#6

Action Potential Waveform Analysis in Human IPSC-Cardiomyocytes Enables Mechanistic Assessment of Multichannel Cardiac Effects

Charlotte Hill and Robert Kirby

Metrion Biosciences Ltd., Cambridge, UK

metrionbiosciences.com

Introduction

Recent regulatory changes, including the FDA Modernisation Act 2.0 and the UK government's Replacing Animals in Science strategy, have accelerated the adoption of human-relevant New Approach Methodologies (NAMs) or Non-Animal Alternatives (NAAs) for nonclinical safety assessment. In cardiac safety evaluation, reliance on single ion channel assays, such as the rapid delayed rectifier potassium channel hERG (K_V11.1), may fail to capture the integrated electrophysiological effects that underlie proarrhythmic risk.

Parameters derived from action potential waveform analysis, including triangulation, engagement of repolarisation reserve and presence of early afterdepolarisations (EADs), are recognised as contributors to arrhythmogenic liability. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a translationally relevant system in which integrated multichannel cardiac effects can be assessed.

Aim

The aim of this study was to evaluate whether optical voltage imaging of hiPSC-CM action potential waveforms can detect compound-induced electrophysiological changes beyond hERG block alone and enable mechanistic interpretation through correlation with ion channel electrophysiology data.

Methods

hiPSC-CMs (iCell[2]; Fujifilm CDI) were seeded into 96-well plates. After 8 days, cells were loaded with voltage-sensitive dye (BeRST[1]) and placed in the Lumencor VOLTA scanner, set to 28°C. Action potentials were measured optically for 40 s using fluorescence (10 kHz; excitation 660 nm/emission 680 nm). Compounds were applied (10 concs; 0.02 nM – 30 µM), incubated for 30 min and 24 h, followed by a 40 s recording. Clinical QTc risk analysis of ycAPD$_{90}$[3] was performed using the thresholds and equations according to Kilfoil *et al.,* 2021[2]. Triangulation was calculated per well as APD$_{30}$/APD$_{90}$ and normalised to vehicle (0.1% DMSO), where a decrease in ratio indicates an increase in triangulation (**Figure 1 a**). Whole-cell voltage clamp experiments were performed at 23°C on CHO cells stably expressing hERG using the Sophion QPatch48 platform. Recording solutions: extracellular (in mM) NaCl 140, KCl 2, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, glucose 5; intracellular (in mM) KF 120, KCl 20, HEPES 10, EGTA 10. Currents were elicited using the voltage protocol shown in **Figure 1 b**.

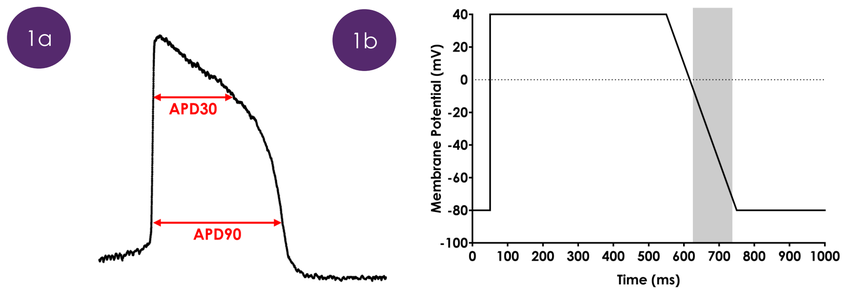
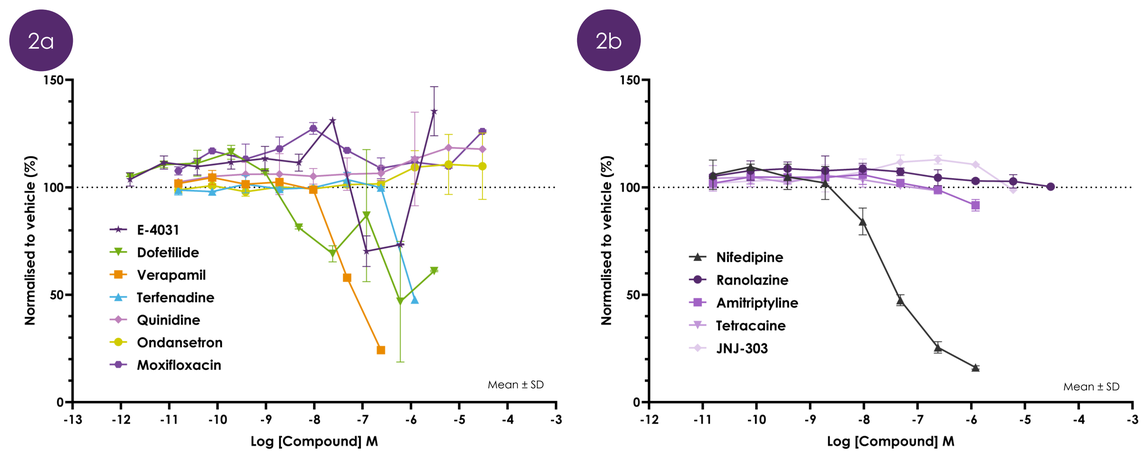


Figure 1. a) Example hiPSC-CM action potential showing APD$_{30}$ and APD$_{90}$. b) QPatch hERG voltage protocol used in this study.

Results

- Compound-mediated effects on APD$_{30}$ were more consistent and more pronounced for some compounds, such as verapamil and nifedipine, indicating reduced inward calcium current (**Figure 2**).

Effect of compounds on APD$_{30}$



- Dofetilide and E-4031 increased APD$_{90}$ and triangulation at low nM concentrations (**Figures 3 a and 4 a**) but also affected APD$_{30}$ at µM concentrations (**Figure 2 a**), which is where EADs were observed in raw traces (**Figure 5**), indicating instability of the action potential.

Effect of compounds on ycAPD$_{90}$[3] after 30 min incubation

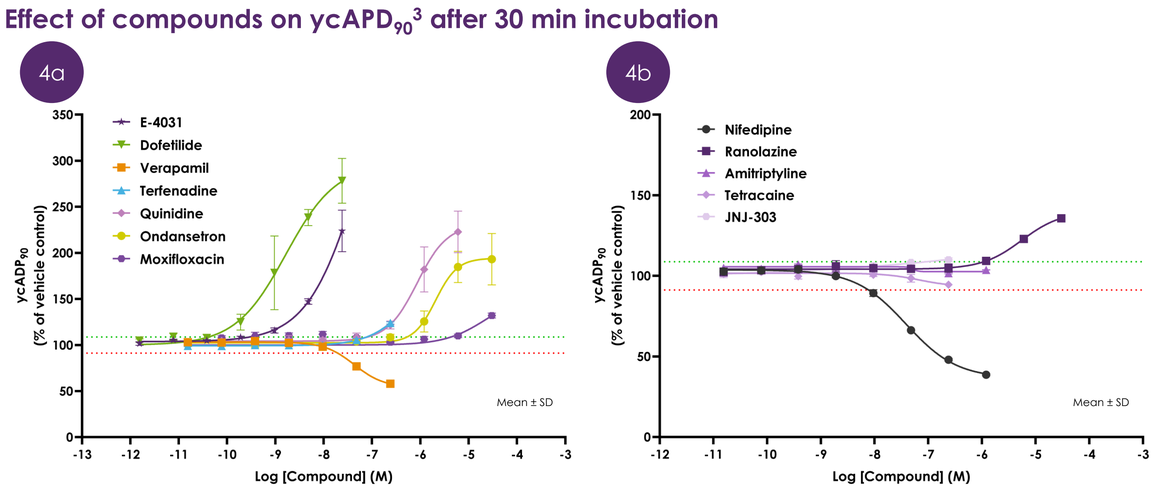


Table 1. Summary of estimated clinical QTc risk, hERG inhibition and EFTPC

Compound	30 min[2] ycAPD$_{90}$[3] – Exposure estimated to produce 10 ms increase in clinical QTc (nM)	24 h[2] ycAPD$_{90}$[3] – Exposure estimated to produce 10 ms increase in clinical QTc (nM)	Mean hERG IC$_{50}$ (nM)	Expected Free Therapeutic Plasma Concentration (EFTPC; nM)
E-4031	0.1	0.9	-	-
Dofetilide	0.1	0.2	14	0.3[4] - 2.1[5]
Verapamil	*10*^	*14*^	674	45[5] - 88[6]
Terfenadine	38	92	166	0.3[5] - 9[6]
Quinidine	47	61	1150	843[5] - 3237[7]
Ondansetron	273	260	5220	178.6[7] - 358.5[5]
Moxifloxacin	3566	8452	82800	2792[4] - 10960[6]
Nifedipine	*3.7*^	*5.2*^	-	8[6] - 14.7[7]
Ranolazine	793	1323	-	1948[5] - 2311[7]
Amitriptyline	-	*657*^	-	36.4[7]
Tetracaine	-	1278	-	-
JNJ-303	30	91	-	-

^*Decrease in ycAPD$_{90}$; - = not available*

Conclusions

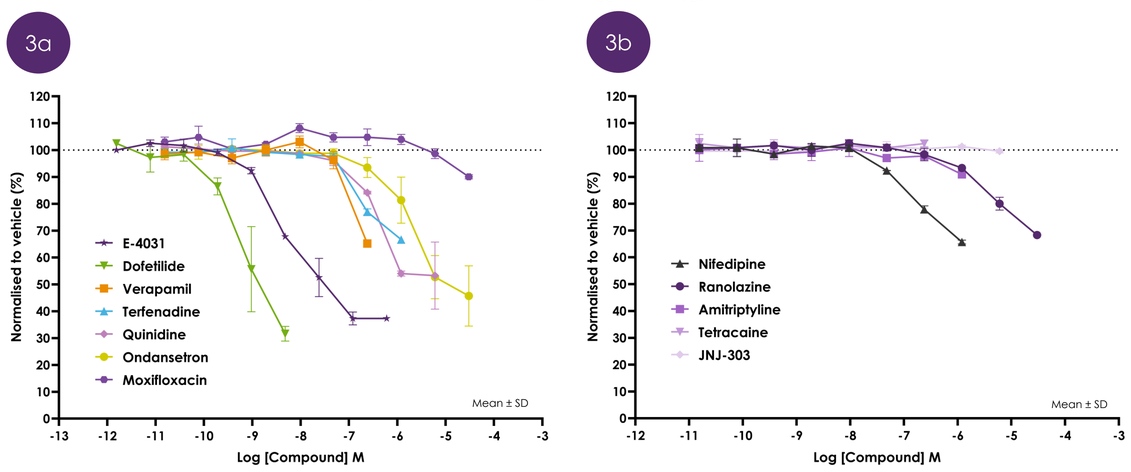
- Action potential waveform analysis in hiPSC-CMs enables mechanistic differentiation of multi-ion channel cardiac effects beyond hERG inhibition and action potential duration, supporting its application in human-relevant cardiac safety assessment.

- These findings demonstrate that optical voltage imaging of hiPSC-CM action potentials provides mechanistic insight into multi-ion channel cardiac effects that cannot be captured by hERG alone.

- The selective hERG inhibitors, dofetilide and E-4031, produced an early and pronounced increase in triangulation (**Figure 3 a**).

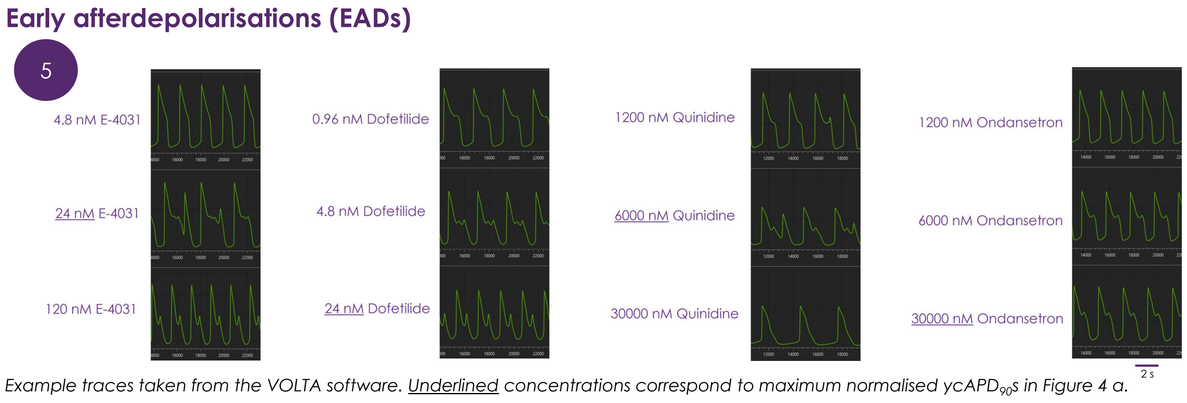
- Multi-ion channel effect compounds, such as verapamil, terfenadine, quinidine and ondansetron, all showed concentration-dependent increases in triangulation at higher exposures (**Figure 3 a**), consistent with modulation of inward (e.g. I_{CaL}) and outward (e.g. I_{Kr}) currents.

Effect of compounds on triangulation ratio (APD$_{30}$/APD$_{90}$)



- Compounds with multi-ion channel effects, such as quinidine and ondansetron showed less pronounced effects on APD$_{30}$ (**Figure 2 a**), despite the presence of EADs (**Figure 5**).

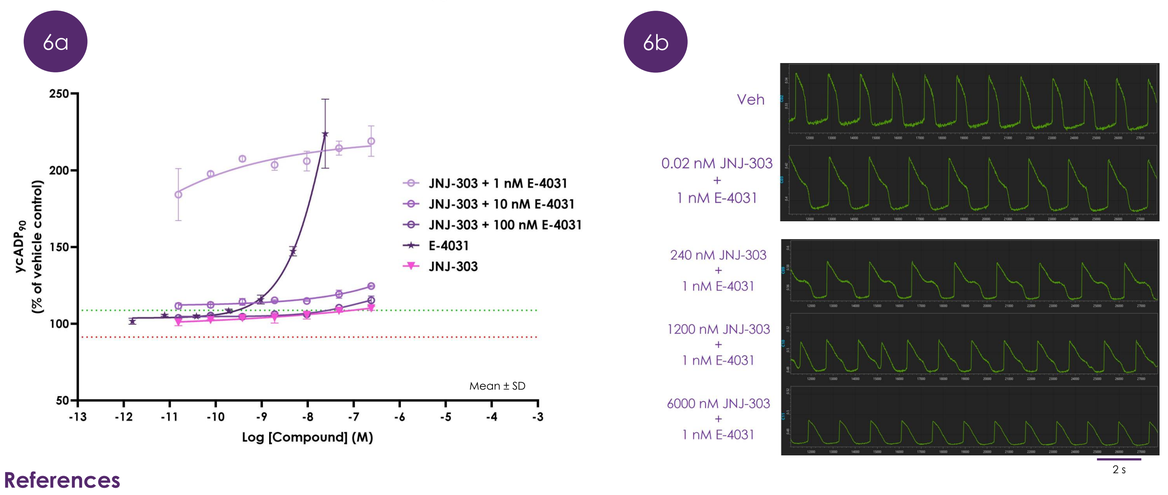
Early afterdepolarisations (EADs)



Example traces taken from the VOLTA software. Underlined concentrations correspond to maximum normalised ycAPD$_{90}$s in Figure 4 a.

- Low concentration of E-4031 resulted in reduction in repolarisation reserve by synergistic inhibition of hERG and hK$_V$LQT1/mink to increase APD (**Figure 6**).
- Higher concentrations of E-4031 resulted in prolongation of the action potential early in phase 2, which can shift voltage-dependent inactivation kinetics of I_{CaL} and counterbalance further APD prolongation (**Figure 6**).

Repolarisation reserve and ycAPD$_{90}$[3]



References

[1] Huang, Y.L., Walker, A.S., & Miller, E.W. (2015). PMID: 26237573.
[2] Kilfoil, P. *et al.,* (2021). PMID: 34678241.
[3] Yamamoto, W. *et al.,* (2016). PMID: 27923051.
[4] ICH E14/S7B Q&As Training Material Examples Supplemental File (2022).
[5] Crumb, W.J. *et al.,* (2022). PMID: 27060526.
[6] Kramer J. *et al.,* (2013). PMID: 23812503.
[7] Heitmann, S., Vandenberg, J.I., Hill, A.P. (2023). PMID: 38079357.