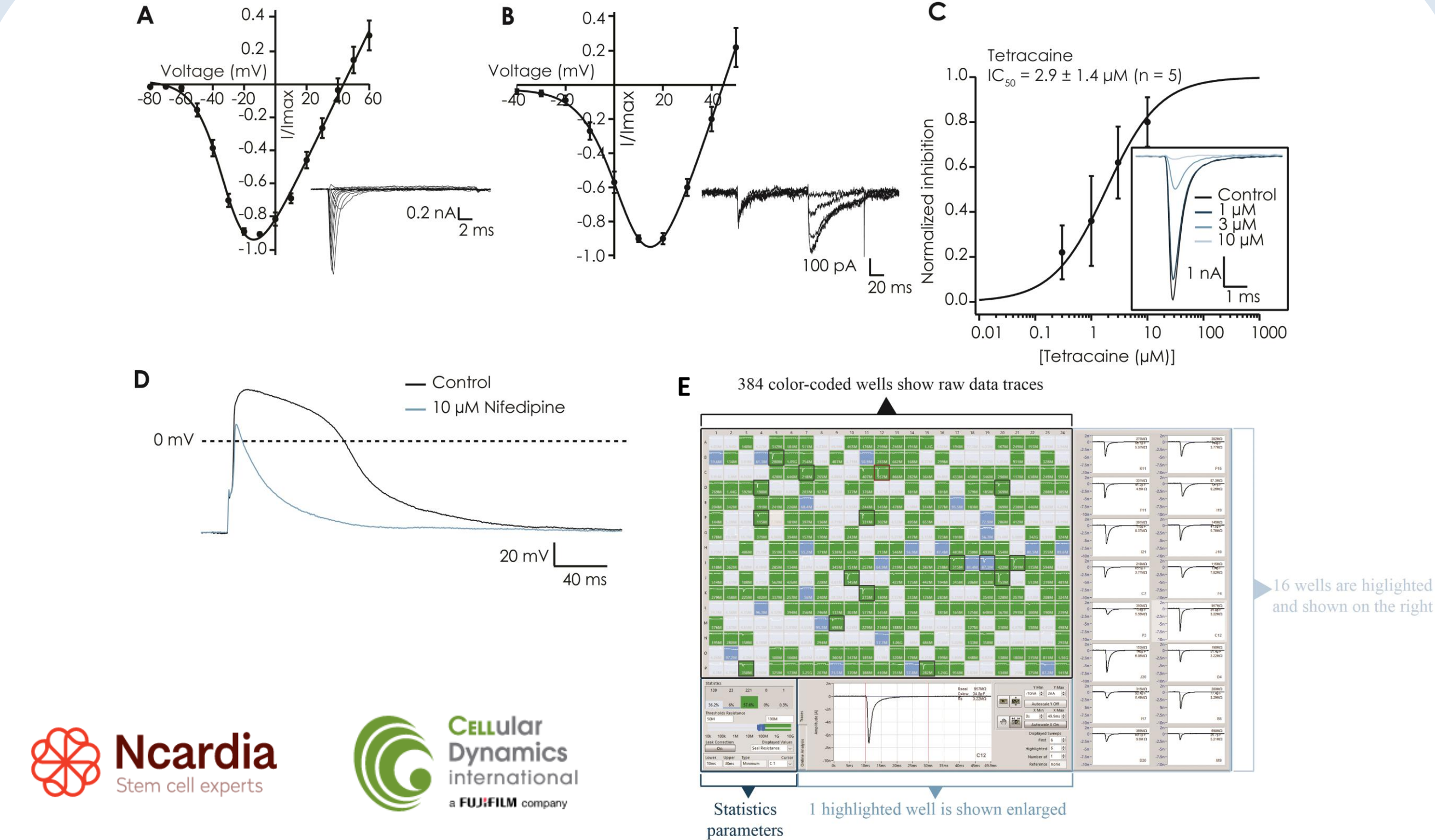
Effect of vandetanib on hERG and Na_V1.5: comparison with hiPSC-CMs



Effect of sotalol on hERG: comparison with hiPSC-CMs

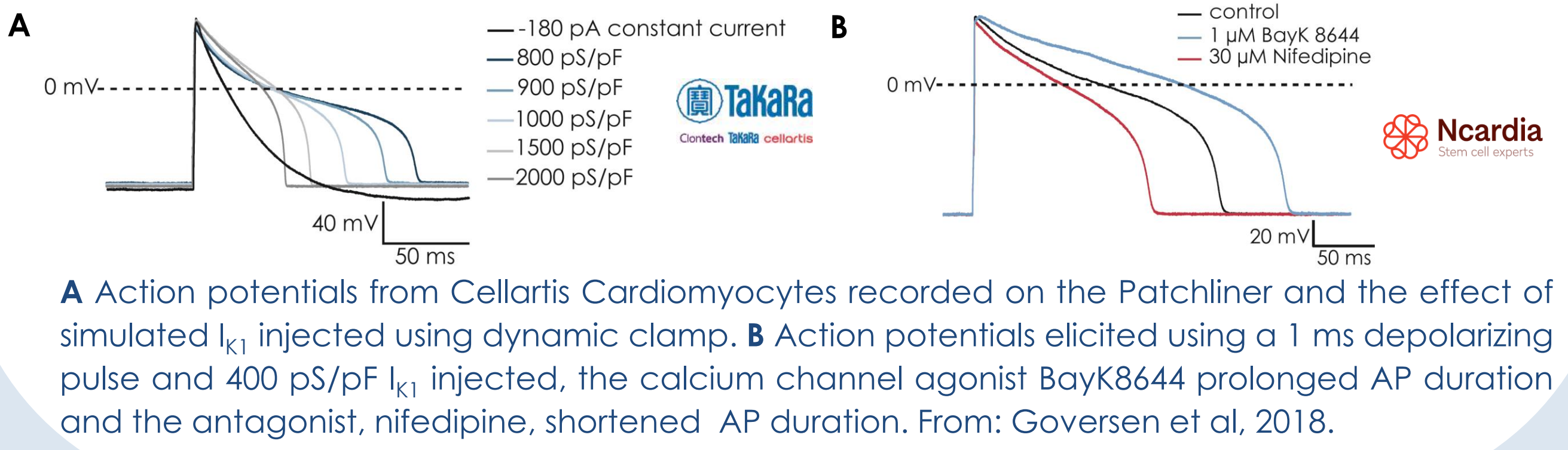


Cardiac ion channels in hiPSC-CMs



iCell® Cardiomyocytes recorded on the Patchliner. (A) Current-voltage relationship of Na_V and (B) Ca_V. V_{half} of activation for NaI_V was -29 mV and for CaI_V was 6.8 mV. (C) Concentration-response curve for tetracaine revealed an IC₅₀ of 2.9 ± 1.4 μM (n = 5). (D) Effect of nifedipine on the action potential. (E) Screenshot of the SyncroPatch 384PE software during a recording of Pluricyte® Cardiomyocytes. From: Obergrussberger et al, 2016.

Action potential duration using dynamic clamp on the Patchliner



Cross-site comparison of CiPA compounds using the CardioExcyte 96

Trend = illustrates trend of average FPD data with min and max values pointed out

FPD<10%

FPD>10%

FPD>10%

FPD>10%

†

††

type A arrhythmia

any other arrhythmia type

Test article concentrations (μM)											
Compound	Site / Cell type	Trend	ETPC-Free (μM)	⚡			⚡			⚡	
				Dose 1	Dose 2	type	Dose 3	type	Dose 4	type	
HIGH RISK											
DJ Sotalol	Site 1, CDI		15	0.1	1	-	10	††	100	††	
DJ Sotalol	Site 2, CDI		15	0.1	1	-	10	††	100	††	
DJ Sotalol	Site 3, CDI		15	0.1	1	-	10	††	100	††	
DJ Sotalol	Site 1, AXG		15	0.1	1	-	10	-	100	††	
DJ Sotalol	Site 2, AXG		15	0.1	1	-	10	-	100	††	
DJ Sotalol	Site 4, AXG		15	0.1	1	-	10	-	100	††	
DJ Sotalol	Site 2, CLS		15	0.1	1	-	10	-	100	††	
DJ Sotalol	Site 4, CLS		15	0.1	1	-	10	-	100	††	
DJ Sotalol	Site 2, PLR		15	0.1	1	-	10	††	100	††	
INTERMEDIATE RISK:											
Domperidone	Site 2, CDI		0.02	0.003	0.03	-	0.3	††	3	††	
Domperidone	Site 2, AXG		0.02	0.003	0.03	-	0.3	††	3	††	
Domperidone	Site 2, CLS		0.02	0.003	0.03	-	0.3	††	3	††	
Domperidone	Site 2, PLR		0.02	0.003	0.03	-	0.3	††	3	††	
LOW RISK											
Ranolazine	Site 2, CDI		1.95	0.1	1	-	10	-	100	quiescence	
Ranolazine	Site 3, CDI		1.95	0.1	1	-	10	-	100	††	
Ranolazine	Site 2, AXG		1.95	0.1	1	-	10	-	100	quiescence	
Ranolazine	Site 2, CLS		1.95	0.1	1	-	10	-	100	††	
Ranolazine	Site 2, PLR		1.95	0.1	1	-	10	-	100	††	

EFP

Impedance

Arrhythmia type

Type A

Type B

Type C

Type D

Type E

Excerpt of cross-site study using the CardioExcyte 96 with example of high, intermediate and low risk compounds on field potential duration (FPD) in EFP mide hiPSC-CMs. From: Bot et al., 2018.

Conclusions

- Cardiac ion channels expressed in cell lines have been measured on the Patchliner and SyncroPatch 384PE as part of the CiPA Ion Channel Work Stream.
- hiPSC-CMs have been used on the Patchliner and SyncroPatch 384PE, voltage-gated ion channels have been recorded and pharmacology performed. Action potentials were recorded in current clamp mode and dynamic clamp combined with the Patchliner.
- hiPSC-derived cardiomyocytes were used on the CardioExcyte 96 as a complementary tool for safety screening. The effect compounds on ion channels is often complex and the overall effect in cardiomyocytes on beating parameters is an important and complementary assay to patch clamp in heterologous expression systems.

References

Obergrussberger, A, et al., 2016. In: Stem cell-derived models in toxicity. Chapter 4. pp. 57-82
Goversen, B, et al., 2018. Front. Physiol. 8:1094
Bot, C, et al., 2018. J. Pharmacol. Toxicol. Methods. doi: 10.1016/j.vascn.2018.06.006

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